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Prevalence and Antimicrobial Resistance of *Salmonella* in Pork, Chicken, and Duck from Retail Markets of China

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Abstract

Salmonella is one of the most important foodborne pathogens associated with animal and human diseases. In this study, 672 samples of fresh meat (pork, 347; chicken, 196; and duck, 129) were collected from retail markets in different provinces of China from 2010 to 2014. We identified 10 different serotypes among 80 *Salmonella* isolates, whereas 12 isolates were nonmotile precluding conventional identification of complete serotype. Among these 92 isolates, *Salmonella enterica* serovar Derby ($n=21$) was the most prevalent serotype, followed by *Salmonella* Enteritidis ($n=17$), *Salmonella* Typhimurium ($n=15$), *Salmonella* Indiana ($n=9$), *Salmonella* Agona ($n=7$), and *Salmonella* Assinie ($n=5$). Antimicrobial resistance testing for 18 antimicrobial agents revealed that all 92 isolates were resistant to at least 1 antimicrobial agent, and 39 different resistance profiles were identified. The highest resistance was to trimethoprim–sulfamethoxazole ($n=87$), followed by tetracycline ($n=51$), carbenicillin ($n=38$), amoxicillin/A.clav ($n=30$), and piperacillin ($n=24$). Our results demonstrated that meats presented a potential public health risk, thereby underlining the necessity for local regulatory enforcement agencies in China to monitor salmonellosis.

Keywords: *Salmonella*, prevalence, antimicrobial resistance, public health risk

Introduction

Salmonella is one of the most important foodborne pathogens worldwide and causes severe public health problems and economic losses (Handeland *et al.*, 2002). Meat products are the most commonly reported vehicles of foodborne pathogens, and the presence of *Salmonella* in these products is a significant food safety risk (Akbar *et al.*, 2013). Pork, chicken, and duck meats are sold in public markets and are traditional staple food in China. Thus, understanding of the prevalence and antimicrobial resistance of *Salmonella* in markets can provide data used for guiding surveillance and controlling activities. One strategy is enhanced biosecurity to avoid food-to-human transmission.

The contamination and antimicrobial resistance of *Salmonella* isolated from food-producing animals are particularly severe in China (Yang *et al.*, 2011; Li *et al.*, 2013b; Kuang *et al.*, 2015; Ren *et al.*, 2016). Moreover, meats remain an important source of human *Salmonella* infections in Canada and the United States (Ray *et al.*, 2007; Imanishi *et al.*, 2014; Sanchez-Maldonado *et al.*, 2017). Indeed, the emergence of multidrug-resistant (MDR) *Salmonella* strains is a new and great threat to public health (Ma *et al.*, 2017). MDR between *Salmonella* strains is frequently isolated from food sources, and infections due to MDR *Salmonella* can increase instances of morbidity and mortality (Song *et al.*, 2018).

In this study, we investigated the presence of *Salmonella* in pork, chicken, and duck samples from retail markets in the southern and northern areas of the Yangtze River (hereafter denoted as Yangtze). We then analyzed the antimicrobial resistance of *Salmonella* isolates and evaluated the risk of transmission of salmonellosis through food chains. Our findings can provide information on the prevalence and antimicrobial resistance of *Salmonella* in meat products and can thus help curb *Salmonella* contamination problems in China.

Materials and Methods

Sample collection

Between 2010 and 2014, meat samples were obtained from eight provinces. Three provinces (Guangdong, Jiangxi, and Hunan) were located south of the Yangtze, and five (Liaoning, Heilongjiang, Jilin, Gansu, and Ningxia) were located in

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the north. Based on the sampling plan, four to five cities were selected per province, two to three markets per city, three retail stalls per market, and two samples per stall. A total of 230 samples were collected north of the Yangtze and 442 samples were collected in the south. Samples of pork ($n=347$), chicken ($n=196$), and duck ($n=129$) were randomly collected from retail markets using sterile gloves and transferred into sterile bags to avoid cross-contamination. Samples were stored in an icebox and immediately transferred to the laboratory for analysis.

Bacterial isolation

All samples were analyzed according to the National Standard GB/T 4789.4. In a typical procedure, 25 ± 0.5 g of each meat was aseptically weighed, transferred into 225 mL of buffered peptone water (Beijing Land Bridge, Beijing, China), and incubated at 37°C for 18 h. Then, 1 mL of the cultures was subcultured in 10 mL of selenite cystine broth (Beijing Land Bridge) at 37°C for 24 h. One loopful of each broth culture was streaked onto BS and Hektoen agar plates (Beijing Land Bridge) and incubated at 37°C for 24–48 h. Biochemical tests of suspected colonies were conducted using API ID 32E test kits following the manufacturer's recommendations (BioMérieux, Marcy-L'Étoile, France).

Serotyping

All *Salmonella* isolates were serotyped by slide agglutination using polyvalent O- and H- antisera (BD, Franklin Lakes) in accordance with the Kauffmann–White scheme (Issenhuth-Jeanjean *et al.*, 2014).

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed on Mueller–Hinton agar plates according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013) with 18 antimicrobial agents selected for ATB® G-5 (BioMérieux, Marcy-L'Étoile, France) used in humans. The corresponding disk concentrations were as follows: meropenem (MER; 10 μg), streptomycin (STR;10 μg), gentamycin (10 μg), amikacin (10 μg), tobramycin (10 μg), trimethoprim–sulfamethoxazole (SXT; 23.75/1.25 μg), ciprofloxacin (CIP; 5 μg), amoxicillin–clavulanic acid (AMC; 20/10 μg), carbenicillin (CB; 100 μg), piperacillin (PIC; 100 μg), ticarcillin–clavulanate (TCC; 75/10 μg), tetracycline (TET; 30 μg), imipenem (IMI; 10 μg), cefuroxime (30 μg), ceftazidime (30 μg), cephalothin (30 μg), kanamycin (30 μg), and cefoxitin (30 μg). Resistance breakpoints were defined by the CLSI. Results were identified as susceptible (S), intermediate (I), or resistant (R). *Escherichia coli* ATCC 25922 was used as the quality control strain according to CLSI guidelines.

Results

Prevalence of Salmonella *in China*

Salmonella was recovered from 13.7% (92/672) of the tested samples. Overall, the prevalence of *Salmonella* in pork was 14.1% (49/347) with *Salmonella* recovered from 12.2% (10/82) of samples collected north of the Yangtze and 14.7% (39/265) of samples collected in the south. The overall prevalence of *Salmonella* in chicken and duck was 14.3% (28/196) and 11.6% (15/129), respectively. *Salmonella* was recovered from 14.9% (22/148) of chicken samples collected north of the Yangtze and 12.5% (6/48) of chicken samples collected in the south (Table 1).

Distribution of Salmonella *serotype*

A total of 80 isolates were divided into 10 distinct serotypes, whereas 12 isolates were nonmotile precluding conventional identification of complete serotype (Table 2). The predominant serotypes were *Salmonella enterica* serovar Derby ($n=21$), followed by *Salmonella* Enteritidis ($n=17$) and *Salmonella* Typhimurium ($n=15$). *Salmonella* Derby and *Salmonella* Typhimurium were isolated most frequently in pork, whereas *Salmonella* Enteritidis ($n=13$) and *Salmonella* Indiana ($n=9$) were the major serotypes in chicken. In duck, *Salmonella* Enteritidis ($n=4$), *Salmonella* Agona ($n=5$), and group C ($n=6$) were identified. The detection rate of *Salmonella* Derby in pork was higher in areas south of the Yangtze ($n=20$) than in areas to the north ($n=1$). In chicken, 12 *Salmonella* Enteritidis strains were isolated from the areas north of the Yangtze, but none was isolated from the areas to the south.

Antimicrobial susceptibility of isolated Salmonella

The antimicrobial susceptibility of all isolates is shown in Tables 3 and 4. All isolates showed resistance to one or more of the tested antimicrobials. Most isolates were resistant to SXT ($n=87$), followed by TET ($n=51$), CB ($n=38$), AMC ($n=30$), STR ($n=25$), and PIC ($n=24$). Resistance to cephem was observed in 60.1% (17/28) of isolates from chicken, 13.3% (2/15) from duck, and 2% (1/49) from pork. Resistance to CIP was also observed in 35.7% (10/28) of isolates from chicken, 0% (0/49) from pork, and 0% (0/15) from duck.

Antimicrobial susceptibility tests resulted in a total of 39 resistance profiles (Table 4). *Salmonella* Newlands, *Salmonella* Rissen, and *Salmonella* Agona were resistant to 1 antimicrobial agent; *Salmonella* Enteritidis ($n=7$) to 2–6 antimicrobial agents; *Salmonella* Derby ($n=8$) to 2–9 agents; *Salmonella* Typhimurium ($n=12$) to 2–10 agents; and *Salmonella* Indiana ($n=9$) to 7–14 agents. Among the three different meat species, the rate of isolates resistant to three or more antimicrobial agents was 64.3% (18/28) from chicken, 40.0% (6/15) from duck, and 36.7% (18/49) from pork.

TABLE 1. PREVALENCE OF *SALMONELLA* FROM PORK, CHICKEN, AND DUCK TO THE NORTH AND SOUTH OF YANGTZE RIVER IN CHINA

Meat category	*Northern (positive samples/total)*	*Southern (positive samples/total)*[a]	*Total (positive samples/total)*
Pork	10/82	39/265	49/347
Chicken	22/148	6/48	28/196
Duck	—	15/129	15/129
Total	32/230	60/442	92/672

[a]One *Salmonella* isolate was collected from each positive sample.

Table 2. Serotyping of *Salmonella* Isolates from Pork, Chicken, and Duck in Northern and Southern China

Group	Serotype	*No. in Pork* North	*No. in Pork* South	*No. in Chicken* North	*No. in Chicken* South	*No. in Duck* South	*Total no. (%)*
C	NA	—	2	—	1	6	9 (9.8)
C1	Rissen	—	1	—	—	—	1 (1.1)
E1	NA	—	3		—	—	3 (3.3)
	Assinie	—	4	—	1	—	5 (5.4)
B	Agona	—	1	—	1	5	7 (7.6)
	Heidelberg	1	—	—	2	—	3 (3.3)
	Indiana	—	—	8	1	—	9 (9.8)
	Typhimurium	7	6	2	—	—	15 (16.3)
D	Enteritidis	1	—	12	—	4	17 (18.5)
	Derby	1	20	—	—	—	21 (22.8)
	London	—	1	—	—	—	1 (1.1)
	Newlands	—	1	—	—	—	1 (1.1)
	Total (%)	10	39	22	6	15	92

Table 3. Antimicrobial Resistance Phenotypes of 92 *Salmonella* Isolates

Antimicrobial agent	*No. of resistant isolates* Pork (n=49)[a]	Chicken (n=28)	Duck (n=15)	Total (n=92)
Aminoglycosides				
AMI	0	5	0	5
KAN	8	6	0	14
TOD	9	7	0	16
GEN	10	7	0	17
STR	11	12	2	25
β-lactams				
TCC	1	1	2	4
AMC	11	14	5	30
Carbactams				
MER	2	1	0	3
IMI	6	7	1	14
Cephems				
CXT	0	0	1	1
CAZ	0	2	0	2
CFT	0	7	1	8
CXM	1	8	0	9
Folate pathway inhibitors				
SXT	45	27	15	87
Penicillins				
PIC	6	14	4	24
CB	14	19	5	38
Fluoroquinolones				
CIP	0	10	0	10
TETs				
TET	32	13	6	51

[a]*n*, number of *Salmonella*-positive isolates tested.

AMI, amikacin; KAN, kanamycin; TOD, tobramycin; GEN, gentamycin; STR, streptomycin; TCC, ticarcillin–clavulanic acid; AMC, amoxicillin–clavulanic acid; MER, meropenem; IMI, imipenem; CXT, cefoxitin; CAZ, ceftazidime; CFT, cephalothin; CXM, cefuroxime; SXT, trimethoprim–sulfamethoxazole; PIC, piperacillin; CB, carbenicillin; CIP, ciprofloxacin; TET, tetracycline.

Discussion

Monitoring the presence of foodborne pathogens in foods is the primary tool for implementing food safety systems. Our study revealed that the prevalence of *Salmonella* contamination had no significant difference among pork (14.1%), chicken (14.3%), and duck (11.6%), similar to the findings of a previous study conducted in the Jiangsu province (Li *et al.*, 2014) but lower than those reported by a study in the Sichuan province (Ma *et al.*, 2017) in China. The *Salmonella* serotypes identified in this study were diverse, that is, 80 isolates belonged to 10 different serotypes, whereas 12 isolates were nonmotile precluding conventional identification of complete serotype (Table 2). *Salmonella* Derby ($n=21$) and *Salmonella* Typhimurium ($n=13$) were the most prevalent serotypes isolated from pork, consistent with previous reports (Li *et al.*, 2014; Lin *et al.*, 2014; Bai *et al.*, 2015; Terentjeva *et al.*, 2017), *Salmonella* Rissen, *Salmonella* Assinie, *Salmonella* Agona, *Salmonella* London, *Salmonella* Newlands, group C, group C1, and group E1 were isolated from the south but not from the north. In chicken, *Salmonella* Enteritidis ($n=12$) and *Salmonella* Indiana ($n=8$) were the most popular serotypes, and the high isolation rates of *Salmonella* Enteritidis in chicken were consistent with previous studies (Bai *et al.*, 2015; Li *et al.*, 2017). *Salmonella* Assinie, *Salmonella* Agona, *Salmonella* Heidelberg, and group C were isolated from chicken in the south but not in the north. More serotypes were detected in pork and chicken south of the Yangtze, which indicated a wider diversity of serotypes in this area possibly due to the rapid animal product circulation. In duck, *Salmonella* Agona ($n=5$), *Salmonella* Enteritidis ($n=4$), and group C ($n=6$) were isolated, and these results were inconsistent with previous reports (Tran *et al.*, 2004; Tsai and Hsiang, 2005; Li *et al.*, 2013a), possibly due to the regional variation of *Salmonella*.

The extensive use of antimicrobial in human and livestock leads to higher exposure to these compounds and consequently promotes the increase in antimicrobial resistance in *Salmonella* (Cruchaga *et al.*, 2001; Antunes *et al.*, 2003; Angkititrakul *et al.*, 2005). In our study, 92 (100%) isolates were resistant to at least one antimicrobial agent, which was much higher than a previous report in Algeria, Senegal, and

Table 4. Diversity Profiles of *Salmonella* Isolates Based on Serotyping and Antimicrobial Resistance

		Serotype (no.)		
Antimicrobial resistance profiles	*No.*	*Pork* (n=*49*)	*Chicken* (n=*28*)	*Duck* (n=*15*)
GEN	1	Typhimurium (1)		
SXT	30	Typhimurium (2), Enteritidis (1), Assinie (1), Derby(3), Heidelberg (1), Newlands (1), Rissen (1), Agona (1), group E1 (2)	Agona (1), Enteritidis (8)	Agona (5), group C (2), Enteritidis (1)
CB SXT	1		Typhimurium (1)	
IMI SXT	1			Enteritidis (1)
IMI TET	1	Group E1 (1)		
SXT TET	16	Assinie (3), Derby (13)		
CB PIC SXT	1		Enteritidis (1)	
CB STR TET	2		Assinie (1), group C (1)	
IMI MER SXT				
IMI SXT TET	1	Typhimurium (1)		
STR SXT TET	1			Group C (1)
STR SXT TET	1	Group C (1)		
AMC CB PIC TET	1	Derby (1)		
AMC CB PIC SXT	1		Heidelberg (1)	
AMC CB STR TET	1	Typhimurium (1)		
CB IMI SXT TET	1	Derby (1)		
CB STR SXT TET	2	Derby (2)		
CB STR SXT TET	1	Typhimurium (1)		
AMC CB PIC SXT TET	1			Group C (1)
AMC CB IMI PIC SXT	1		Enteritidis (1)	
AMC CB CFT CXT SXT	1			Enteritidis (1)
AMC CB PIC SXT TCC	1		Heidelberg (1)	
CB CIP STR SXT TET	1		Typhimurium (1)	
IMI MER STR SXT TET	1	Derby (1)		
AMC CB PIC SXT TCC TET	2			Group C (2)
AMC CB IMI PIC STR SXT	1		Enteritidis (1)	

(*continued*)

Table 4. (Continued)

		Serotype (no.)		
Antimicrobial resistance profiles	*No.*	*Pork* (n = *49*)	*Chicken* (n = *28*)	*Duck* (n = *15*)
AMC CB GEN KAN SXT TOD	2	Typhimurium (2)		
AMC PIC PIC STR SXT TET	1		Enteritidis (1)	
AMC PIC PIC STR SXT TET	1			Enteritidis (1)
AMC CB CIP GEN IMI SXT TOD	1		Indiana (1)	
AMC CB GEN KAN SXT TET TOD	1	Typhimurium (1)		
AMC CB GEN PIC STR SXT TET TOD	1	London (1)		
AMC CB GEN KAN PIC STR SXT TET TOD	4	Typhimurium (3), Derby (1)		
AMC CB CIP IMI KAN PIC STR SXT TET	1		Indiana (1)	
AMC CB CXM GEN IMI KAN STR SXT TET TOD	1	Typhimurium (1)		
AMC CAZ CB CFT CIP CXM KAN PIC STR SXT TET	1		Indiana (1)	
AMC CB CIP CXM GEN IMI KAN MER PIC STR SXT TOD	1		Indiana (1)	
AMC AMI CB CFT CIP CXM GEN KAN PIC STR SXT TET TOD	3		Indiana (3)	
AMC AMI CB CFT CIP CXM GEN IMI KAN PIC STR SXT TET TOD	2		Indiana (2)	

AMI, amikacin; KAN, kanamycin; TOD, tobramycin; GEN, gentamycin; STR, streptomycin; TCC, ticarcillin–clavulanic acid; AMC, amoxicillin–clavulanic acid; MER, meropenem; IMI, imipenem; CXT, cefoxitin; CAZ, ceftazidime; CFT, cephalothin; CXM, cefuroxime; SXT, trimethoprim–sulfamethoxazole; PIC, piperacillin; CB, carbenicillin; CIP, ciprofloxacin; TET, tetracycline.

Vietnam (Stevens *et al.*, 2006; Van *et al.*, 2007; Elgroud *et al.*, 2009). Higher degrees of resistance to SXT (87/92) and TET (51/92) were found, that is, the percentage of resistance to SXT in pork, chicken, and duck was 91.8% (45/49), 96.4% (27/28), and 100% (15/15), and the percentage of resistance to TET in pork, chicken, and duck was 65.3% (32/49), 64.4% (13/28), and 40% (6/15), respectively. These results indicated that farmers should reduce their use of SXT and TET to ensure their effectiveness in treating human infections and limit selective pressure, which drives resistance in an array of organisms.

The antimicrobial resistance profiles differed among serotypes. A total of 42 isolates were resistant to 3 or more antimicrobials, including 77.8% (7/9) in *Salmonella* Indiana, 66.7% (10/15) in *Salmonella* Typhimurium, 29.4% (5/17) in *Salmonella* Enteritidis, and 28.6% (6/21) in *Salmonella* Derby. *Salmonella* Indiana displayed resistance to the largest number of tested antimicrobials (including five isolates exhibiting resistance to IMI and/or MER). This degree of resistance is consistent with previous reports (Lu *et al.*, 2011; Moe *et al.*, 2017). In this study, resistance appeared to vary by serovar, and MDR *Salmonella* was isolated in higher numbers from chicken than from pork and duck. Apparently, serovars circulating in poultry environment were more resistant than serovars circulating in pig and duck farms, possibly due to differences in antimicrobial usage between chicken and pork farms. These findings highlighted the enormous challenges associated with the treatment of *Salmonella* infections in humans and animals and the importance of implementing legislation about antimicrobial use by authorities in China. Therefore, more investigations should be conducted and the antimicrobial resistance of foodborne pathogens from extensive sources should be continuously monitored.

Conclusion

We examined *Salmonella* contamination in fresh meat, provided data for foodborne pathogen risk assessment, and elucidated the differences in *Salmonella* distribution and antimicrobial susceptibility to control the significant threat of clinical *Salmonella* infection in humans and animals. Our results indicated a need to further investigate the prevalence and antimicrobial susceptibility pattern of *Salmonella* by considering it a potential foodborne pathogen from farm to table.

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Disclosure Statement

No competing financial interests exist.

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