

Enumeration and Characterization of *Salmonella* Isolates from Retail Chicken Carcasses in Beijing, China

Yeru Wang,^{1,2,*} Qian Chen,^{3,*} Shenghui Cui,² Xiao Xu,² Jianghui Zhu,¹
Haipeng Luo,² Di Wang,³ and Fengqin Li¹

Abstract

Epidemiological reports have implicated contaminated raw or undercooked chicken as primary vehicles of *Salmonella* transmission to human beings. Risk assessments relating to *Salmonella* contamination of poultry products in China are frequently hampered by the lack of quantitative data. In this study, whole chicken carcasses ($n=395$) were collected from the retail markets of Beijing, and the level of *Salmonella* contamination was enumerated by most probable number (MPN) analysis and all *Salmonella* isolates were further characterized for their serotypes and antimicrobial resistance. Overall, 49.9% (197/395) of the retail whole chicken carcasses were contaminated by *Salmonella* and the MPN values ranged from 1.5 to >550 MPN/100 g. The 50% percentile of *Salmonella* MPN value was 7.5 MPN/100 g in chicken carcass. The predominant serotypes isolated were *Salmonella* Enteritidis ($n=309$, 94 samples), *Salmonella* Indiana ($n=205$, 54 samples) and *Salmonella* Infantis ($n=89$, 23 samples). Multidrug-resistant *Salmonella* isolates were recovered from 100 chicken carcass samples; 102 isolates (from 41 chicken carcasses) even showed resistance to both ciprofloxacin and cefotaxime. Our findings showed a high prevalence of *Salmonella* contamination in retail chicken carcasses, which could be a source of exposure for consumers to multidrug-resistant isolates. This study provided baseline enumeration data for the risk assessment and evaluation of new control measures of *Salmonella* contamination in retail chicken products.

Introduction

NONTYPHOIDAL *SALMONELLA* *SPP.* are one of the leading causes of foodborne bacterial gastroenteritis worldwide, and the incidence of salmonellosis in China is ranked fourth among foodborne diseases caused by microbial agents (Todd, 1997; Zhu *et al.*, 2012). Epidemiological reports have implicated contaminated raw or undercooked chicken as primary vehicles of *Salmonella* transmission to human beings (M'Ikanatha *et al.*, 2010; Fearnley, *et al.*, 2011). Numerous studies have investigated the prevalence and characteristics of *Salmonella* in retail chicken carcasses, and high prevalence has been observed worldwide because of inevitable cross-contamination during the slaughtering process (Meldrum *et al.*, 2007; Zhao *et al.*, 2001; Olsen *et al.*, 2003). Besides high *Salmonella* prevalence, chicken products have become a major source of multidrug-resistant (resistant to three or more categories of antimicrobials) *Salmonella* isolates in recent years because of the prophylactic or therapeutic application of antimicrobials in broiler farms (Ahmed *et al.*, 2009; Thai

et al., 2012). *Salmonella* isolates in chicken products have raised great concerns because of the limited salmonellosis treatment alternatives, especially with the spreading of fluoroquinolone and/or third-generation cephalosporin-resistant *Salmonella* isolates (Lestari *et al.*, 2009; Lu *et al.*, 2011).

Risk assessments relating to food safety in China are frequently hampered by the lack of quantitative data. As a key element of *Salmonella* transmission to the consumers, retail chicken products have been recognized as priority for the risk assessment in different agencies to get an estimate of the risk of human salmonellosis due to consumption of chicken (Marcus *et al.*, 2007; Smadi *et al.*, 2013). The prevalence and numbers of *Salmonella* in retail chicken are a clear reflection of consumer exposure and are required for quantitative microbial risk assessment models and the development of strategies to reduce risk from this pathogen. To the present time, however, no study has been conducted for *Salmonella* enumeration in retail chicken products in China.

In order to provide the scientific data for quantitatively assessing the impact of *Salmonella* on Chinese public health

¹Key Laboratory of Food Safety Risk Assessment, Ministry of Health, China National Center for Food Safety Risk Assessment, Beijing, China.

²National Institutes for Food and Drug Control, Beijing, China.

³Beijing Municipal Center for Disease Prevention and Control, Beijing, China.

*These two authors contributed equally to this work.

and safety resulting from consumption of retail poultry products, whole chicken carcasses were collected from 39 retail markets in Beijing, and the level of *Salmonella* contamination was enumerated by most probable number (MPN) analysis; the isolates were further characterized for their serotypes and antimicrobial resistance.

Materials and Methods

Sample collection and preparation

From August 2010 to March 2012, fresh ($n=243$) or frozen ($n=152$) retail whole chicken carcasses were randomly collected from 31 supermarkets ($n=266$) and eight farmer's markets ($n=129$) every 2 weeks in Beijing city. From supermarkets, individually packed chicken carcasses were collected and from farmer's markets, birds were processed and packaged on site. On each sampling day, no more than five chicken carcasses were randomly selected from each sampling site and transported on ice to the laboratory within 1 h. Each sample was immediately aseptically removed from the package and placed in a 3500 stomach bag (Seward, UK) followed by the addition of 500-mL buffered peptone water (BPW; Becton-Dickinson, Beijing, China) per kilogram of carcass. The bag was manually massaged for 3–5 min and the rinse was used for *Salmonella* MPN analysis; the whole analysis process must be finished within 2 h.

Salmonella enumeration and serotyping

Salmonella MPN in chicken carcass rinse was determined by using a modified method based on the United States Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) Microbiology Laboratory Guidebook (USDA/FSIS, 2008). Briefly, triplicate 10 mL of BPW chicken rinses were taken directly from each stomach bag and placed into three empty sterile test tubes, and then 1 mL of sample rinse was transferred to triplicate 9-mL BPW dilution tubes followed by 1:10 dilution in triplicate tubes of BPW. All tubes were pre-enriched in a shaking incubator at 100 rpm/min for 22–24 h at 37°C. From each tube, 0.5 mL or 0.1 mL of the pre-enrichment culture were transferred to 10 mL tetrathionate broth (TT; Becton-Dickinson) or Rappaport-Vassiliadis (RV; Becton-Dickinson) broth and incubated at 42±1°C with shaking at 100 rpm for 22–24 h. After selective enrichment, a loopful of TT or RV broth culture was streaked on xylose lysine tergitol 4 (XLT4; Becton-Dickinson) agar, and incubated at 37±1°C overnight. No more than one presumptive *Salmonella* colony on each XLT4 plate was inoculated onto triple sugar iron slant (Becton-Dickinson) and incubated at 35±1°C for 24 h. Isolates with typical *Salmonella* phenotypes were confirmed by amplification of the *invA* gene by polymerase chain reaction (Malorny *et al.*, 2003) and the API 20E test (BioMérieux, Beijing, China). For each of nine pre-enrichment tubes from one chicken carcass rinse, *Salmonella* was counted as positive if *Salmonella* was recovered from either TT or RV selective-enrichment broth. Since each kilogram of chicken sample was rinsed in 500 mL BPW, MPN/100 g of in chicken carcass was equal to the MPN of 50-mL chicken rinses. For all confirmed *Salmonella* isolates, serotypes were determined by slide agglutination with commercial *Salmonella* antisera (Statens Serum Institute, Denmark) following the Kauffmann–White–Le Minor scheme. All *Salmonella* isolates obtained from

a single sample were tested for serotypes and antimicrobial susceptibility.

Antimicrobial susceptibility testing

Minimal inhibitory concentrations to 10 antimicrobials were determined via the agar microdilution method for all *Salmonella* isolates, including ampicillin (AMP), ampicillin-sulbactam (SAM), ceftazidime (CAZ), cefotaxime (CTX), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), imipenem (IMP), nalidixic acid (NAL) and tetracycline (TET). All susceptibility results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) interpretive standards (CLSI, 2012). *Escherichia coli* ATCC 25922 and *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603 were used as quality-control organisms in antimicrobial susceptibility experiments.

Statistical analysis

Factors related to the frequencies and MPN of *Salmonella* in chicken carcasses were statistically analyzed. The differences in frequencies were analyzed by chi-square, and the differences in MPN were analyzed by the nonparametric test using SPSS version 17.0.

Results

Sample collection and MPN determination

A total of 874 *Salmonella* isolates were recovered from 197 (197/395, 49.9%) whole chicken carcasses (Table 1). On average, 86.5 MPN/100 g of *Salmonella* were detected in the chicken carcass rinse and 4.43 isolates were actually recovered for each positive sample. Six samples showed *Salmonella* loads greater than 550 MPN/100 g. The 50% percentile of *Salmonella* MPN value was 7.5 MPN/100 g in chicken carcass. Chicken carcasses in summer showed the highest *Salmonella* load (152.9 MPN/100 g), but samples collected in winter showed the highest contamination rate (64.4%). Fresh chicken carcasses showed significantly higher *Salmonella* MPN value and contamination rate than frozen chicken carcasses ($p<0.05$). Chicken carcasses collected from the farmer's market also showed a significantly higher *Salmonella* MPN value and contamination rate than samples from the supermarkets ($p<0.01$). Using TT broth as the selective enrichment media, a significantly higher *Salmonella* recovery rate (42.3%) was obtained than using RV broth (30.9%) from chicken carcass rinse ($p<0.01$), but 180 *Salmonella* isolates were only recovered by using RV broth, instead of TT broth (Table 2).

Salmonella serotyping

After serotyping, 24 distinct serotypes were identified, including six dominant serotypes and 18 minor serotypes (Table 2). Isolates of more than one distinct *Salmonella* serotype were recovered in 55 chicken carcasses and Enteritidis–Indiana (11 samples), Enteritidis–Infantis (five samples), and Enteritidis–Typhimurium (four samples) ranked as the top three serotype combinations. For *Salmonella* Enteritidis and minor serotypes, using TT broth for selective enrichment showed a significantly higher recovery rate than using RV broth ($p<0.01$).

TABLE 1. MOST PROBABLE NUMBER (MPN) ESTIMATES OF *SALMONELLA* CONTAMINATION IN CHICKEN CARCASS RINSE IN BEIJING, CHINA

Characteristics	No. of positive samples	MPN values (MPN/100 g)					
		Mean \pm SD	MPN _{Min}	MPN _{10%}	MPN _{50%}	MPN _{90%}	MPN _{Max}
Total (<i>n</i> =395)	197 (49.9%)	86.5 \pm 169.8	1.5	1.8	7.5	550	> 550
Seasons ^a							
Spring (<i>n</i> =113)	58 (51.3%)	48.9 \pm 138.7	1.5	1.7	4.6	55.1	> 550
Summer (<i>n</i> =88)	41 (46.6%)	152.9 \pm 196.8	1.5	4.6	75	550	> 550
Autumn (<i>n</i> =107)	42 (39.3%)	57.6 \pm 130.5	1.5	1.8	4.6	230	550
Winter (<i>n</i> =87)	56 (64.4%)	98.6 \pm 190.7	1.5	1.7	6.1	550	> 550
Sample categories							
Fresh chicken (<i>n</i> =243)	158 (65.0%)	91.6 \pm 170.6	1.5	1.8	11.5	550	> 550
Frozen chicken (<i>n</i> =152)	39 (25.7%)	66.2 \pm 170.0	1.5	1.8	3.7	206	> 550
Market categories							
Supermarkets (<i>n</i> =266)	119 (44.7%)	66.3 \pm 153.3	1.5	1.5	4.7	230	> 550
Farmers' markets (<i>n</i> =129)	78 (60.5%)	117.4 \pm 189.2	1.5	1.8	12.8	550	> 550
Enrichment broths							
TT broth (<i>n</i> =395)	167 (42.3%)	57.5 \pm 135.0	1.5	1.8	4.6	179	> 550
RV broth (<i>n</i> =395)	122 (30.9%)	51.9 \pm 132.6	1.5	1.5	4.7	120	> 550

^aArbitrary definition of seasons: Spring (March, April, May), Summer (June, July, August), Autumn (September, October, November), Winter (December, January, February).

SD, standard deviation; MPN_{Min}, minimum MPN value; MPN_{10%}, 10th percentile of MPN value; MPN_{50%}, 50th percentile of MPN value; MPN_{90%}, 90th percentile of MPN value; MPN_{Max}, maximum MPN value; TT, tetrathionate; RV, Rappaport-Vassiliadis.

Antimicrobial susceptibility

Among 874 *Salmonella* isolates, 183 (20.9%) isolates were susceptible to all tested antimicrobials, and all isolates were susceptible to imipenem. Resistance to nalidixic acid was the most common (580/874, 66.4%), followed by ampicillin (501/874, 57.3%), ampicillin-sulbactam (357/874, 40.9%), tetracycline (314/874, 35.9%), chloramphenicol (217/874, 24.8%), and gentamicin (211/874, 24.1%). Among 199 ciprofloxacin-resistant isolates (from 65 samples), 191 isolates were resistant to two or more additional categories of antimicrobials. Out of 188 cefotaxime-resistant isolates (from 61 samples), 144 isolates were resistant to two or more additional categories of

antimicrobials. One hundred two isolates (from 41 samples) were resistant to both ciprofloxacin and cefotaxime and 99 isolates being *Salmonella* Indiana (Table 3).

In total, 71 antimicrobial resistance profiles were identified among 874 *Salmonella* isolates. And 305 isolates (from 100 chicken carcasses) showed multidrug resistant profiles. The most common antimicrobial resistance profiles were NAL (*n*=124), AMP-SAM-NAL (*n*=118), AMP-SAM-CHL-CIP-CTX-GEN-NAL-TET (*n*=52), AMP-NAL (*n*=38), and TET (*n*=34) (Table 4). Nine *Salmonella* Indiana isolates (from five samples) recovered through RV broth showed resistance to nine antimicrobials: CAZ-CTX-CIP-NAL-AMP-SAM-CHL-GEN-TET (Table 4).

TABLE 2. PREVALENCE OF *SALMONELLA* SEROTYPES FROM CHICKEN RINSES IN BEIJING, CHINA

Salmonella serotypes	No. of Salmonella isolates/no. of chicken samples					
	TT broth only ^a	RV broth only ^b	Both TT and RV broth ^c	Farmers' market	Supermarket	Total
Enteritidis	159/73	50/29	100/23	96/35	213/59	309/94
Indiana	58/28	57/21	90/19	90/23	115/31	205/54
Infantis	23/11	20/11	46/10	46/10	43/13	89/23
Senftenberg	24/10	5/4	18/4	35/9	12/3	47/12
Typhimurium	13/8	9/3	22/3	36/8	8/2	44/10
Montevideo	10/6	4/3	24/4	19/4	19/4	38/8
Minor serotypes ^d	57/32	35/22	50/10	81/20	61/26	142/46
Total	344/135	180/82	350/67	403/78	471/119	874/197

^a*Salmonella* isolates recovered only in tetrathionate (TT) broth, not in Rappaport-Vassiliadis (RV) broth from a buffered peptone water (BPW) pre-enrichment culture.

^b*Salmonella* isolates recovered only in RV broth, not in TT broth from a BPW pre-enrichment culture.

^c*Salmonella* isolates recovered in both TT and RV broth from a BPW pre-enrichment culture.

^dMinor serotypes included *Salmonella* Kentucky (*n*=25, 7 samples), *Salmonella* Muenster (*n*=22, 4 samples), *Salmonella* Westhampton (*n*=17, 3 samples), *Salmonella* Rissen (*n*=17, 7 samples), *Salmonella* II-O67 (*n*=14, 9 samples), *Salmonella* Mississippi (*n*=10, 1 sample), *Salmonella* Newport (*n*=8, 6 samples), *Salmonella* Uppsala (*n*=5, 3 samples), *Salmonella* Blegdam (*n*=4, 1 sample), *Salmonella* Thompson (*n*=3, 2 samples), *Salmonella* II-O7 (*n*=3, 1 sample), *Salmonella* Derby (*n*=3, 2 samples), *Salmonella* Saintpaul (*n*=2, 2 samples), *Salmonella* Mbandaka (*n*=2, 1 sample), *Salmonella* II-O30 (*n*=2, 2 samples), *Salmonella* Assinie (*n*=2, 2 samples), *Salmonella* Legon (*n*=1, 1 sample) and *Salmonella* II-O39 (*n*=1, 1 sample). One *Salmonella* isolate could not be typed by the available antisera.

TABLE 3. RESISTANCE PHENOTYPES OF *SALMONELLA* ISOLATES RECOVERED FROM CHICKEN CARCASSES IN BEIJING, CHINA

Antimicrobial agents	MIC mg/L	No. of resistant isolates/no. of chicken samples							
		Enteritidis (N = 309/94)	Indiana (N = 205/54)	Infantis (N = 89/23)	Senftenberg (N = 47/12)	Typhimurium (N = 44/10)	Montevideo (N = 38/8)	Other serotypes (N = 142/46)	Total (N = 874/197)
Ampicillin	≥32	233/71	158/44	42/13	4/2	19/5	3/2	42/18	501/137
Ampicillin-sulbactam	≥32/16	176/59	129/41	8/4	0/0	19/5	2/1	23/14	357/111
Cefotaxime	≥4	0/0	121/40	38/10	4/2	0/0	1/1	24/10	188/61
Ceftazidime	≥16	1/1	29/8	1/1	0/0	0/0	0/0	13/2	44/12
Chloramphenicol	≥32	0/0	138/42	32/6	0/0	19/5	3/2	25/11	217/66
Ciprofloxacin	≥4	28/12	141/42	0/0	2/1	18/5	3/2	7/5	199/65
Gentamicin	≥16	41/19	116/40	38/10	0/0	5/4	2/1	9/7	211/78
Nalidixic acid	≥32	296/92	164/46	13/6	3/3	22/7	22/6	60/26	580/160
Tetracycline	≥16	73/33	148/49	35/8	0/0	19/5	5/3	34/20	314/108

MIC, minimal inhibitory concentration.

TABLE 4. TOP 10 ANTIMICROBIAL RESISTANCE PROFILES OF *SALMONELLA* ISOLATES RECOVERED FROM CHICKEN CARCASSES IN BEIJING, CHINA

Resistance profiles	No. of resistant isolates/no. of chicken samples							Total (N=874/197)
	Enteritidis (N = 309/94)	Indiana (N = 205/54)	Infantis (N = 89/23)	Senftenberg (N = 47/12)	Typhimurium (N = 44/10)	Montevideo (N = 38/8)	Other serotypes (N = 142/46)	
AMP-SAM-CHL-CIP-CTX-GEN-NAL-TET	0	52/27	0	0	0	0	0	52/27
AMP-SAM-CHL-CIP-CTX-NAL-TET	0	16/6	0	0	0	0	1/1	17/7
AMP-SAM-CHL-CIP-NAL-TET	0	6/3	0	0	13/3	0	2/1	21/7
AMP-SAM-CIP-GEN-NAL-TET	14/8	1/1	0	0	0	2/1	0	17/10
AMP-CHL-CTX-GEN-TET	0	2/1	24/3	0	0	0	0	26/4
AMP-SAM-NAL-TET	24/14	0	0	0	0	0	2/2	26/15
AMP-SAM-NAL	114/34	0	0	0	0	0	4/3	118/37
AMP-NAL	35/17	0	0	0	0	0	3/1	38/18
NAL	65/34	1/1	9/4	3/3	2/1	17/3	27/11	124/55
TET	1/1	28/7	0	0	0	0	5/4	34/12

AMP, ampicillin; SAM, ampicillin-sulbactam; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; GEN, gentamicin; NAL, nalidixic acid; TET, tetracycline.

The antimicrobial resistant profiles differed among serotypes. Among 205 *Salmonella* Indiana isolates, 163 isolates (from 46 samples) were multidrug resistant. Out of 141 ciprofloxacin-resistant isolates, 138 isolates were resistant to two or more additional categories of antimicrobials. Out of 121 cefotaxime resistant isolates, 104 isolates were resistant to two or more additional categories of antimicrobials. The dominant resistance profile was AMP-SAM-CHL-CIP-CTX-GEN-NAL-TET ($n=52$, 27 samples), which was only found among *Salmonella* Indiana isolates, whereas among 309 *Salmonella* Enteritidis isolates, 79 isolates were multidrug resistant and 28 isolates were resistant to ciprofloxacin. One isolate was resistant to ceftazidime and all isolates were susceptible to ceftriaxone. The dominant resistant profile was AMP-SAM-NAL ($n=114$).

All 89 *Salmonella* Infantis isolates were susceptible to ciprofloxacin and 37 isolates were susceptible to all tested antimicrobials. Thirty-three isolates were multidrug resistant and 38 isolates were resistant to cefotaxime. The dominant resistant profile was AMP-CHL-CTX-GEN-TET ($n=24$).

Discussion

In this study, approximately half of the retail whole chicken carcasses collected from the retail markets of Beijing were contaminated by *Salmonella* and the MPN values ranged from 1.5 to >550 MPN/100 g. Furthermore, multidrug-resistant *Salmonella* isolates were recovered from more than a quarter of chicken carcasses. More than 100 isolates even showed resistance to both ciprofloxacin and cefotaxime, which are the first-rank antimicrobials in salmonellosis treatment (Guerrant *et al.*, 2001). To the best of our knowledge, this is the first surveillance report of *Salmonella* enumeration study of retail chicken carcasses in China. Our study provided baseline enumeration data for the food safety risk assessment of *Salmonella* contamination and antimicrobial-resistant isolates from retail chicken carcasses.

The data showed that retail chicken carcasses were important vehicles of *Salmonella* transmission. *Salmonella* isolates are recovered from approximately 50% of chicken carcass samples, which is comparable to other studies in China and other developing countries (Ta *et al.*, 2012; Yang *et al.*, 2010, 2011), but much higher than the contamination rate in developed countries at retail level (Lestari *et al.*, 2009). In developed countries, good manufacturing processes, which have been developed for *Salmonella* control on broiler farms and in slaughtering houses, might contribute to the lower *Salmonella* contamination rate at the retail level (USDA/FSIS, 2010). The average load of *Salmonella* in chicken carcasses in this study was 86.5 MPN/100 g, which was lower than *Salmonella* loads in another study (Shashidhar *et al.*, 2011) but was much higher than *Salmonella* loads of pork products (Prendergast *et al.*, 2009). Although *Salmonella* MPN values of most chicken samples were not high, cross-contamination to other food and temperature abuse would offer an opportunity to proliferate to hazardous numbers. Samples collected from farmer's markets in summer showed the highest *Salmonella* loads, which further indicated the necessity of good manufacturing process application, such as good refrigeration control during shipment and in retail stores.

The data showed that methodology used in *Salmonella* recovery was an important factor affecting MPN analysis re-

sults. In this study, the 50% percentile of *Salmonella* MPN value was 0.15 MPN/mL, and the minimum MPN value was 0.03 MPN/mL in chicken carcass rinse, which indicated that a minimum 10-mL chicken carcass rinse in triplicate should be taken in the pre-enrichment step for *Salmonella* recovery. If little volume of chicken rinses was taken for pre-enrichment as in a mini-modified semi-solid Rappaport Vassiliadis (MINI-MSRV) MPN method (Pavic *et al.*, 2010; Shashidhar *et al.*, 2011), high false-negative results should be expected because of the low *Salmonella* contamination level. Besides the sample volume, these data showed that both TT and RV broth should be used for *Salmonella* MPN analysis, instead of one broth as in other reports (Pavic *et al.*, 2010; Shashidhar *et al.*, 2011). Although a better *Salmonella* recovery ratio was observed using TT broth in this study, 180 isolates were only recovered by using RV broth, which indicated the necessity of using two selective broths for *Salmonella* recovery from chicken samples. These data also showed that selective enrichment broth might affect the recovery of certain serotypes, as shown in another recent study, which indicated that different *Salmonella* serotypes could exhibit distinct growth characteristics in the same selective media (Singer *et al.*, 2009). Using two selective enrichment broths as in this study could not only avoid biased conclusions about the dominant strain present in a sample with mixed *Salmonella* populations, but also increase sensitivity for detecting *Salmonella* strains as in this study.

Our study showed that the dominant serotypes in chicken carcasses were Enteritidis, Indiana, and Infantis, which have been observed in other independent studies of chicken-originated isolates (Yang *et al.*, 2010; Lu *et al.*, 2011). These serotypes were also frequently isolated in human infections in China (Xia *et al.*, 2009; Ran *et al.*, 2011), and the contribution of retail chicken-originated *Salmonella* in the community infections has been established (Padungtod *et al.*, 2006; Kim *et al.*, 2008). More than one third of *Salmonella* isolates in this study were multidrug resistant, which has also been reported in other studies in China (Yan *et al.*, 2010; Lu *et al.*, 2011). Besides these, more than 20% of *Salmonella* isolates showed resistance to ciprofloxacin or third-generation cephalosporin. Widespread fluoroquinolone resistance in *Salmonella* has also been documented as a unique characteristic in other studies of China and some Asia countries that do not use strict antimicrobial application control (Von Seidlein *et al.*, 2006; Cui *et al.*, 2009). However, in countries with strict antimicrobial controls, such as the United States, less than 3% of *Salmonella* isolates were considered nonsusceptible to ciprofloxacin (Medalla *et al.*, 2013). In the United States, third-generation cephalosporin-resistant isolates have also been observed because of the spreading of plasmid-encoded *bla*_(CMY) β -lactamase and the application of ceftiofur in chicken farms (Folster *et al.*, 2012; Zhao *et al.*, 2008). These data further emphasized the necessity of strict antimicrobial application control in food production animals.

Furthermore, more than 100 isolates were resistant to both fluoroquinolones and third-generation cephalosporins, which were the first-rank antimicrobials of salmonellosis treatment, and most isolates were *Salmonella* Indiana, which have also been reported in another surveillance study of chicken carcasses in China (Yang *et al.*, 2010). Why most of the ciprofloxacin and cefotaxime co-resistant isolates were *Salmonella* Indiana was not clear, but multidrug resistant *Salmonella* Indiana isolates have also been reported

in human isolates in China (Xia *et al.*, 2009). The relationship between chicken- and human-originated ciprofloxacin and cefotaxime co-resistant *Salmonella* Indiana isolates should be fully characterized to study their genetic relationship. Since cefotaxime-resistance determinants were usually plasmid mediated and fluoroquinolone-resistance determinants were chromosomal or plasmid mediated (Li, 2005; Zhao *et al.*, 2013), these resistance determinants should be characterized to explore why the resistance was more likely observed among certain serotypes. The surveillance agencies should stay alert for the spreading of these cefotaxime and ciprofloxacin co-resistant isolates and the resistance mechanisms, since their transmission directly endangered the optimal treatment alternatives of salmonellosis in the community.

Conclusions

Our findings showed a high prevalence of *Salmonella* contamination in retail chicken carcasses, which could be a source of exposure for consumers to multidrug-resistant isolates. Hazard analysis critical control point systems for *Salmonella* control in poultry production at the farm, processing, and retail level should be implemented. These data provided a baseline that can be used to further validate risk assessment predictions, and to determine the effectiveness of new control measures of retail chicken products.

Acknowledgments

This research was supported by grant (2012AA101603) from the Ministry of Science and Technology, China.

Disclosure Statement

All authors listed in the manuscript contributed to the conception, acquisition, analysis, and interpretation of data, design and critical revision of the manuscript, and approval of the final submitted version. The authors have no conflict of interest to declare.

References

- Ahmed AM, Shimabukuro H, Shimamoto T. Isolation and molecular characterization of multidrug-resistant strains of *Escherichia coli* and *Salmonella* from retail chicken meat in Japan. *J Food Sci* 2009;74:405–410.
- [CLSI] Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty Second Informational Supplement*. CLSI Document M100-S22. Wayne, PA: CLSI, 2012.
- Cui S, Li J, Sun Z, Hu C, Jin S, Li F, Guo Y, Ran L, Ma Y. Characterization of *Salmonella enterica* isolates from infants and toddlers in Wuhan, China. *J Antimicrob Chemother* 2009;63:87–94.
- Fearnley E, Raupach J, Lagala F, Cameron S. *Salmonella* in chicken meat, eggs and humans; Adelaide, South Australia, 2008. *Int J Food Microbiol* 2011;146:219–227.
- Folster JP, Pecic G, Singh A, Duval B, Rickert R, Ayers S, Abbott J, McGlinchey B, Bauer-Turpin J, Haro J, Hise K, Zhao S, Fedorka-Cray PJ, Whichard J, McDermott PF. Characterization of extended-spectrum cephalosporin-resistant *Salmonella enterica* serovar Heidelberg isolated from food animals, retail meat, and humans in the United States 2009. *Foodborne Pathog Dis* 2012;9:638–645.
- Guerrant RL, Van Gilder T, Steiner TS, Thielman NM, Slutsker L, Tauxe RV, Hennessy T, Griffin PM, DuPont H, Sack RB, Tarr P, Neill M, Nachamkin I, Reller LB, Osterholm MT, Bennish ML, Pickering LK. Practice guidelines for the management of infectious diarrhea. *Clin Infect Dis* 2001;32:331–351.
- Kim SH, Kim S, Chun SG, Park MS, Park JH, Lee BK. Phage types and pulsed-field gel electrophoresis patterns of *Salmonella enterica* serovar Enteritidis isolated from humans and chickens. *J Microbiol* 2008;46:209–213.
- Lestari SI, Han F, Wang F, Ge B. Prevalence and antimicrobial resistance of *Salmonella* serovars in conventional and organic chickens from Louisiana retail stores. *J Food Prot* 2009;72:1165–1172.
- Li XZ. Quinolone resistance in bacteria: Emphasis on plasmid-mediated mechanisms. *Int J Antimicrob Agents* 2005;25:453–463.
- Lu Y, Wu CM, Wu GJ, Zhao HY, He T, Cao XY, Dai L, Xia LN, Qin SS, Shen JZ. Prevalence of antimicrobial resistance among *Salmonella* isolates from chicken in China. *Foodborne Pathog Dis* 2011;8:45–53.
- Malorny B, Hoorfar J, Bunge C, Helmuth R. Multicenter validation of the analytical accuracy of *Salmonella* PCR: Towards an international standard. *Appl Environ Microbiol* 2003;69:290–296.
- Marcus R, Varma JK, Medus C, Boothe EJ, Anderson BJ, Crume T, Fullerton KE, Moore MR, White PL, Lyszkowicz E, Voetsch AC, Angulo FJ. Re-assessment of risk factors for sporadic *Salmonella* serotype Enteritidis infections: A case-control study in five FoodNet Sites, 2002–2003. *Epidemiol Infect* 2007;135:84–92.
- Medalla F, Hoekstra RM, Whichard JM, Barzilay EJ, Chiller TM, Joyce K, Rickert R, Krueger A, Stuart A, Griffin PM. Increase in resistance to ceftriaxone and nonsusceptibility to ciprofloxacin and decrease in multidrug resistance among *Salmonella* strains, United States, 1996–2009. *Foodborne Pathog Dis* 2013;10:302–309.
- Meldrum RJ, Wilson IG. *Salmonella* and *Campylobacter* in United Kingdom retail raw chicken in 2005. *J Food Prot* 2007;70:1937–1939.
- M'ikanatha NM, Sandt CH, Localio AR, Tewari D, Rankin SC, Whichard JM, Altekruze SF, Lautenbach E, Folster JP, Russo A, Chiller TM, Reynolds SM, McDermott PF. Multidrug-resistant *Salmonella* isolates from retail chicken meat compared with human clinical isolates. *Foodborne Pathog Dis* 2010;7:929–934.
- Olsen JE, Brown DJ, Madsen M, Bisgaard M. Cross-contamination with *Salmonella* on a broiler slaughterhouse line demonstrated by use of epidemiological markers. *J Appl Microbiol* 2003;94:826–835.
- Padungtod P, Kaneene JB. *Salmonella* in food animals and humans in northern Thailand. *Int J Food Microbiol* 2006;108:346–354.
- Pavic A, Groves PJ, Bailey G, Cox JM. A validated miniaturized MPN method, based on ISO 6579:2002, for the enumeration of *Salmonella* from poultry matrices. *J Appl Microbiol* 2010;109:25–34.
- Prendergast DM, Duggan SJ, Gonzales-Barron U, Fanning S, Butler F, Cormican M, Duffy G. Prevalence, numbers and characteristics of *Salmonella* spp. on Irish retail pork. *Int J Food Microbiol* 2009;131:233–239.
- Ran L, Wu S, Gao Y, Zhang X, Feng Z, Wang Z, Kan B, Klena JD, Lo Fo Wong DM, Angulo FJ, Varma JK. Laboratory-based surveillance of nontyphoidal *Salmonella* infections in China. *Foodborne Pathog Dis* 2011;8:921–927.
- Shashidhar R, Srivastava I, Bandekar JR. Quantification of *Salmonella* in food samples from India using the MINI-MSRV

- MPN and modified MINI-MSRV MPN methods. *J Food Sci* 2011;76:M564–M567.
- Singer RS, Mayer AE, Hanson TE, Isaacson RE. Do microbial interactions and cultivation media decrease the accuracy of *Salmonella* surveillance systems and outbreak investigations? *J Food Prot* 2009;72:707–713.
- Smadi H, Sargeant JM. Quantitative risk assessment of human salmonellosis in Canadian broiler chicken breast from retail to consumption. *Risk Anal* 2013;33:232–248.
- Ta YT, Nguyen TT, To PB, Pham da X, Le HT, Alali WQ, Walls I, Lo Fo Wong DM, Doyle MP. Prevalence of *Salmonella* on chicken carcasses from retail markets in Vietnam. *J Food Prot* 2012;75:1851–1854.
- Thai TH, Hirai T, Lan NT, Yamaguchi R. Antibiotic resistance profiles of *Salmonella* serovars isolated from retail pork and chicken meat in North Vietnam. *Int J Food Microbiol* 2012;156:147–151.
- Todd EC. Epidemiology of foodborne diseases: A worldwide review. *World Health Stat Q* 1997;50:30–50.
- [USDA/FSIS] United States Department of Agriculture Food Safety and Inspection Service, Most Probable Number Procedure and Tables. 2008. Available at: http://www.fsis.usda.gov/PDF/MLG_Appendix_2_03.pdf, accessed August 13, 2010.
- [USDA/FSIS]. Compliance Guideline for Controlling *Salmonella* and *Campylobacter* in Poultry. 2010, Third Edition. Available at: http://www.fsis.usda.gov/PDF/Compliance_Guide_Controling_Salmonella_Campylobacter_Poultry_0510.pdf, accessed October 7, 2012.
- Von Seidlein L, Kim DR, Ali M, Lee H, Wang X, Thiem VD, Canh do G, Chaicumpa W, Agtini MD, Hossain A, Bhutta ZA, Mason C, Sethabutr O, Talukder K, Nair GB, Deen JL, Kotloff K, Clemens J. A multicentre study of *Shigella* diarrhoea in six Asian countries: Disease burden, clinical manifestations, and microbiology. *PLoS Med* 2006;3:e353.
- Xia S, Hendriksen RS, Xie Z, Huang L, Zhang J, Guo W, Xu B, Ran L, Aarestrup FM. Molecular characterization and antimicrobial susceptibility of *Salmonella* isolates from infections in humans in Henan Province, China. *J Clin Microbiol* 2009;47:401–409.
- Yan H, Li L, Alam MJ, Shinoda S, Miyoshi S, Shi L. Prevalence and antimicrobial resistance of *Salmonella* in retail foods in northern China. *Int J Food Microbiol* 2010;143:230–234.
- Yang B, Qu D, Zhang X, Shen J, Cui S, Shi Y, Xi M, Sheng M, Zhi S, Meng J. Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. *Int J Food Microbiol* 2010;141:63–72.
- Yang B, Xi M, Wang X, Cui S, Yue T, Hao H, Wang Y, Cui Y, Alali WQ, Meng J, Walls I, Wong DM, Doyle MP. Prevalence of *Salmonella* on raw poultry at retail markets in China. *J Food Prot* 2011;74:1724–1728.
- Zhao C, Ge B, De Villena J, Sudler R, Yeh E, Zhao S, White DG, Wagner D, Meng J. Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area. *Appl Environ Microbiol* 2001;67:5431–5436.
- Zhao WH, Hu ZQ. Epidemiology and genetics of CTX-M extended-spectrum β -lactamases in Gram-negative bacteria. *Crit Rev Microbiol* 2013;39:79–101.
- Zhao S, White DG, Friedman SL, Glenn A, Blickenstaff K, Ayers SL, Abbott JW, Hall-Robinson E, McDermott PF. Antimicrobial resistance in *Salmonella enterica* serovar Heidelberg isolates from retail meats, including poultry, from 2002 to 2006. *Appl Environ Microbiol* 2008;74:6656–6662.
- Zhu M, Cui S, Lin L, Xu B, Zhao J, Xia S, Deng W, Xie Z, Zhang J, Wang Z, Feng Z, Yang W, Ran L. Analysis of the aetiology of diarrhoea in outpatients in 2007, Henan province, China. *Epidemiol Infect* 2012;June 7:1–9. DOI: <http://dx.doi.org/10.1017/S0950268812000970> [E-pub ahead of print].

Address correspondence to:

Fengqin Li, PhD

Key Laboratory of Food Risk Assessment

Ministry of Health

China National Center for Food Safety Risk Assessment

7# Panjiayuan Nanli

Chaoyang District

Beijing, 100021, China

E-mail: lifengqin@cfssa.net.cn