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Antibiotic Resistance in *Salmonella* from Retail Foods of Animal Origin and Its Association with Disinfectant and Heavy Metal Resistance

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This study aims to demonstrate the antibiotic resistance and its association with disinfectant and heavy metal resistance in 152 *Salmonella* isolates recovered from retail foods of animal origins. Susceptibility testing demonstrated that 92.8% isolates were resistant to at least one antibiotic, and the resistance was highest to oxytetracycline (80.9%), followed by trimethoprim (64.5%), amoxicillin (28.9%), ampicillin (28.3%), levofloxacin (21.7%), ciprofloxacin (16.4%), and gentamicin (10.5%), respectively. The bla_{TEM} and *tet*A genes (44.7%) were commonly present. The *qacF* and *qacEΔ1* genes were detected in 18.4% and 8.6% of all isolates. The Cu-resistance genes *pco*R, *pco*C, and *pco*A were the most prevalent (20.4–40.8%), followed by Hg-resistance gene *mer*A (17.8%) and As-resistance genes *ars*B (6.6%). The antibiotic resistance was highly associated with disinfectant or certain heavy metal resistance genes. Most notably, the association among Cu-resistance genes (*pco*C, *pco*R), disinfectant resistance genes (*qacF*, *qacEΔ1*), and tetracycline and sulfonamide resistance genes (*tet*, *sul*) was significant ($p < 0.05$). Pulsed-field gel electrophoresis revealed that *Salmonella* isolates was associated with supermarkets indicating the possibility of crosscontamination in farms or processing environment. This study indicated that retail meats may be a reservoir for the dissemination of antibiotic-resistant *Salmonella* and using disinfectants for decontamination or metals in livestock may provide a pressure for coselecting strains with acquired resistance to other antimicrobials.

Keywords: antibiotic, disinfectant, heavy metal, resistance, *Salmonella*

Introduction

Salmonella is recognized as a common bacterial cause of foodborne diarrheal illness worldwide.[1–3] Every year, ~42,000 cases of salmonellosis are reported through the Centers for Disease Control and Prevention in the United States; these cases have resulted in high morbidity and economic costs.[4,5] In China, the increase in consumption of food products of animal origin also increased potential exposure to *Salmonella*.[6,7] The genus *Salmonella* encompasses a large taxonomic group with more than 2,600 different serotypes.[8] Although all serotypes of *Salmonella* may be regarded as potential human pathogens, the vast majority of infections are caused by a limited number of serotypes, of which *Salmonella* Enteritidis and *Salmonella* Typhimurium are the two most common serotypes associated with gastrointestinal disease in humans.[9,10]

Approaches to prevent and control salmonellosis in livestock have been dependent on the use of antibiotics for many years. However, numerous antibiotic-resistant bacteria have been reported in different countries.[11–13] Most of the antibiotic-resistant *Salmonella* are of zoonotic origin and acquire their resistance in food animal hosts, which might cause human infections through the food chain.[14] Therefore, antibiotic resistance in pathogenic bacteria from animals can be a serious threat to public health.[15]

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Disinfectants are extensively used to control infection and/or microbial contamination in food manufacturing facilities and environments. Among the different disinfectants currently available, quaternary ammonium compounds (QACs), such as benzalkonium chloride (BC) and cetylpyridinium chloride (CTPC), are used extensively because these compounds are nonirritating and noncorrosive with little toxicity and high antimicrobial efficacy over a wide pH range.[16,17] The wide use and misuse of QACs in food environments can impose a selective pressure for bacteria and contribute to the emergence of disinfectant-resistant microbes.[18]

Metal-containing compounds with antimicrobial or growth-promoting activity are also widely used as feed additive in food animals.[19,20] Copper sulfate and organic arsenicals (*e.g.*, phenylarsonic acid) are agents for therapy and growth, and mercury compounds have been usually used as disinfectants in food animal-producing environment for a number of years.[20,21] Due to its stable and persistent features, when heavy metals accumulate to critical concentrations, they potentially trigger resistance to emerge within the food animal hosts. The heavy metal resistance genes (HMRGs) have also been identified in different environments. The genes that are responsible for resistance to arsenic (*ars*A), copper (*cop*B), and zinc (*czr*C) have been observed in methicillin-resistant *Staphylococcus aureus* isolated from livestock.[20] Meanwhile, the genes *czc*A and *ars*B for cobalt and arsenic resistance, respectively, have been found in municipal wastewater treatment plants and associated with antibiotic resistance genes (ARGs).[22] Furthermore, the HMRGs appear to be predominantly plasmid mediated.[21,23]

Previously studies demonstrated that a relationship between the acquisition of HMRGs and ARGs, and antibiotic resistance may arise through coresistance or crossresistance to metals or coregulation of resistance pathways.[20,24,25] The disinfectant resistance genes, as well as HMRGs, are commonly located in mobile genetic elements (MGEs).[23,26,27] The widespread use of disinfectants has raised concerns over their possible involvement in the development of antimicrobial resistance, particularly coresistance to antibiotics.[27,28] Therefore, under the pressure of concomitant use of antibiotics, heavy metals, and disinfectants, the potential coselection of resistance genes and the spread of acquired resistance is enhanced.[22,29] However, little information was known about the occurrence of disinfectant and heavy metal resistance in *Salmonella* isolated from retail foods of animal origin. The current study investigated the prevalence of antibiotic, disinfectant, and heavy metal resistance in *Salmonella* isolates, determined the associations between antibiotic resistance and the presence of disinfectant (QACs) and/or HMRGs, and explored the genetic relatedness of *Salmonella* from retail foods of animal origin.

Materials and Methods

Sampling

A total of 327 raw meat samples, including pork ($n=137$), chicken ($n=91$), and beef ($n=99$) were purchased from supermarkets in Sichuan Province between July 2013 and December 2014. The samples were aseptically collected in sterilized plastic bags and kept cold during transport from the supermarket to the laboratory.

Salmonella *isolation and serotyping*

The methods described by the United States Department of Agriculture Food Safety and Inspection Service were used to isolate *Salmonella* from retail foods of animal origin.[30] Briefly, 25 g portions of the products were used for culturing. Each sample was placed in separate sterile Erlenmeyer flasks with 225 ml buffered peptone water and incubated at 37°C in a water bath with shaking at 120 rpm for 6 h. After pre-enrichment, 10 and 1 ml of pre-enriched rinses were transferred to 100 ml each of the tetrathionate (TT; Beijing Land Bridge Technology Co., Ltd., Beijing, China) and Rappaport-Vassiliadis (RV; Beijing Land Bridge Technology Co., Ltd.) broths, respectively, and incubated at 42°C in a water bath with shaking at 160 rpm for 24 h. One loopful of overnight TT broth culture was streaked onto xylose lysine tergitol agar plates (Beijing Land Bridge Technology Co., Ltd.), whereas the RV broth was streaked onto agar of xylose lysine deoxycholate (Beijing Land Bridge Technology Co., Ltd.) and incubated at 37°C for 24 h.

Three presumptive *Salmonella* colonies from each plate were inoculated onto triple sugar iron (Beijing Land Bridge Technology Co., Ltd.) and urea agar slants (Beijing Land Bridge Technology Co., Ltd.).[30] After 24 h of incubation at 35°C, isolates with typical *Salmonella* phenotypes were confirmed by polymerase chain reaction (PCR). The PCR assays for identification of *Salmonella* were described previously.[30] A 284 bp PCR product targeting *invA* was amplified using the primers *invA* 139 (5′-GTGAAATTATCGCCA CGTTCGGGCAA-3′) and *invA* 141 (5′-TCATCGCACCGT CAAAGGAACC-3′). Amplicons were sequenced by Shanghai Sangon Bioengineering Co., Ltd. Nucleotide sequences were analyzed using BLAST software, which is available at the National Center for Biotechnology Information web-site (www.ncbi.nlm.nih.gov). If more than one isolate from each sample was *Salmonella* positive, only one was randomly selected and included in this study.[31] Confirmed isolates were stored in Tryptone Soya Broth (Hangzhou Microbial Reagent Co., Ltd.) containing 20% glycerol at −80°C until use. All isolates were further serotyped using commercial antisera purchased from Statens Serum Institute, Denmark, as described by the manufacturer.

Antibiotic resistance in Salmonella

The tested antibiotics were as follows: amoxicillin (AMX), amoxicillin/clavulanic acid (AMC), ampicillin (AMP), ceftiofur (EFT), oxytetracycline (OTC), ciprofloxacin (CIP), levofloxacin (LEV), trimethoprim (TMP), and gentamicin (GEN), all of which were purchased from Hangzhou Microbial Reagent Co., Ltd. The minimum inhibitory concentrations (MICs) were determined by using the agar dilution method, and breakpoints for antibiotic susceptible and/or resistant were determined as recommended by the Clinical and Laboratory Standards Institute (CLSI).[32] *Escherichia coli* ATCC 25922 and *E. coli* ATCC 35218 were used as the quality control strains. The resistance genes were examined through PCR using specific oligonucleotide primers as described previously.[33–39] All results were confirmed by at least two independent experiments.

Disinfectant resistance in Salmonella

The disinfectants tested were BC (Chengdu Best-Reagent Company, Chengdu, China; ≥98% purity) and CTPC (J&K

Chemical; ≥98% purity). The MICs of disinfectants were determined using the agar dilution method recommended by the CLSI.[24,32] The range of concentrations used to determine the MICs of both disinfectants were 0.125 to 1,024 mg/L. *E. coli* ATCC 10536 was used as the quality control strain. The disinfectants' resistance genes [*sugE(p)*, *qacEΔ1*, *qacE*, *qacF*, and *qacG*] were amplified and sequenced as described previously.[27]

Detection of HMRGs

The 17 different HMRGs encoding for 9 heavy metal resistance were detected by PCR based on published methods.[19–21,23,29,40–46] The positive controls that carried the resistance genes were confirmed using PCR followed by sequence analysis (Sangon Biotech, Shanghai, China).

Pulsed-field gel electrophoresis

All the isolates were selected for pulsed-field gel electrophoresis (PFGE) analysis using the PulseNet protocol (www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf). The *Xba*I-digested DNA fragments were analyzed using 1% agarose gels and a CHEF MAPPER electrophoresis system (Bio-Rad, Hercules, CA). The electrophoresis conditions were as previously described.[47] *Salmonella enterica* serovar, Braenderup H9812, was used as a marker. PFGE results were analyzed by BioNumerics software, and banding patterns were compared using Dice coefficients with a 1.5% band position tolerance.

Data analysis

Chi-squared test of independence or Fisher's exact test was performed to analyze data using SPSS v.12 (SPSS, Inc., 1989–2003). A *p*-value less than 0.05 was considered statistically significant for comparison.

Results

Prevalence and serotypes of Salmonella

Of the 327 retail meat samples, 46.5% ($n=152$) were contaminated with *Salmonella*. The most common prevalence was observed in pork ($n=75$, 54.7%), followed by chicken ($n=43$, 47.3%) and beef ($n=34$, 34.3%), respectively.

Among the 152 *Salmonella* isolates, 21 *Salmonella* serotypes were detected. *Salmonella* Derby was the most prevalent (28.9%, $n=44$), followed by *Salmonella* Typhimurium (15.8%, $n=24$), *Salmonella* Rissen (15.8%, $n=24$), *Salmonella* Enteritidis (9.9%, $n=15$), and *Salmonella* London (5.9%, $n=9$). The top five serotypes accounted for more than 75% of the strains.

The distribution of serotypes varied by meat types (Table 1). In chicken, the predominant serotypes were *Salmonella* Enteritidis (34.9%, $n=15$), *Salmonella* Derby (18.6%, $n=8$), and *Salmonella* Typhimurium (9.3%, $n=4$). Six serotypes, including *Salmonella* Enteritidis, *Salmonella* Agona, *Salmonella* Corvallis, *Salmonella* Hadar, *Salmonella* Indiana, and *Salmonella* Kouka, were only isolated from chicken. For pork samples, most isolates were contaminated with *Salmonella* Derby (32.0%, $n=24$), followed by *Salmonella* Rissen (24.0%, $n=18$), and *Salmonella* Typhimurium (14.7%, $n=11$). The Uganda and Waycross serotypes were only detected in pork. In beef samples, the top three serotypes were *Salmonella* Derby (35.3%, $n=12$), *Salmonella* Typhimurium (26.5%, $n=9$), and *Salmonella* Rissen (8.8%, $n=3$), whereas *Salmonella* Albany and *Salmonella* Give were only detected in beef.

Phenotype and genotype of antibiotic resistance in Salmonella

In general, 92.8% ($n=141$) *Salmonella* isolates demonstrated resistance to at least one antibiotic and 43.4% ($n=66$) were multidrug resistant (MDR, resistance to at least three classes of antibiotics). As shown in Fig. 1, of all the resistant isolates, 80.9% ($n=123$) were resistant to OTC, followed by TMP (64.5%, $n=98$), AMX (28.9%, $n=44$), AMP (28.3%, $n=43$), LEV (21.7%, $n=33$), CIP (16.4%, $n=25$), and GEN (10.5%, $n=16$). Moreover, 48 resistance profiles were observed in the resistant isolates, and the top three frequent resistance profiles were OTC (17.7%, $n=25$), OTC–TMP (17.7%, $n=25$), and AMX–OTC–TMP (11.3%, $n=16$). The frequency of antibiotic resistance varied depending on meat types (Fig. 1) and serotypes (Fig. 2). Notably, of the isolates from pork, 97.3% ($n=73$) showed resistance to antibiotics, followed by 94.1% ($n=32$) in beef, and 83.7% ($n=36$) in chicken. The prevalence of MDR *Salmonella* also was highest in pork (49.3%, $n=37$), followed by chicken (44.2%, $n=19$) and beef ($n=10$, 29.4%). Besides, the prevalence of

Table 1. Serotypes of *Salmonella* from Different Retail Meats

Chicken	% (n)	*Pork*	% (n)	*Beef*	% (n)
Salmonella Enteritidis	34.9 (15)	*Salmonella* Derby	32.0 (24)	*Salmonella* Derby	35.3 (12)
Salmonella Derby	18.6 (8)	*Salmonella* Rissen	24.0 (18)	*Salmonella* Typhimurium	26.5 (9)
Salmonella Typhimurium	9.3 (4)	*Salmonella* Typhimurium	14.7 (11)	*Salmonella* Rissen	8.8 (3)
Salmonella Agona	7.0 (3)	*Salmonella* London	9.3 (7)	*Salmonella* London	5.9 (2)
Salmonella Rissen	7.0 (3)	*Salmonella* Anatum	8.0 (6)	*Salmonella* Kumas	5.9 (2)
Salmonella Hadar	4.7 (2)	*Salmonella* Clackamas	4.0 (3)	*Salmonella* Clackamas	3.0 (1)
Salmonella Anatum	2.3 (1)	*Salmonella* Meleagridis	2.7 (2)	*Salmonella* Albany	3.0 (1)
Salmonella Indiana	2.3 (1)	*Salmonella* Norwich	1.3 (1)	*Salmonella* Bareilly	3.0 (1)
Salmonella Kouka	2.3 (1)	*Salmonella* Bareilly	1.3 (1)	*Salmonella* Kedougou	3.0 (1)
Salmonella Meleagridis	2.3 (1)	*Salmonella* Uganda	1.3 (1)	*Salmonella* Meleagridis	3.0 (1)
Salmonella Kedougou	2.3 (1)	*Salmonella* Waycross	1.3 (1)	*Salmonella* Give	3.0 (1)
Salmonella Norwich	2.3 (1)				
Salmonella Corvallis	2.3 (1)				
Salmonella Bareilly	2.3 (1)				

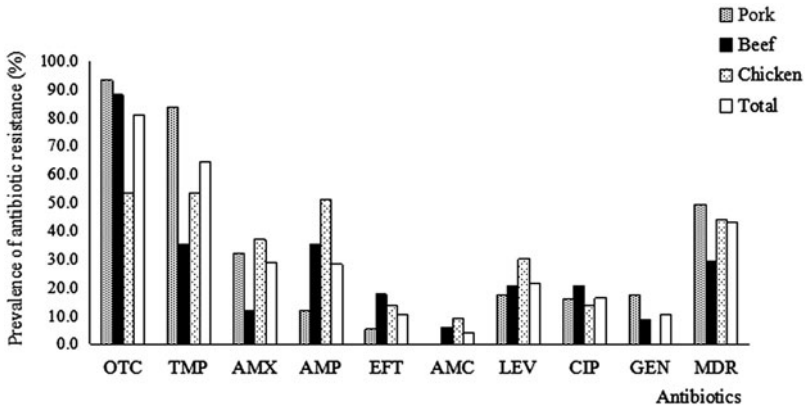

FIG. 1. Antibiotic resistance of *Salmonella* isolated from different meat types. OTC, oxytetracycline; TMP, trimethoprim; AMX, amoxicillin; AMP, ampicillin; EFT, ceftiofur; AMC, amoxicillin/clavulanic acid; LEV, levofloxacin; CIP, ciprofloxacin; and GEN, gentamicin; MDR, multidrug resistance.

resistance to OTC and TMP was observed significantly higher in pork isolates than in beef and chicken isolates ($p<0.001$), whereas a higher prevalence of resistance to AMP was observed in the isolates from chicken than from pork and beef isolates ($p<0.001$). Interestingly, all *Salmonella* Typhimurium ($n=24$) were resistant to at least one antibiotic, and high frequency of antibiotic resistance to LEV ($p<0.001$) and CIP ($p<0.05$) was observed in *Salmonella* London compared with the other serotypes.

As shown in Fig. 3a, the bla_{TEM} (44.7%, $n=34$) gene was most common in β-lactam-resistant isolates. Of the aminoglycoside-resistant isolates, the *ant(3")-Ia* gene was detected in highest frequency (50%, $n=8$). In tetracycline-resistant isolates, *tet*A (44.7%, $n=55$) was the most prevalent. Notably, the prevalence of *sul*1, *sul*2, and *sul*3 were the same (20.4%, $n=20$) in trimethoprim-resistant isolates. Only the *qnr*A gene was detected in 40% ($n=14$) of quinolone-resistant isolates. Moreover, 68 ARGs combinations were observed in *Salmonella*, and the top three frequent resistance gene combinations were *tet*A (5.3%, $n=8$), *tet*C (4.6%, $n=7$), *tet*G (3.3%, $n=5$), and *tet*A-*tet*G (3.3%, $n=5$).

The prevalence of ARGs also varied by meat types and serotypes. The tetracycline-resistant genes, *tet*A, *tet*G, and *tet*C, were the most common genes in pork (46.7%, $n=35$), beef (35.3%, $n=12$), and chicken (37.3%, $n=16$) isolates. Among different meat types, the prevalence of trimethoprim resistance genes and aminoglycoside resistance genes in pork isolates was the highest. Among different serotypes, *Salmonella* Rissen contained all ARGs genes tested and the prevalence of *tet*A, *sul*1, *sul*3, *bla*CTX-M, and *ant(3")-Ia* were the highest. However, most ARGs were absent in the isolates of *Salmonella* Enteritidis.

Phenotype and genotype of disinfectant resistance in Salmonella

Figure 4 showed the distribution of the MICs of disinfectants in *Salmonella* isolates from different retail meats. Our results revealed that the MICs of CTPC were 8 to 256 mg/L and BC were 8 to 128 mg/L. Generally, most *Salmonella* isolates exhibited MICs of 128 mg/L for CTPC (79.4%, $n=102$) and BC (58.6%, $n=89$). The chicken isolates (90.7%, $n=39$) had higher MICs (128 mg/L) for BC than those from the beef (79.4%, $n=27$) and pork (48.0%, $n=36$) isolates ($p<0.001$). Meanwhile, 76.0% ($n=57$) of the isolates that originated from pork had higher MICs (128 mg/L) for CTPC than the 53.5% ($n=23$) from chicken and the 20.6% ($n=9$) from beef ($p<0.001$). The MIC_{50} values and MIC_{90} values were the same in the isolates from different meat types (128 mg/L). In addition, the MIC ranges varied in the top five serotypes.

The *qacF* and *qacEΔ1* gene was detected in 18.4% ($n=28$) and 8.6% ($n=13$) of all the isolates, whereas the *qacE*, *qacG*, and *sugE(p)* genes were not detected in any isolates (Fig. 3b). The *qacF* was found the highest frequency in pork (24%, $n=18$) and *Salmonella* Rissen (26.1%, $n=6$). The *qacEΔ1* gene was found the highest frequency in chicken (16.3%, $n=7$) and *Salmonella* Rissen (26.1%, $n=6$).

Prevalence of HMRGs

Totally, 58.55% ($n=89$) of the isolates carried at least one HMRG. The Cu-resistance genes *pco*R, *pco*C, and *pco*A were the most common, accounting for 43.4% ($n=66$), 40.8% ($n=62$), and 20.4% ($n=31$), respectively (Fig. 3c). Besides, 17.8% ($n=27$) and 6.6% ($n=10$) of the isolates

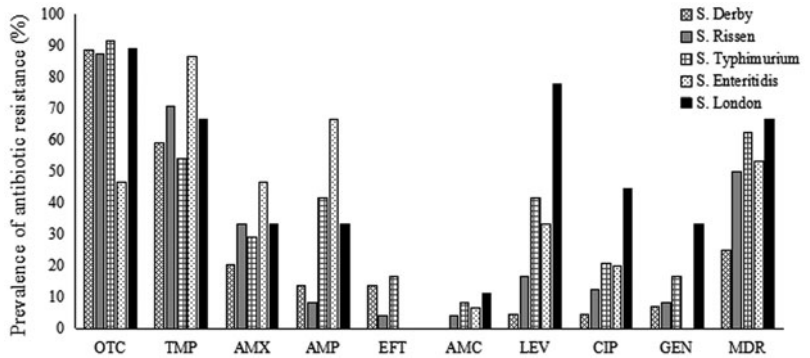

FIG. 2. Antibiotic resistance of *Salmonella* isolated from different serotypes.

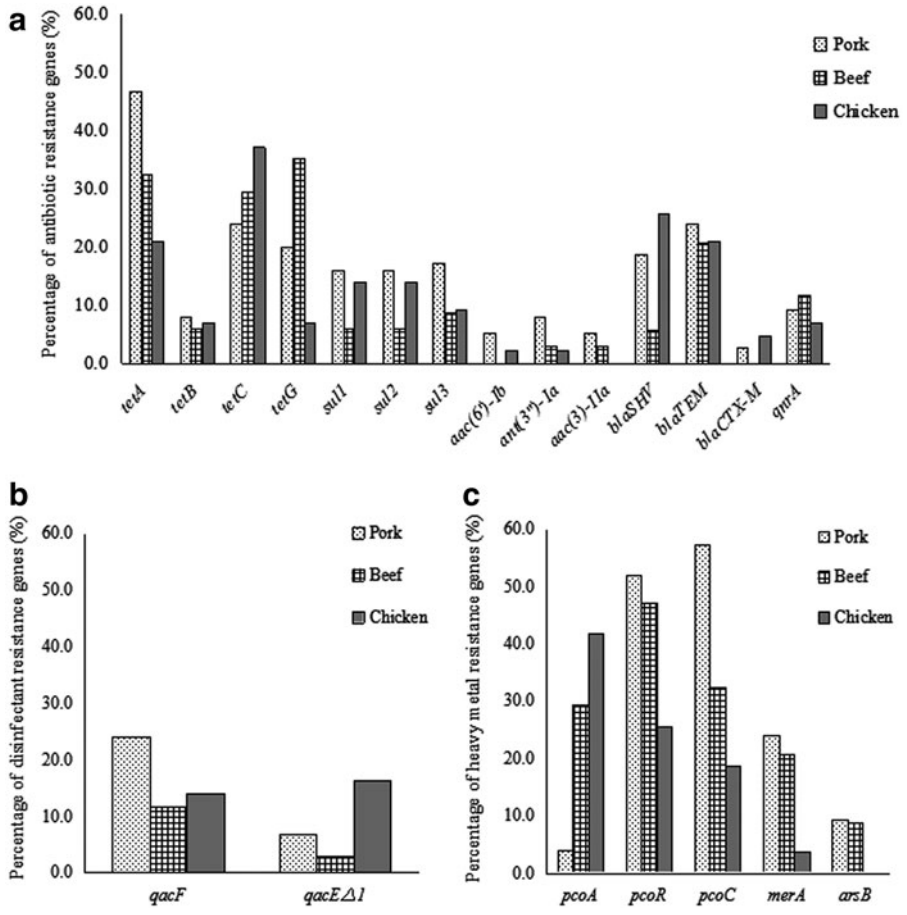

FIG. 3. **(a)** Frequency of antibiotic resistance genes of *Salmonella* in different retail meats. **(b)** Frequency of disinfectant resistance genes of *Salmonella* in different retail meats. **(c)** Frequency of heavy metal resistance genes of *Salmonella* in different retail meats.

carried the Hg-resistance gene *mer*A, and As-resistance gene *ars*B, respectively. A total of 14 gene combinations were found in all isolates. The top three resistance gene combinations were *pco*C ($n=14$), *pco*R ($n=14$), and *pco*C-*pco*R ($n=14$) (Supplementary Table S1; Supplementary Data are available online at www.liebertpub.com/mdr).

The distribution of the gene combinations varied by meat types and serotypes. The pork isolates possessed most types of the gene combinations, in which *pco*C (14.7%, $n=11$) and *pco*C-*pco*R (13.3%, $n=10$) were the main genotypes. The *pco*R (11.6%, $n=5$) and *pco*C-*pco*R (7.0%, $n=3$) were the top two gene combinations in chicken isolates. The *pco*R (14.7%, $n=5$) and *pco*R-*pco*A-*pco*C (14.7%, $n=5$) were the top two gene combinations in beef isolates. The *pco*C and *pco*R were found in high prevalence in *Salmonella* Rissen (75.0%, $n=18$). The *mer*A and *ars*B were found in a frequency of 34.9% ($n=8$) in *Salmonella* Typhimurium, and *pco*A was detected in 47.8% ($n=11$) of *Salmonella*

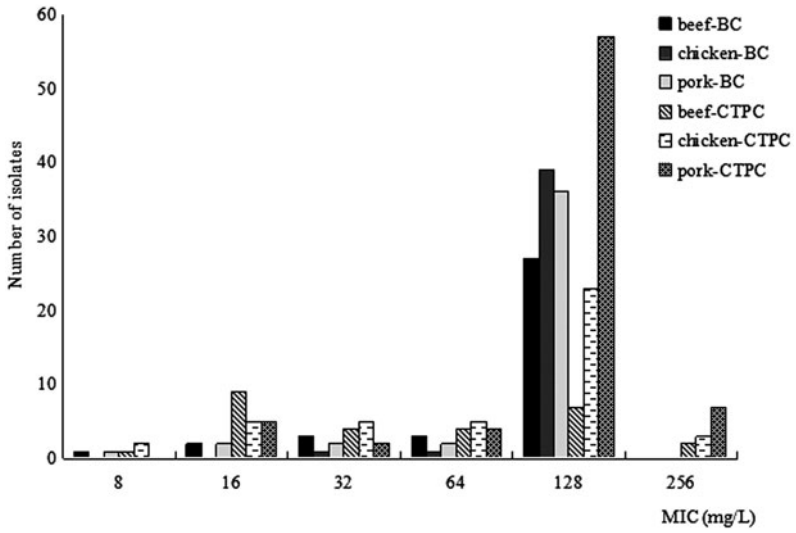

FIG. 4. Distribution of quaternary ammonium compound MICs of disinfectants in *Salmonella* isolates from different retail meats. The corresponding values of BC and CTPC for the control strains were 16 and 16 mg/L, respectively. BC, benzalkonium chloride; CTPC, cetylpyridinium chloride; MIC, minimum inhibitory concentration.

Typhimurium. In *Salmonella* Rissen, only Cu-resistance genes were found, and only one isolate of *Salmonella* Enteritidis had the *mer*A gene.

Association between disinfectant and antibiotic resistance

Most notably, the presence of disinfectant resistance genes was significantly associated with the trimethoprim and the related resistance genes *sul* ($p < 0.05$) (Supplementary Table S2). Among the *qacF*- and *qacEΔ1*-positive isolates, 100% ($n = 28$) and 93.3% ($n = 12$) of the isolates were resistant to at least one antibiotic, and 64.3% ($n = 18$) and 46.2% ($n = 6$) of the isolates were MDR, respectively. All the *qacF*-positive isolates contained β-lactam resistance genes and 85.7% ($n = 24$) had trimethoprim resistance genes. Meanwhile, 84.6% ($n = 11$) and 61.5% ($n = 8$) of the *qacEΔ1*-positive isolates possessed β-lactam and sulfonamide resistance genes, respectively.

Association between HMRGs and antibiotic resistance

In general, we found association between the presence of HMRGs and antibiotic resistance or ARGs ($p < 0.05$ or <0.001) (Supplementary Table S2). The gene *pco*A was significantly associated with antibiotic resistance and the genes, including β-lactam and *bla*TEM, tetracycline and *tet*A, *tet*B, *tet*C, and aminoglycosides and *ant(3")-Ia*. The *pco*C gene was significantly associated with the resistance to trimethoprim and *sul3*, and tetracycline and *tetG*. The *pco*R gene was associated with tetracycline resistance and the genes *tet*A, *tet*C, and *tet*G. Moreover, the association among *mer*A and tetracycline resistance and the gene *tet*G was significant. No significant association was observed between the presence of *ars*B and antibiotic resistance.

More particularly, the association among tetracycline resistance genes, disinfectant resistance genes, and Cu-resistance genes *pco*C or *pco*R, and sulfonamide resistance genes, disinfectant resistance genes, and Cu-resistance genes, *pco*C or *pco*R, was significant ($p < 0.05$).

PFGE typing

Up to 41 distinct PFGE clusters (using a cutoff value of 70%) were identified among the 152 *Salmonella* isolates (Supplementary Fig. S1). The dendrogram showed that isolates of the same serotypes were clustered together, except for a few individual isolates. The most common cluster 21 was comprised of 16 isolates of *Salmonella* Derby. Similar results were obtained in cluster 9 (*Salmonella* Typhimurium) and cluster 6 (*Salmonella* Rissen). PFGE clusters were also associated with supermarkets. In cluster 41, two *Salmonella* Anatum isolates originated from the same supermarket (WH). Moreover, 75% ($n = 12$) of the *Salmonella* Derby isolates (cluster 21) were obtained from the same supermarket located in different areas (including QJ, CJ, JJ, JJ1, and WJ). Similarly, cluster1, 12, 25, and 37 were clearly associated with the sampling supermarkets regardless of location. In addition, some PFGE clusters were also associated with meat types. For example, PFGE clusters 10, 22, and 40 were comprised of isolates recovering from pork, whereas clusters 1 and 28 were from chicken, and cluster 17 from beef. Thus, PFGE revealed that the *Salmonella* isolates were associated with the meat types and sampling supermarkets, indicating the possibility of crosscontamination in farms or processing environments. PFGE results also indicated that the resistance genes were widely distributed in isolates with different meat types and serotypes.

Discussion

Generally, *Salmonella* Derby, *Salmonella* Typhimurium, and *Salmonella* Rissen were the most common serotypes in our study, followed by *Salmonella* Enteritidis and *Salmonella* London. *Salmonella* Enteritidis and *Salmonella* Typhimurium were of the most common serotypes associated with human infections from food of animal origins and have been found among human isolates in Italy,[48] Spain,[49] and United States.[50,51] Gantzhorn *et al.*[52] reported that *Salmonella* Derby and *Salmonella* Typhimurium were the most prevalent serotypes in Danish pig slaughterhouses. *Salmonella* Enteritidis and *Salmonella* London were the common prevalent serotypes in retail meats as well.[7,13] In our study, *Salmonella* Enteritidis was only identified with high frequency in chicken, which was also the most prevalent serotype in chicken meat in the Shandong and Shaanxi province, China.[7,53] Higher carriage of these *Salmonella* serotypes by animals may contribute to the contamination of retail meats in human food supply.

The increase of antimicrobial resistance in *Salmonella* from retail meats has become a common problem worldwide.[13,53–55] Antibiotic resistance was common among the *Salmonella* isolates, particularly a high level of resistance to tetracycline (80.9%) and trimethoprim (64.5%). Similarly, the most frequent resistance profile of *Salmonella* isolated from pork and chicken meats in North Vietnam was tetracycline (58.5%), followed by sulfonamides (58.1%).[54] The Food and Drug Administration (FDA) has confirmed that *Salmonella* isolated from food animals showed highest resistance to tetracycline and sulfonamides.[56] The globalization of trade in retail meats could allow resistant *Salmonella* spread to different countries, which might increase the risk of the emergence and accumulation of antibiotic resistance worldwide. The *Salmonella* isolates from pork in our study showed higher resistance to antibiotics than from beef and chicken. All *Salmonella* Typhimurium isolates were resistant to at least one antibiotic. The results showed that the frequency of antibiotic resistance may be related with meat types and serotypes.[54,55]

The bla_{TEM}, *ant(3")-Ia*, and *tet*A genes were the most common in β-lactam-, aminoglycoside-, and tetracycline-resistant genes in our isolates, respectively. A similar result was reported by Yahiaoui *et al.*,[57] in which bla_{TEM} was the most frequent β-lactam-resistant gene in *E. coli*. Moreover, a high prevalence of *tet*A (55.6%), *tet*B (91.7%), and *ant(3")-Ia* (67.5%) genes were observed in the *S. enterica* isolated from chicken and quail carcasses.[58] The distribution of ARGs varied by meat types. The genes *tet*A, *tet*G, and *tet*C, were the most common genes in pork, beef, and chicken isolates, respectively. There was a higher prevalence of trimethoprim- and gentamicin-resistant genes found in pork isolates than in chicken and beef isolates. Bacci *et al.*[58] also observed that a higher percentage of the *ant(3")-Ia* gene in *Salmonella* spp. isolated from quail and chicken carcasses than in those isolated from chicken meat, and the

*tet*A and *tet*B genes were more frequent in chicken meat and carcasses isolates, respectively.

The majority of the *Salmonella* isolates showed MICs of 128 mg/L for CTPC (79.4%) and BC (58.6%). Our previous study demonstrated that 67.5% and 52.6% of the *E. coli* isolated from retail meats exhibited MICs of 4 to 128 and 32 mg/L for CTPC and BC, respectively.[59] In China, the disinfection requirements for a slaughterhouse (SB/T-10660-2012) stipulated the dosage of QACs in the range of 0.015% to 0.1% for slaughterhouses and meat production facilities. However, a previous study reported the increasing use of QACs with high concentration (10%), and susceptibilities to QACs have apparently increased among the bacteria isolated from retail meats or their production environments.[18,52,59] The disinfectant resistance genes *qacF* and *qacEΔ1* were detected in 18.4% and 8.6% of all the isolates, respectively. The *qacF* and *qacEΔ1* genes were less common in *E. coli* isolated from retail meats.[27,59] However, these two genes were located in MEGs and linked (coexisted) with different ARGs showing coresistance.[27] The widespread use of disinfectant may provide a selective environment for different degrees of adaptive resistance to *Salmonella*.

To best of our knowledge, limited research focused on the prevalence of varied HMRGs in *Salmonella* isolated from retail meats. The copper (228 mg/kg) and zinc (297 mg/kg) concentrations in feed of livestock and poultry were excessive in Liaoning, China. Comparing with the maximum limit value in Chinese standard (GB15199-94, GB13106-91), the copper concentration in chicken was twice as the standards and the zinc concentration obviously exceeded the limit.[60] While in Jiangsu province, concentrations of zinc and copper in animal feeds were 15.9–2041.8 and undetected–392.1 mg/kg, respectively.[61] Moreover, unlike antibiotics and QACs that are biodegradable through microorganisms,[62–64] heavy metals are not subject to degradation and can subsequently represent a long-term selection pressure, which potentially contributes to the development and maintenance of resistance genes.[65] The Cu-resistance genes *pco*R (43.42%), *pco*C (40.79%), and *pco*A (20.39%) were relatively higher than the Hg resistance gene *mer*A (17.76%) and As resistance genes *ars*B (6.58%). Argudín *et al.*[20] found that among the methicillin-resistant *S. aureus* isolated from livestock, 4.1%, 0.3%, 23.4%, and 62.7% were positive for the genes *ars*A (As), *cad*D (Ca), *cop*B (Cu), and *czr*C (Zn/Ca), respectively. The difference of genes in frequency may be related to the use of metal compounds as feed supplements at different farms. In addition, HMRGs have been observed in other environments. The genes of *mer* operon (*mer*RTPABDE) were detected in *Aeromonas salmonicida* isolates recovered from juvenile Atlantic salmon (*Salmo salar*) aquaculture.[66] Besides, Roosa *et al.*[29] observed seven HMRGs (*ars*B, *cop*A, *czc*A, *czc*C, *czc*D, *ncc*A, and *pbr*T) among the DNA of sediments. The environment of origin (*e.g.*, animal husbandry, sediments, or slaughter house) of the bacterial community might be a factor that contributes to the spread of HMRGs.[22]

In general, we found significant associations among HMRGs, disinfectant resistance genes, antibiotic resistance, and related genes ($p < 0.05$). The HMRGs and ARGs, as well as the disinfectant resistant genes we tested, were usually located on MGEs, such as plasmid pRJ1004, transposon Tn4380, and Tn501.[21,23,27,67,68] Besides, a higher proportion of plasmids hosted by *Salmonella* tended to carry the three classes of genes on the same plasmids.[69] The MGEs may potentially accrue different resistance genes through coresistance [22,62] and could be efficiently spread through bacteria community. We investigated the disinfectant resistance genes that were significantly associated with the sulfonamide resistance genes ($p < 0.05$). The class 1 integron contains the gene *qacE*Δ1 and *sul*1 resistance,[70] and coresistance may also occur involving the *qacE*Δ1, *aadA*2, and *sul*1 located in Tn5045.[68]

Besides, the association between the presence of HMRGs and antibiotic resistance and related ARGs was significant ($p < 0.05$). Osman *et al.*[71] isolated an aquatic bacterium harboring a plasmid which contained genes conferring resistance to antibiotics and metals Cr and Co. The IncA/C plasmid isolated from *Aeromonas salmonicida* subsp., *salmonicida* carried *mer* and multiple ARGs.[66] The association among Cu-resistant genes (*pco*A and *pco*R), disinfectant-resistant genes, and tetracycline- and sulfonamide-resistant genes was significant ($p < 0.05$). Both Hg-resistance genes (*mer*RTPCADE) and several antimicrobial-encoding genes (*sul*1, *qacE*Δ1, *aadA*1, and *bla*OXA-1) have been identified on plasmid pUO-StVR2 in *Salmonella* Typhimurium and these are linked with Transposon 21.[72] Whole genome sequencing was performed in our study and will confirm the location of these genes in the near future.

Conclusions

In conclusion, this study demonstrated that antimicrobial resistance was common among the *Salmonella* isolates from retail food of animal origin. Antibiotic, disinfectant, and heavy metal resistance varied by meat types and serotypes. The antibiotic resistance was highly associated with disinfectant or HMRGs. The farms or processing environment may be a major source for crosscontamination with *Salmonella*. Therefore, the retail meats may be a reservoir for the dissemination of antibiotic-resistant *Salmonella* and using disinfectants for decontamination or metals in livestock may provide a pressure for coselecting strains with acquired resistance to other antimicrobials.

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Disclosure Statement

No competing financial interests exist.

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