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Prevalence and characterization of *Salmonella enterica* serovar in retail meats in market place in Uighur, Xinjiang, China

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ABSTRACT

Ninety nine *Salmonella* isolates recovered from 1414 retail meats were characterized by serotyping, antimicrobial susceptibility, pulsed-field gel electrophoresis (PFGE), presence of amino acid mutation in quinolone resistance determination region (QRDR) of DNA gyrase subunit A (GyrA) and topoisomerase IV subunit C (ParC), and of *qnr*A, *qnr*B, *qnr*S, *aac(6′)-Ib*, *qep*A, and *oqx*AB. Among 1414 retail meat samples, 96 (6.8%) including 49 (9.0%) chickens, 22(6.8%) lambs, 10 (4.8%) beefs, 13 (6.8%) porks and 2(1.4%) horse meats were positive to *Salmonella*. The commonly detected *Salmonella* serotypes were *S.* Hadar (n = 21, 21.2%), *S.* Enteritidis (n = 17, 17.2%), *S.* London (n = 17, 17.2%), and *S.* Havana (n = 11, 11.1%). Eighty four (84.8%) isolates were simultaneously resistant to more than three antimicrobial agents. Antibiotic resistance was most commonly found to trimethoprim (100%), and a less extent to chloramphenicol (88.9%), tetracycline (63.6%), nalidixic acid (58.6%), sulfisoxazole (57.6%), streptomycin (43.4%), trimethoprim/tulfisoxazole (41.4%), ampicillin (25.6%), amoxicillin/clavulanate (25.6%), kanamycin (6.1%), ceftriaxone (5.1%), gentamicin (3.0%), cefoxitin (2.0%), and amikacin (1.0%). *qnr*A (11.1%), *qnr*B (34.3%), *qnr*S (8.1%), *aac(6′)-Ib* (7.1%), *qep*A (7.1%), *oqx*A (10.1%) and *oqx*B (9.1%) were detected from the 99 isolates. Amino acid substitutions of Asp87Asn (4.8%), Asp87Tyr (28.6%), Asp87Val (4.8%), Ser83Phe (52.4%), Ser83Tyr (7.1%) and Gly75Phe (2.4%) in GyrA were detected, as well as Thr57Ser (98.6%) and Gly53Val (1.4%) in ParC. Mutations of Ser83Phe (GyrA)/Thr57Ser (ParC) that simultaneously detected in GyrA and ParC were found in 22 isolates. Totally 82 different DNA patterns generated after the 99 isolates were subtyped using PFGE. The results demonstrated that the prevalence of *Salmonella* in retail meats in Uighur of Xinjiang province were not common, however, the isolates exhibited multidrug resistance, phenotypical and genotypical diverse.

1. Introduction

Foodborne *Salmonella* has been recognized as significant pathogen associated with public health worldwide. In the United States, approximately 1 million cases, 19,336 hospitalizations, 378 deaths, and $600 million to $3.5 billion medical expenditures were caused by non-typhoidal *Salmonella* annually (Scallan et al., 2011; Yang et al., 2010). In European, in 2009 and 2010, 108,614 and 99,020 salmonellosises were confirmed, respectively (Bonardi et al., 2013; De, Bravo, & Medina, 2012). Meanwhile, in Southeast Asia, an unofficial *Salmonella* surveillance indicated that approximate 22.8 million cases were occurred yearly with 37,600 deaths (Van, Nguyen, Smooker, & Coloe, 2012). As those uncovered, *Salmonella* infections commonly associated with consumption of contaminated foods that were mainly processed from food animals including poultry, pig, beef and lamb, which all are original sources to *Salmonella* (Aslam et al., 2012; Boonmar et al., 2013; Liu, Chen, Huang, Liu, & Shi, 2010; Mąka, Maćkiw, Ścieżyńska, Pawłowska, & Popowska, 2014; Wouafo et al., 2010).

Up to present, more than 2500 serovars have been identified among *Salmonella* (Son et al., 2013), while majorities of human infections were caused by limited number of serovars, among

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which, *S.* Enteritidis has been recognized as the most common serovar to human salmonellosis, other serovars including *S.* London, *S.* Hadar and *S.* Derby that involved in salmonellosis were reported to be prevalent in certain foods as well (Dogru, Ayaz, & Gencay, 2010; Hendriksen et al., 2011; Thong & Modarressi, 2011). With the antibiotics were widely used in food animal production, more and more *Salmonella* strains were identified as multiple resistant (MDR) ones, and the increasing MDR *Salmonella* were of great concern for food safety and public health worldwide (Boonmar et al., 2012; Kim, Park, Kwak, & Woo, 2011). Presence and dissemination of plasmid-mediated quinolone resistance (PMQR) genes (*qnr*A, *qnr*B, *qnr*S, *aac(6′)*-Ib, *qep*A, *oqx*AB) were regarded as one of the important mechanisms that contributed to quinolone resistance. Meanwhile, Amino acid alterations in GyrA and ParC of the quinolone resistance determining region (QRDR) have been demonstrated to result in decreased susceptibility to fluoroquinolones (Shariat et al., 2013; Zhang et al., 2014).

In this study, 99 *Salmonella* isolates recovered from retail meats in 2013 and 2014 in Xinjiang Uighur were characterized for better understand food safety situation in China to ensure public health.

2. Materials and methods

2.1. Isolates

A total of 1414 retail raw meat samples including 542 chickens, 325 lambs, 210 beefs, 192 porks and 145 horse meats were collected from 23 wet markets in seven districts of Uighur city of Xinjiang province, China, during 2013–2014. Detailed information for sample collection and *Salmonella* isolation were as previously described (Yin et al., 2014). Isolates with typical *Salmonella* phenotypes on XLT4 (Beijing Land Bridge Technology Co Ltd., Beijing, China) and XLD (Beijing Land Bridge Technology Co Ltd.) plates were finally identified and confirmed by O hypersera A-F (S&A Reagent Lab, Bangkok, Thailand). All isolates were stored at −80 °C in Luria–Bertani broth (LB; Difco, Maryland, USA)/glycerol (50%/50%, V/V) until use.

Salmonella isolates were serotyped by slide agglutination method (GB 4789.4-2010; Yang et al. 2013) using specific O and H antisera (S&A Reagent Lab, Bangkok, Thailand) in Henan Center for Disease Control and Prevention, Zhengzhou, Henan, China.

2.2. Antimicrobial susceptibility test

All isolates were examined for their susceptibility to 15 antibiotics including ampicillin (AMP), amoxicillin/clavulanic (AMC), amikacin (AMK), cefoxitin (CFX), ceftriaxone (CRO), chloramphenicol (CHL), nalidixic acid (NAL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), tetracycline (TET), trimethoprim (TIO), sulfisoxazole (FIS) and trimethoprim/sulfamethoxazole (SXT). The minimum inhibitory concentrations (MICs) of the antibiotics were determined by agar dilution method that described by the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2013). *Escherichia coli* ATCC25922 and *Enterococcus faecalis* ATCC29212 were used as quality control organisms in MICs determinations. The breakpoints for antimicrobial susceptible and/or resistant were interpreted and determined by CLSI guidelines except streptomycin, the breakpoint of which was interpreted according to that of the National Antimicrobial Resistance Monitoring System (NARMS) managed by the Food and Drug Administration (FDA), the U.S. Centers for Disease Control and Prevention (CDC) and the United States Department of Agriculture (USDA) (U.S. Food and Drug Administration, 2013).

2.3. Pulse field gel electrophoresis (PFGE)

PFGE was carried out for *Salmonella* genetic subtyping according to the protocol developed by the CDC (Ribot et al., 2006). Briefly, *Salmonella* isolates were cultured on Luria–Bertani agar (Beijing Land Bridge Technology Co Ltd.) at 37 °C overnight, the genomic DNA was prepared by embedding the isolates in agarose plugs, after cells were lysised, the embedded DNA was digested with 50 U of XbaI(TaKaRa, Dalian, China) for 1.5–2 h in a water bath at 37 °C. The DNA fragments were subsequently separated by electrophoresis in 0.5 × TBE buffer at 14 °C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA, USA) with pulse times of 2.16–63.8 s. *Salmonella* Braenderup H9812 was used as the standard control strain. The gels were stained with ethidium bromide and the DNA bands were visualized using UV transillumination (Bio-Rad). Fingerprinting profiles were analyzed using the BioNumerics software (Version 3.0; Applied-Maths, Kortrijk, Belgium) manually, the genotype was determined by a cutoff value of 90% similarity based on the unweighted pair group method with arithmetic mean (UPGMA).

2.4. Detection of PMQR genes (qnrA, qnrB, qnrS, aac(6′)-Ib-cr, qepA and oqxAB) and QRDR (GyrA and ParC) mutations

All nalidixic acid resistant isolates were screened for presence of PMQR genes and amino acid substitutions in GyrA and ParC by polymerase chain reaction (PCR) using the primers and annealing temperatures listed in Table 1. PCRs were carried out in a 25 μL PCR mixture that contained 0.5 μM of each primer, 250 μM of each dNTP, 2.5 μL of 10 × PCR buffer, 0.5 U of Taq DNA polymerase (TaKaRa), 1.5 mM $MgCl_2$ and 5 μL of sample template DNA, with predenaturation at 94 °C for 10 min; 35 cycles of denaturation at 94 °C for 30 s, at annealing temperatures for 30 s and a final extension at 72 °C for 7 min. Primers were synthesized by TaKaRa Biotechnology Co., Ltd. PCR products were stained with ethdium bromide and visualized under UV light after gel electrophoresis in 1% agarose. For *gyr*A and *par*C analysis, PCR products were sequenced in Shanghai Sunny Biotechnology Co, Ltd (Shanghai, China) and the sequence were aligned using BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

3. Results

3.1. Isolation and identification of Salmonella

Among 1414 retail meat samples, 96 (6.8%) including 49 (9.0%) of 542 chickens, 22 (6.8%) of 325 lambs, 10 (4.8%) of 210 beefs, 13 (6.8%) of 192 porks and 2 (1.4%) of 145 horse meats were detected *Salmonella* positive (Table 2). Totally 99 *Salmonella* isolates (1–2 isolates per sample) including 52 from chicken, 22 from lamb, 13 from pork, 10 from beef and 2 from horsemeat were recovered (see Fig. 1).

3.2. Serotype distribution

A total of 18 different serotypes were identified among 99 *Salmonella* isolates. Nine, 10, 8, 8, and 1 serotypes were identified among isolates recovered from retail chicken, lamb, beef, pork and horsemeat, respectively. The commonly prevalent serotypes were *Salmonella* Hadar (n = 21, 21.2%), *S.* Enteritidis (n = 17, 17.2%), *S.* London (n = 17, 17.2%) and *S.* Havana (n = 11, 11.1%). *Salmonella* Enteritidis, *S.* London, and *S.* Havana were simultaneously identified from retail chickens, lambs, beefs and porks. *Salmonella* Enteritidis, *S.* Hadar, *S.* London, *S.* Paratyphi B and *S.* Havana were commonly identified among the isolates recovered from retail chickens.

Table 1
Primers used for target genes detection and sequencing.

Target gene	Primer	Sequence (5′-3′)	Annealing temperature (°C)	Product size (bp)	Reference
Amplification and sequencing primers					
*gyr*A	*gyr*A-F	ACGTACTAGGCAATGACTGG	56	190	Eaves, Liebana, Woodward, & Piddock, 2002
	*gyr*A-R	AGAAGTCGCCGTCGATAGAA			
*par*C	*par*C-F	CTATGCGATGTCAGAGCTGG	54	270	Eaves et al., 2004
	*par*C-R	TAACAGCAGCTCGGCGTATT			
Detection primers					
*qnr*A	*qnr*A-F	AGAGGATTTCTCACGCCAGG	60	580	Cattoir, Poirel, Rotimi, Soussy, & Nordmann, 2007
	*qnr*A-R	TGCCAGGCACAGATCTTGAC			
*qnr*B	*qnr*B-F	GGMATHGAAATTCGCCACTG	56	264	Cattoir et al., 2007
	*qnr*B-R	TTTGCYGYYCGCCAGTCGAA			
*qnr*S	*qnr*S-F	GCAAGTTCATTGAACAGGGT	57	428	Cattoir et al., 2007
	*qnr*S-R	TCTAAACCGTCGAGTTCGGCG			
aac(6′)-Ib	*aac(6′)-Ib*-F	TTGCGATGCTCTATGAGTGGCTA	55	482	Park, Robicsek, Jacoby, Sahm, & Hooper, 2006
	aac(6′)-Ib-R	CTCGAATGCCTGGCGTGTTT			
*qep*A	*qep*A-F	CTGCAGGTACTGCGTCATG	60	403	Chen, Zhang, et al., 2012; Chen, Shao, et al., 2012
	*qep*A-R	CGTGTTGCTGGAGTTCTTC			
*oqx*A	*oqx*A-F	GACAGCGTCGCACAGAATG	62	339	Chen, Zhang, et al., 2012; Chen, Shao, et al., 2012
	*oqx*A-R	GGAGACGAGGTTGGTATGGA			
*oqx*B	*oqx*B-F	CGAAGAAAGACCTCCCTACCC	62	240	Chen, Zhang, et al., 2012; Chen, Shao, et al., 2012
	*oqx*B-R	CGCCGCCAATGAGATACA			

Table 2
Prevalence of *Salmonella* in different source of retail meats during 2013–2014.

Retail meat	No. sample	No.(%) samples positive to *Salmonella*	No. *Salmonella* isolates recovered[a]
Chicken	542	49 (9.0)	52
Lamb	325	22 (6.8)	22
Beef	210	10 (4.8)	10
Pork	192	13 (6.8)	13
Horsemeat	145	2 (1.4)	2
Total	1414	96 (6.8)	99

[a] 1–2 *Salmonella* isolates were recovered from each positive sample.

Salmonella Schwarzengrund II, *S.* Agona, *S.* Assinie, *S.* Braenderup, *S.* Goldcoast, *S.* Infantis, *S.* Thompson, *S.* Typhimurium, *S.* Tennessee, and *S.* Bloomsbury were detected from retail lambs, beefs, and porks. Two *Salmonella* isolates recovered from horsemeat were all *S.* London (Table 3).

3.3. Antimicrobial susceptibility testing

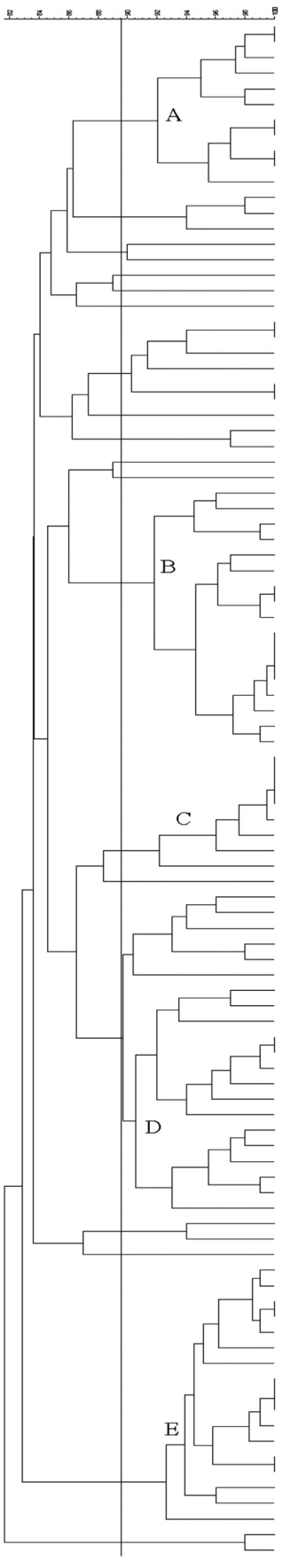
Ninety nine *Salmonella* isolates were all susceptible to ciprofloxacin (see Table 4). Antibiotic resistance was commonly observed to trimethoprim (100%), then to chloramphenicol (88.9%), tetracycline (63.6%), nalidixic acid (58.6%), sulfisoxazole (57.6%), streptomycin (43.4%) and trimethoprim/sulfisoxazole (41.4%), and a less extent to ampicillin (25.6%), amoxicillin/clavulanate (25.6%), kanamycin (6.1%), ceftriaxone (5.1%), gentamicin (3.0%), cefoxitin (2.0%) and amikacin (1.0%).

All isolates were resistant to at least one antibiotic, no *Salmonella* can resist to more than 10 antimicrobial agents (Table 5). Thirty (30.3%) *Salmonella* isolates were resistant to 1–3 antibiotics, 34 (34.3%) to 4–6 antibiotics, 31 (31.3%) to 7–9 antibiotics and 4 (4.0%) to 10 antibiotics. When analyzed by retail meat types, resistance was most frequently detected in chicken-derived *Salmonella* isolates, 23 (44.3%) of which were resistant to at least 7 antimicrobials, and 3 (5.8%) to 10 antimicrobials. *Salmonella* isolates derived from beefs (n = 7, 70.0%) and lambs (n = 10, 45.5%) were most commonly resistant to 4–6 antimicrobials, those from porks (n = 10, 7.7%) were to 10 antimicrobials. *Salmonella* isolates (n = 2) derived from horsemeats was relatively susceptible.

When analyzed by serovar, 33.3% of *Salmonella* Havana and 29.6% of *S.* London isolates frequently exhibited resistance to 1–3 antibiotics. 50.0% of *S.* Hadar and 25.0% of *S.* London isolates were simultaneously resistant to 4–6 antibiotics. Some of *S.* Enteritidis (n = 12, 46.1%), *S.* Hadar (n = 7, 26.9%) and *S.* Derby (n = 4, 15.4%) isolates could resist more than 7 antibiotics. Two *S.* Enteritidis and two *S.* London isolates could resist 10 antibiotics (Table 6). Generally, antibiotic resistance level of *Salmonella* Enteritidis, *S.* Hadar and *S.* Derby isolates was much higher than that of isolates of other serotypes.

3.4. PFGE

Genetic relatedness of 99 *Salmonella* isolates was analyzed using PFGE with XbaI Fig. 1. A total of 82 different PFGE patterns were observed. Using a cutoff value of 90% similarity, five typical clusters (named as A, B, C, D, and E, respectively) and several individual profiles were observed. Among each cluster, DNA profiles of the isolates exhibited high homology, although difference still could be found. Eleven isolates were grouped in cluster A, and 10 of them were *S.* Havana. Seventeen *S.* Enteritidis isolates were grouped in cluster B. Eight isolates were grouped in cluster C, and six of them were *S.* Paratyphi B. Twenty one isolates were grouped in cluster D, 20 of them were *S.* Hadar. Seventeen isolates were grouped in cluster E, and isolates in this cluster were all *S.* London. Three pairs of *S.* Havana isolates in cluster A, four *S.* Enteritidis isolates in Cluster B, four *S.* Paratyphi B isolates in Cluster C, two *S.* Hadar isolates in Cluster D and three *S.* London isolates in Cluster E, were detected 100% similarity. DNA profiles of some isolates with same serotype in same PFGE cluster were the same or similar, although these isolates were recovered from different times, different sampling places and different retail meats.



strian	serotype	year	district	origin	antibiotic resistence profile
ymy055	Havana	2014	SY	Lamb	Tio,Fox
ymy056	Havana	2014	CJ	Chicken	Chl,Tio,Tcy
ymy076	Havana	2014	XS	Pork	Chl,Tio,Tcy
ymy044	Havana	2014	SM	Chicken	Chl,Tio
ymy108	Havana	2013	SM	Chicken	Tio,Fis
ymy078	Havana	2014	MD	Chicken	Chl,Tio,Fis,Tcy
ymy014	Havana	2013	SY	Chicken	Tio
ymy107	Havana	2013	SM	Chicken	Chl,Tio,Sxt,Fis,Nal,Tcy,Str,Kan
ymy017	Havana	2013	SM	Chicken	Tio,Fis
ymy018	Havana	2013	SM	Beef	Chl,Tio
ymy011	Derby	2013	TS	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy012	Havana	2013	SY	Beef	Chl,Tio,Fis
ymy013	Paratyphi B	2013	SY	Chicken	Chl,Tio
ymy057	Derby	2014	CJ	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy034	Goldcoast	2013	MD	Pork	Chl,Tio
ymy094	Tennessee	2014	XS	Lamb	Chl,Tio,Sxt,Fis,Nal,Tcy,Kan
ymy030	Mbandaka	2013	SM	Beef	Chl,Tio,Fis,Nal
ymy104	Oran II	2014	SY	Beef	Chl,Tio,Sxt,Fis,Nal,Tcy,Kan
ymy033	Assinie	2013	SM	Beef	Chl,Tio,Sxt,Nal
ymy088	Mbandaka	2014	MD	Lamb	Tio,Fis,Nal,Tcy
ymy087	Mbandaka	2014	MD	Chicken	Tio,Fis,Tcy
ymy086	Mbandaka	2014	MD	Chicken	Tio,Fis,Tcy
ymy051	Mbandaka	2014	TS	Lamb	Chl,Tio
ymy089	Schwarzengrundll	2014	MD	Beef	Chl,Tio,Fis,Tcy
ymy090	Schwarzengrundll	2014	MD	Beef	Chl,Tio,Fis,Nal,Tcy
ymy080	Bloomsbury	2014	XS	Pork	Chl,Tio,Tcy
ymy042	Derby	2014	XS	Pork	Tio,Sxt,Fis,Tcy,Str
ymy043	Derby	2014	XS	Pork	Chl,Tio,Sxt,Fis,Cro,Tcy,Str
ymy036	Infantis	2013	SY	Pork	Amp,Amc,Tio,Sxt,Fis,Gen,Amk,Kan
ymy065	Braenderup	2014	SY	Lamb	Chl,Tio
ymy082	Enteritidis	2014	SY	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Cro,Tcy,Str
ymy003	Enteritidis	2013	SY	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Str
ymy019	Enteritidis	2013	SY	Lamb	Chl,Tio,Nal
ymy021	Enteritidis	2013	XS	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy004	Enteritidis	2013	SY	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Str
ymy029	Enteritidis	2013	SM	Beef	Chl,Tio,Fis,Nal
ymy035	Enteritidis	2013	MD	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Str
ymy040	Enteritidis	2014	SY	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Str
ymy041	Enteritidis	2014	SY	Chicken	Chl,Tio,Nal
ymy038	Enteritidis	2014	CJ	Pork	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Str
ymy039	Enteritidis	2014	CJ	Lamb	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Str
ymy045	Enteritidis	2014	SM	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Str
ymy084	Enteritidis	2014	SY	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy085	Enteritidis	2014	SY	Pork	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy109	Enteritidis	2014	SY	Chicken	Amp,Amc,Chl,Tio,Fis,Cro,Tcy
ymy046	Enteritidis	2014	TT	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Str
ymy047	Enteritidis	2014	TT	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Cro,Tcy,Str
ymy052	Paratyphi B	2014	TS	Lamb	Tio,Nal
ymy008	Paratyphi B	2013	TS	Chicken	Chl,Tio,Fis
ymy010	Derby	2013	TS	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy015	Paratyphi B	2013	SY	Chicken	Chl,Tio,Fis
ymy058	Paratyphi B	2014	TT	Chicken	Chl,Tio,Fis,Nal
ymy002	Paratyphi B	2013	SY	Chicken	Chl,Tio
ymy001	Paratyphi B	2013	SY	Chicken	Chl,Tio,Sxt,Nal
ymy102	Oran II	2014	MD	Chicken	Chl,Tio,Nal,Tcy
ymy083	Agona	2014	SY	Chicken	Amp,Amc,Chl,Tio,Fis,Cro,Tcy
ymy005	Hadar	2013	SY	Lamb	Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy031	Hadar	2013	SY	Lamb	Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy007	Hadar	2013	SY	Pork	Chl,Tio,Sxt,Nal,Tcy,Str
ymy028	Hadar	2013	SM	Chicken	Chl,Tio,Nal,Tcy,Str
ymy032	Hadar	2013	SY	Lamb	Chl,Tio,Sxt,Nal,Tcy,Str
ymy077	Thompson	2014	MD	Lamb	Chl,Tio,Fis,Fox,Tcy
ymy026	Hadar	2013	SM	Chicken	Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy110	Hadar	2014	XS	Chicken	Chl,Tio,Fis,Nal,Tcy,Str
ymy064	Hadar	2014	SY	Lamb	Chl,Tio,Nal,Tcy,Str
ymy060	Hadar	2014	TT	Lamb	Chl,Tio,Nal,Tcy,Str
ymy061	Hadar	2014	TT	Lamb	Chl,Tio,Nal,Tcy,Str
ymy091	Hadar	2014	XS	Chicken	Chl,Tio,Nal,Tcy,Str
ymy092	Hadar	2014	XS	Lamb	Chl,Tio,Nal,Tcy,Str
ymy023	Hadar	2013	XS	Pork	Chl,Tio,Fis,Nal,Tcy,Str
ymy027	Hadar	2013	SM	Chicken	Amp,Amc,Chl,Tio,Fis,Nal,Tcy,Str
ymy025	Hadar	2013	CJ	Chicken	Chl,Tio,Nal,Tcy,Str
ymy006	Hadar	2013	SY	Lamb	Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy020	Hadar	2013	SY	Lamb	Chl,Tio,Nal,Tcy,Str
ymy016	Hadar	2013	SM	Chicken	Chl,Tio,Sxt,Fis,Nal,Tcy,Str
ymy024	Hadar	2013	CJ	Chicken	Chl,Tio,Nal,Tcy,Str
ymy101	Hadar	2014	MD	Chicken	Chl,Tio,Fis,Nal,Tcy,Str
ymy097	Hadar	2014	CJ	Chicken	Chl,Tio,Sxt,Fis,Nal,Tcy,Kan
ymy111	Oran II	2014	CJ	Lamb	Chl,Tio,Sxt,Fis,Nal,Tcy,Kan
ymy096	Typhimurium	2014	XS	Beef	Amp,Amc,Chl,Tio,Nal,Tcy
ymy059	London	2014	TT	Chicken	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Tcy,Str,Gen
ymy022	London	2013	XS	Chicken	Chl,Tio,Sxt,Fis,Tcy
ymy049	London	2014	XS	Chicken	Chl,Tio
ymy062	London	2014	XS	Lamb	Chl,Tio
ymy048	London	2014	XS	Pork	Chl,Tio,Sxt
ymy079	London	2014	MD	Chicken	Chl,Tio,Fis,Tcy
ymy112	London	2014	MD	Lamb	Tio,Fis,Nal,Tcy
ymy071	London	2014	SY	Lamb	Chl,Tio,Fis,Tcy
ymy072	London	2014	SY	Pork	Chl,Tio,Fis,Tcy
ymy075	London	2014	TS	Chicken	Chl,Tio,Tcy
ymy074	London	2014	SY	Horsemeat	Chl,Tio,Tcy
ymy073	London	2014	SY	Horsemeat	Chl,Tio,Tcy
ymy068	London	2014	SY	Chicken	Chl,Tio
ymy069	London	2014	SY	Chicken	Chl,Tio,Tcy
ymy037	London	2013	SY	Pork	Amp,Amc,Chl,Tio,Sxt,Fis,Nal,Tcy,Str,Gen
ymy070	London	2014	SY	Beef	Chl,Tio,Sxt,Fis,Tcy
ymy009	London	2013	TS	Chicken	Chl,Tio,Sxt,Fis
ymy066	Paratyphi B	2014	SY	Chicken	Amp,Amc,Chl,Tio,Nal,Tcy,Str
ymy067	Paratyphi B	2014	SY	Chicken	Amp,Amc,Chl,Tio,Nal,Tcy,Str

Table 3
Distribution of *Salmonella* serovars in retail meats during 2013–2014.

Serotype	No. (%) isolates (n = 99)	No. (%) type of meat				
		Chicken (n = 52)	Lamb (n = 22)	Beef (n = 10)	Pork (n = 13)	Horsemeat (n = 2)
Hadar	21 (21.2)	10 (19.2)	9 (40.9)	2 (15.4)		
Enteritidis	17 (17.2)	12 (23.1)	2 (9.1)	2 (15.4)	1 (10.0)	
London	17 (17.2)	8 (15.4)	3 (13.6)	3 (23.1)	1 (10.0)	2 (100)
Havana	11 (11.1)	7 (13.5)	1 (4.5)	1 (9.1)	2 (20.0)	
Paratyphi B	9 (9.1)	8 (15.4)	1 (4.5)			
Derby	5 (5.1)	3 (5.8)		2 (15.4)		
Mbandaka	5 (5.1)	2 (3.8)	2 (9.1)		1 (10.0)	
Oran II	3 (3.0)	1 (1.9)	1 (4.5)		1 (10.0)	
Schwarzengrund II	2 (2.0)				2 (20.0)	
Agona	1 (1.0)	1 (1.9)				
Assinie	1 (1.0)				1 (10.0)	
Braenderup	1 (1.0)		1 (4.5)			
Goldcoast	1 (1.0)			1 (9.1)		
Infantis	1 (1.0)			1 (9.1)		
Thompson	1 (1.0)		1 (4.5)			
Typhimurium	1 (1.0)				1 (10.0)	
Tennessee	1 (1.0)		1 (4.5)			
Bloomsbury	1 (1.0)			1 (9.1)		
Total	99 (100)	52 (52.5)	22 (22.2)	13 (13.1)	10 (10.1)	2 (2.0)

Table 4
Antimicrobial resistance of *Salmonella* isolated from retail meats during 2013–2014.

Antimicrobial agent	No. of isolates (%)						
	Resistant breakpoint[a] (μg/ml)	Chicken (n = 52)	Lamb (n = 22)	Pork (n = 13)	Beef (n = 10)	Horsemeat (n = 2)	Total (n = 99)
Trimethoprim	R ≥ 16	52 (100)	22 (100)	13 (100)	10 (100)	2 (100)	99 (100)
Chloramphenicol	R ≥ 32	47 (90.4)	18 (81.8)	11 (84.6)	10 (100)	2 (100)	88 (88.9)
Tetracycline	R ≥ 16	32 (61.5)	15 (68.2)	9 (69.2)	5 (50.0)	2 (100)	63 (63.6)
Nalidixic acid	R ≥ 32	31 (59.6)	16 (72.7)	5 (38.5)	6 (60.0)		58 (58.6)
Sulfisoxazole	R ≥ 512	33 (63.5)	10 (45.5)	8 (61.5)	6 (60.0)		57 (57.6)
Streptomycin[b]	R ≥ 64	26 (50.0)	10 (45.5)	7 (53.8)			43 (43.4)
Trimethoprim/Sulfisoxazole	R ≥ 4/76	22 (42.3)	7 (31.8)	8 (61.5)	4 (40.0)		41 (41.4)
Ampicillin	R ≥ 32	19 (36.5)	1 (4.5)	4 (30.8)	1 (10.0)		25 (25.6)
Amoxycillin/Clavulanate	R ≥ 32/16	19 (36.5)	1 (4.5)	4 (30.8)	1 (10.0)		25 (25.6)
Kanamycin	R ≥ 64	2 (3.8)	2 (9.1)	1 (7.7)	1 (10.0)		6 (6.1)
Ceftriaxone	R ≥ 64	4 (7.7)		1 (7.7)			5 (5.1)
Gentamicin	R ≥ 16	1 (1.9)		2 (15.4)			3 (3.0)
Cefoxitin	R ≥ 32		2 (9.1)				2 (2.0)
Amikacin	R ≥ 64			1 (7.7)			1 (1.0)
Ciprofloxacin	R ≥ 8						

[a] MICs (μg/ml) were determined via agar dilution in accordance with CLSI.
[b] The breakpoint was interpreted according to that of the NARMS.

3.5. Detection of GyrA and ParC mutation and qnrA, qnrB, qnrS, aac(6′)-Ib-cr, qepA, oqxAB

Mutations of GyrA and ParC were detected in 89 of the 99 *Salmonella* isolates, and a total of 113 amino acid substitutions including 42 in GyrA and 71 in ParC were detected. The frequently detected mutations in GyrA were Ser83Phe (n = 22, 52.4%), Asp87Tyr (n = 12, 28.6%), Ser83Tyr (n = 3, 7.1%), Asp87Val (n = 2, 4.8%) and Gly75Phe (n = 1, 2.4%). Thr57Ser (n = 69, 97.2%) was most commonly detected in ParC in addition to Gly53Val/Thr57Ser (n = 1, 1.4%). Simultaneously mutations of Thr57Ser(ParC)-Ser83Phe(GyrA) (n = 22, 95.7%) and Thr57Ser(ParC)-Ser83Tyr(GyrA) (n = 1, 4.3%) detected in GyrA and ParC in 23 isolates. Sixty one isolates were detected harboring PMQR genes, the most prevalent one of which was *qnr*B (34.3%), followed ones were *oqx*AB (19.2%), *qnr*A (11.1%), *qnr*S (8.1%), *aac(6′)-Ib* (7.1%) and *qep*A (7.1%). Forty there (43.4%), 12 (12.1%), 3 (3.0%) and 2 (2.0%) isolates were detected simultaneously carrying 1, 2, 3 and 4 of the seven PMQR genes, respectively.

4. Discussion

In present study, retail meats collected from seven districts of Uighur, Xinjiang in China were identified for the presence of *Salmonella*, and *Salmonella* isolates were further characterized via serotyping, antimicrobial susceptibility, and PFGE.

Our results indicated that *Salmonella* was prevalent in retail meats in Xinjiang, and was more prevalent in retail chickens (9.0%)

Fig. 1. Dendrogram of PFGE patterns of 99 *Salmonella* isolates from retail meats in Xinjiang Province, China. Antimicrobial agents including Trimethoprim(TIO), Chloramphenicol(CHL), Tetracycline(TET), Nalidixic acid(NAL), Sulfisoxazole(FIS), Streptomycin(STR), Trimethoprim/Sulfisoxazole(SXT), Ampicillin(AMP), Amoxycillin/Clavulanate(AMC), Kanamycin(KAN), Ceftriaxone(CRO), Gentamicin(GEN), Cefoxitin(FOX), Amikacin(AMK), Ciprofloxacin(CIP). Districts including Shayibake(SY), Shuimogou(SM), Xinshiqu(XS), Toutunhe(TT), Changji(CJ), Midong (MD), Tianshan(TS).

Table 5
Multidrug resistance (MDR) among *Salmonella* isolates recovered from retail meats during 2013–2014.

Retail meats (no. isolates)	No. (%) isolates resisted to indicated number of antimicrobials				
	1–3	4–6	7–9	10	Total resistance (≥1)
Chicken (n = 52)	16 (30.8)	13 (25.0)	20 (38.5)	3 (5.8)	52 (100)
Lamb (n = 22)	6 (27.3)	10 (45.5)	6 (27.3)		22 (100)
Pork (n = 13)	4 (30.8)	4 (30.8)	4 (30.8)	1 (7.7)	13 (100)
Beef (n = 10)	2 (20.0)	7 (70.0)	1 (10.0)		10 (100)
Horsemeat (n = 2)	2 (100)				2 (100)
Total (n = 99)	30 (30.3)	34 (34.3)	31 (31.3)	4 (4.0)	99 (100)

than in lambs (6.8%), porks (6.8%), beefs (4.8%) and horsemeats (1.4%). Previous studies indicated that *Salmonella* in retail meats in Shaanxi (9.15%), Shandong (5.63%), and Xinjiang (9.14%) was similar to the results acquired in this study as well as a survey carried by the Chinese National Centers for Disease Control and Prevention (9.35%) (Chen, Shao, Guan, Hu, & Dong, 2010; Wang et al., 2004; Yin et al., 2014; Zhang, Zhu, & Li, 2008). Among various retail meats, chicken was regarded as one of the dominant *Salmonella* vectors all over the world. Compared to previous studies that overall 52.2% of retail chickens were observed to be positive to *Salmonella* in six provinces and two national cities of China including Guangxi (65.3%), Guangdong (64.6%), Beijing (63.9%), Shaanxi (50.7%), Henan (47.9%), Shanghai (44.4%), Fujian (42.4%), and Sichuan Province (38.9%) in 2010 (Yang et al., 2011), and those were 31% in pork, 17% in beef, and 20% in lamb samples in supermarkets and free markets in Shaanxi Province during 2007–2008 (Yang et al., 2010), while 28.3% in retail chicken and 10% in pork meat in Sichuan during 2010–2011 (Li et al., 2013), we thought the prevalence of *Salmonella* in retail meat in Xinjiang province was much lower, and the reason to which may associate with its special geographical position.

In this study, 18 serotypes were identified among 99 *Salmonella* isolates. *Salmonella* Hadar was commonly found in the previous investigations (Mąka et al., 2014; Thai and Yamaguchi, 2012; Thong & Modarressi, 2011; Wouafo, et al, 2014) and was detected to be the predominant serovar, particularly in lamb meats, the results of our study were agreement with those of the previous ones. Other than *Salmonella* Hadar, *S.* Enteritidis was verified as the most common serotype that identified in epidemiological investigations associated with consumption of contaminated livestock and poultry products (Laconcha, Baggesen, Rementeria, & Garaizar, 2000; Wang et al., 2015), while it was only identified to be the predominant serovar in chicken meats, and was the second serovar together with *Salmonella* London in this study. Meanwhile, other serovars recovered in the present study included *S.* Thompson, *S.* Agona, *S.* Infantis and *S.* Derby were often detected in other surveys (Bonardi et al., 2013; Mąka et al., 2014; Wouafo et al., 2010). The prevalence of meat-derived *Salmonella* may vary from the differences in country, types of meat sample, sampling seasons and isolation methods.

Table 6
Multidrug resistance (MDR) observed among commonly detected *Salmonella* serovars during 2013–2014.

Serotype (no. isolates)	No. isolates (%) resisted to indicated number of antimicrobials			
	1–3	4–6	7–9	10
Hadar (n = 21)		14 (50.0)	7 (26.9)	
Enteritidis (n = 17)	2 (7.4)	1 (3.6)	12 (46.1)	2 (50.0)
London (n = 17)	8 (29.6)	7 (25.0)		2 (50.0)
Havana (n = 11)	9 (33.3)	1 (3.6)	1 (3.8)	
Paratyphi B (n = 9)	5 (18.5)	2 (7.1)	2 (7.7)	
Derby (n = 5)		1 (3.6)	4 (15.4)	
Mbandaka (n = 5)	3 (11.1)	2 (7.1)		
Total (n = 85)	27 (31.8)	28 (32.9)	26 (30.6)	4 (4.0)

Antimicrobial resistance in foodborne pathogens has been a global problem. Surveillance data demonstrated an obvious increase in overall antimicrobial resistance among foodborne *Salmonella* isolates from 20% to 30% in the 1990s to 70% at the beginning of the century (Su, Chiu, Chu, & Jonathan, 2004). Our results indicated that none of the isolates were susceptible to the antimicrobial agents tested in addition to ciprofloxacin, however, all the isolates were resistant to at least one antimicrobial agent, 35.3% of the *Salmonella* isolates were resistant to more than 7 antibiotics, but none was resistant to more than 10 antibiotics, and only 4.0% of the isolates were resistant to 10 antibiotics, which indicated that the antibiotic resistance situation in retail meats in Xinjiang province was better than that in other districts (Shao, 2011; Wang et al., 2007; Yang et al., 2010).

Antimicrobial resistant *Salmonella* isolates were also detected in previous investigations and were found commonly resistant to the traditional antibiotics that early applied in disease prevention and health treatment, such as amoxicillin, ampicillin, streptomycin, penicillin, tetracycline, chloramphenicol (Shao, 2011; Wang et al., 2007; Yang et al., 2010). Our study was consistent with previous ones that *Salmonella* isolates could resist the most commonly used antibiotics. The reasons may be due to the selection pressure caused by the usage of traditional antimicrobials in food animal production for therapy, prophylaxis and growth promotion for a long time.

Cephalosporins and fluoroquinolones were effectively used to treat invasive infections, particularly in *Salmonella* infections (White et al., 2001). Our study indicated that just a few *Salmonella* isolates were resistant to cefoxitin (3.0%) and ceftriaxone (5.1%), and no isolate was resistant to ciprofloxacin. A previous study on meat-derived *Salmonella* in 10 provinces of China during 1999–2000 indicated that resistance to cephalosporin was similar to that in other provinces (Chen et al., 2004; Yang, 2010). Nalidixic acid was the first generation of quinolones applied in the treatment of *Salmonella* disease after chloramphenicol. In present study, resistance of *Salmonella* to nalidixic acid (58.6%) was very common, particularly of isolates recovered from lamb (72.7%), beef (60.0%) and chicken (59.6%), which indicated an increased nalidixic acid-resistant *Salmonel*la and this result was consistent with other reports from China (Chen et al., 2010; Pan et al., 2009; Zhang et al., 2009; Zhu et al., 2014). Ciprofloxacin-resistant *Salmonella* usually associated with infections and was resistant to multiple drugs (Cui et al., 2008), fortunately, all isolates recovered from retail meats in Xinjiang province were susceptible to ciprofloxacin.

Resistance to fluoroquinolones was partly due to amino acid substitutions of DNA gyrase and topoisomerase IV. In this study, majorities (89 of 99) of isolates were detected harbored amino acid substitutions in their QRDR (GyrA and/or ParC), and 53.9% of these isolates were resistant to nalidixic acid, which was in agreement with the findings that point mutations in QRDR of GyrA and/or ParC

could mediate resistance to the non fluorinated quinolone (Lee et al., 2009).

Quinolone resistance was thought to be acquired only by chromosomal mutations, until plasmid-mediated quinolone resistance (PMQR) was described in 1998 (Chen, Zhang, et al., 2012; Chen, Shao, et al., 2012). A penta-peptide repeated protein that encoded by plasmid-mediated *qnr* gene accompanied with two additional PMQR determinants, *qep*A and *oqx*AB, have also been described to involved in quinolone resistance (Chen, Zhang, et al., 2012; Chen, Shao, et al., 2012; Wang et al., 2003). Meanwhile, *aac(6′)-Ib* was also described to encode aminoglycoside acetyltransferase to reduce susceptibility to ciprofloxacin (Cui et al., 2008). Although aminoglycoside acetyltransferase encoded by *aac(6′)-Ib* itself was not enough to cause complete ciprofloxacin resistance, when combined with gyrase mutation, over expressed efflux pump, or *qnr*, the expression of *aac(6′)-Ib* could result in strong resistance to ciprofloxacin (Robicsek, Jacoby, & Hooper, 2006). Three major groups of *qnr* determinants (*qnr*A, *qnr*B and *qnr*S) had been identified in several bacterial species such as *Escherichia* coli and *Klebsiella* pneumoniae in Europe, the United States, Africa, Australia, and Asia (Robicsek et al., 2006; Wang et al., 2003; Yang, 2010). In our study, *qnr* was commonly detected in *Salmonella* isolates recovered in different districts and meats in Xinjiang province, which were highly consistent with previous studies (Robicsek et al., 2006; Wang et al., 2003). According to the previous results that *qnr* only confered low-level quinolone resistance and could facilitate the development of QRDR mutations, interactions between QRDR mutations and *qnr* can result in higher-level fluoroquinolone resistance (Jacoby, Chow, & Waites, 2003; Wu et al., 2010). Our results partly agreed with and strengthened previous studies. However, no *qnr* genes, amino acid substitutions of GyrA and ParC were detected in some isolates that exhibited nalidixic acid resistance, the reason of which should be further studied.

PFGE was considered a gold-standard method for *Salmonella* molecular subtyping, and was commonly used for relatedness determination to the isolates in outbreaks and for epidemiology understanding of the sporadic cases acquired from various sources (Pang et al., 2007; Yang et al., 2010). In this study, several typical PFGE clusters were found among 99 isolates, and some isolates with identical PFGE patterns were grouped in a typical PFGE cluster, which suggested that *Salmonella* isolates in each certain serovar were genetically diverse.

In a word, results of this study demonstrated that the prevalence of *Salmonella* in retail meats in Uighur of Xinjiang province were not common, however, the isolates exhibited multidrug resistance, phenotypical and genotypical diverse.

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