



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



Mingyuan Yin<sup>a,2</sup>, Baowei Yang<sup>b,1,2</sup>, Yun Wu<sup>a,c,\*</sup>, Lu Wang<sup>c</sup>, Haotian Wu<sup>a</sup>, Tao Zhang<sup>c</sup>, Gulnazi Tuohetaribayi<sup>a</sup>

<sup>a</sup> College of Food Science and Pharmaceutical Science, Xinjiang Agricultural University, Urumqi, Xinjiang, 830052, China

<sup>b</sup> College of Food Science and Engineering, Northwest A&F University, Yangling, 712100, China

<sup>c</sup> Xinjiang Agricultural University Institute of Science and Technology, Urumqi, Xinjiang, 830052, 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 *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib*, *qepA*, and *oqxAB*. 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%). *qnrA* (11.1%), *qnrB* (34.3%), *qnrS* (8.1%), *aac(6′)-Ib* (7.1%), *qepA* (7.1%), *oqxA* (10.1%) and *oqxB* (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.

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### 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

salmonellosis 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

\* Corresponding author. College of Food Science and Pharmaceutical Science, Xinjiang Agricultural University, 311# Road, Urumqi, Xinjiang, 830052, China.

E-mail address: [wuyunster@sina.com](mailto:wuyunster@sina.com) (Y. Wu).

<sup>1</sup> Co-first author.

<sup>2</sup> The first two authors contributed equally to this paper.

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 (*qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib*, *qepA*, *oqxAB*) 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  $-80^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  overnight, the genomic DNA was prepared by embedding the isolates in agarose plugs, after cells were lysed, the embedded DNA was digested with 50 U of XbaI (TaKaRa, Dalian, China) for 1.5–2 h in a water bath at  $37^{\circ}\text{C}$ . The DNA fragments were subsequently separated by electrophoresis in  $0.5 \times \text{TBE}$  buffer at  $14^{\circ}\text{C}$  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  $\mu\text{L}$  PCR mixture that contained 0.5  $\mu\text{M}$  of each primer, 250  $\mu\text{M}$  of each dNTP, 2.5  $\mu\text{L}$  of  $10 \times$  PCR buffer, 0.5 U of Taq DNA polymerase (TaKaRa), 1.5 mM  $\text{MgCl}_2$  and 5  $\mu\text{L}$  of sample template DNA, with predenaturation at  $94^{\circ}\text{C}$  for 10 min; 35 cycles of denaturation at  $94^{\circ}\text{C}$  for 30 s, at annealing temperatures for 30 s and a final extension at  $72^{\circ}\text{C}$  for 7 min. Primers were synthesized by TaKaRa Biotechnology Co., Ltd. PCR products were stained with ethidium bromide and visualized under UV light after gel electrophoresis in 1% agarose. For *gyrA* and *parC* 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.

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