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Molecular characterization of *Salmonella enterica* serovar Enteritidis on retail raw poultry in six provinces and two National cities in China

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ABSTRACT

One hundred and twenty six *Salmonella* Enteritidis isolates recovered from 1152 retail raw poultries were characterized by antimicrobial susceptibility test, pulsed-field gel electrophoresis (PFGE), presence of quinolone resistance (Qnr) associated genes, Class I integron, extended spectrum beta-lactmases (ESBLs) encoding genes, and mutations in quinolone resistance-determining region (QRDR) of *Gyr*A and *Par*C. Resistance was most frequently found to nalidixic acid (88.1%), followed by to tetracycline (65.9%), sulfisoxazole (65.1%), and ampicillin (61.9%), and a less extent to cefoxitin (8.7%), gatifloxacin (8.7%), levofloxacin (7.9%), ceftriaxone (7.1%), and ceftiofur (6.3%). One hundred and twenty three (98.4%) isolates were resistant to at least one antibiotic, and 93 (74.4%) to at least four antibiotics. *aac(6′)-Ib-cr*, *qnr*B, *qnr*A and *qnr*S genes were detected in 15 (11.9%), 11 (8.7%), 6 (4.8%) and 1 (0.8%) isolates, respectively. Amino acid substitutions of Ser83Tyr, Asp87Asn, Asp87Tyr, Asp87Gly and Ser83Phe/Asp87Asn were detected in QRDR of *Gyr*A, Arg80Ser was the unique mutation in *Par*C. Eight isolates were detected with amino acid substitution both in *Gyr*A and *Par*C. Three isolates carried Class I integron that harboring *dfr*A17-*aad*A5, *dhf*R1-*aad*A1, and *dfr*A1, respectively. Five isolates were detected carrying bla_{TEM}–bla_{ACC} ($n = 1$), bla_{TEM} ($n = 1$), bla_{TEM}–bla_{OxA} ($n = 3$), respectively. Genetic diversities ($D = 0.9255$) were found among isolates based on PFGE analysis.

1. Introduction

Salmonella is one of the leading causes of foodborne illness worldwide (Rivoal et al., 2009; Tauxe, 2002; White et al., 2001; Yang et al., 2010). Nearly 1.4 million salmonellosis occurred each year in the United States, and 95% of which were foodborne ones (http://wwwnc.cdc.gov/eid/article/17/1/p1-1101_article.htm). *Salmonella enterica* serovar Enteritidis, defined as one of the predominant *Salmonella* serovars, has comprehensive animal reservoirs and potential to disseminate among poultry and poultry products (Aktas et al., 2007; Gatto et al., 2006). Recent studies indicated that *S.* Enteritidis was the most frequently serovar that was responsible for illness with incidences from 60% to 86% (Aktas et al., 2007). Data from Asia, Europe and Latin America also showed *S.* Enteritidis was the most prevalent serovar among human salmonellosis and clinical settings (Galanis et al., 2006).

Massive usage of antimicrobial agents in livestock production and human disease increased and favored the survival of multidrug resistant (MDR) pathogens (Ahmed et al., 2009a; Araque, 2009; Hur et al., 2011; Zhang et al., 2010; Zhao et al., 2003). Fluoroquinolones are drugs widely recommended for life-threatening salmonellosis treatment, and resistance to which commonly associated with amino acid substitutions in gyrase subunit A (*Gyr*A) and B (*Gyr*B), and topoisomerase subunit C (*Par*C) and E (*Par*E), and quinolone resistant proteins (Qnr) encoded by *qnr*A, *qnr*B, and *qnr*S (Chen et al., 2004; Gebreyes and Thakur, 2005; Hur et al., 2011). Furthermore, class I integron and other mobile DNA elements are also well documented for their ability to transfer antimicrobial resistance among bacteria (Yang et al., 2010). In this study, 126 *S.* Enteritidis isolates were characterized to better understand *Salmonella* in retail raw poultry in China.

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2. Materials and methods

2.1. Salmonella Enteritidis isolates

One hundred and twenty six *Salmonella* Enteritidis isolates were recovered from 1152 retail raw chickens collected from 192 supermarkets and 96 wet markets in Shaanxi, Henan, Sichuan, Fujian, Guangxi, Guangdong Province, and Beijing and Shanghai City, China, from March to December, in 2010. Detailed information for sample collection, *Salmonella* isolation and identification were as previously described (Yang et al., 2011). *Salmonella* isolates were serotyped in Henan Center for Disease Control and Prevention, Zhengzhou, Henan, China. O and H hyperimmune seras (S&A Reagent Lab, Bangkok, Thailand; Statens Serum Institut, Artilerivej, Denmark) and slide agglutination method were employed according to the manufacturer's instructions. All isolates were stored at −80 °C in Luria–Bertani broth (LB; Beijing Land Bridge Technology Co., Ltd, Beijing, China)/glycerol (50%/50%, V/V) until use.

2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined by using agar dilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI; Clinical and Laboratory Standards Institute, 2010). Breakpoints for antimicrobials were interpreted by CLSI standards (Clinical and Laboratory Standards Institute, 2010) except streptomycin, which was determined according to the breakpoints used for susceptibility testing of *Salmonella* and *Escherichia coli* of the National Antimicrobial Resistance Monitoring System (NARMS) of the United States (http://www.fda.gov/AnimalVeterinary/SafetyHealth/AntimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm334828.htm). Antimicrobial agents including amikacin (AK), gentamicin (GE), kanamycin (KA), streptomycin (ST), ampicillin (AP), amoxicillin/clavulanic acid (AC), ceftiofur (TI), ceftriaxone (CR), cefoperazone (CEFO), cefoxitin (CEFOX), sulfisoxazole (FI), trimethoprim-sulfamethoxazole (SX), chloramphenicol (CH), ciprofloxacin (CI), nalidixic acid (NA), levofloxacin (LE), gatifloxacin (GA), and tetracycline (TE). *E. coli* ATCC 25922 and ATCC35218, *Enterococcus faecalis* ATCC 29212 were used as quality control organisms in MICs determinations.

2.3. Detection of amino acid substitution of GyrA and ParC, and presence of aac(6′)-Ib-cr, qnrA, qnrB and qnrS, class I integron and extended-spectrum beta-lactamases encoding genes

PCR was used to amplify QRDR genes of *gyr*A and *par*C, quinolone resistance associated genes of *qnr*A, *qnr*B, *qnr*S, and *aac(6′)-Ib-cr*, class I integron, and ESBLs encoding genes. Primers used for amplification and sequencing were listed in Table 1. PCRs were carried out in a 25 μl PCR mixture contained 0.5 μM of each primer, 250 μM of each dNTP, 1 × PCR buffer, 1.5 mM $MgCl_2$, 0.5 U of ExTaq DNA polymerase (TaKaRa, Dalian, China) and 5 μl of sample DNA with incubation at 94 °C for 10 min, followed by 35 cycles of 94 °C for 30 s, at annealing temperatures (Table 1) for 30 s and 72 °C for 30 s, and a final extension of 72 °C for 7 min. PCR products were stained with ethdium bromide and visualized under UV light after gel electrophoresis in 1% agarose.

2.4. Sequencing analysis

PCR products were purified with a kit (TaKaRa Agarose Gel DNA Purification Kit Ver. 2.0, TaKaRa) and sequenced at Beijing AuGCT

Table 1
Primers used for *aac(6′)-Ib-cr*, *qnr*A, *qnr*B, *qnr*S, and ESBLs encoding genes amplification, and for class I integron, *gyr*A and *parC* amplification and sequencing.

Gene	Primer	Sequence (5′–3′)	Products size (bp)	Annealing temperature (°C)	Reference
aac(6′)-Ib-cr	*aac(6′)-Ib*-F	TTGCGATGCTCTATGAGTGGCTA	482	55	Cattoir et al., 2007
	aac(6′)-Ib-R	CTCGAATGCCTGGCGTGTTT			
*qnr*A	*qnr*A-F	AGAGGATTTCTCACGCCAGG	580	60	Cattoir et al., 2007
	*qnr*A-R	TGCCAGGCACAGATCTTGAC			
*qnr*B	*qnr*B-F	GGMATHGAAATTCGCCACTG	264	56	Cattoir et al., 2007
	*qnr*B-R	TTTGCYGYYCGCCAGTCGAA			
*qnr*S	*qnr*S-F	GCAAGTTCATTGAACAGGGT	428	57	Cattoir et al., 2007
	*qnr*S-R	TCTAAACCGTCGAGTTCGGCG			
*gyr*A	*gyr*A-F	ACGTACTAGGCAATGACTGG	190	56	Eaves et al., 2002
	*gyr*A-R	AGAAGTCGCCGTCGATAGAA			
*par*C	*par*C-F	CTATGCGATGTCAGAGCTGG	270	54	Eaves et al., 2002
	*par*C-R	TAACAGCAGCTCGGCGTATT			
class I integron	*int-F*	GGCATCCAAGCAGCAAGC		56	Yang et al., 2009
	int-R	AAGCAGACTTGACCTGAT			
bla_{TEM}	bla_{TEM}-F	ATGAGTATTCAACATTTCCG	964	50	Archambault et al., 2006
	bla_{TEM}-R	ACCAATGCTTAATCAGTGAG			
bla_{CTx}	bla_{CTx}-F	GAGTTTCCCCATTCCGTTTC	909	50	Kiratisin et al., 2008
	bla_{CTx}-R	CAGAATAAGGAATCCCATGGTT			
bla_{ACC}	bla_{ACC}-F	AGCCTCAGCAGCCGGTTAC	818	55	Archambault et al., 2006
	bla_{ACC}-R	GAAGCCGTTAGTTGATCCGG			
bla_{SHV}	bla_{SHV}-F	TTCGCCTGTGTATTATCTCCCTG	854	50	Archambault et al., 2006
	bla_{SHV}-R	TTAGCGTTGCCAGTGCTCG			
bla_{OxA}	bla_{OxA}-F	ACCAGATTCAACTTTCAA	590	55	Usha et al., 2008
	bla_{OxA}-R	TCTTGGCTTTTATGCTTG			
bla_{PSE}	bla_{PSE}-F	AATGGCAATCAGCGCTTCCC	598	55	Shahada et al., 2006
	bla_{PSE}-R	GGGGCTTGATGCTCACTACA			
bla_{VEB}	bla_{VEB}-F	GATAGGAGTACAGACATATG	914	60	Kiratisin et al., 2008
	bla_{VEB}-R	TTTATTCAAATAGTAATTCCACG			
bla_{PER}	bla_{PER}-F	ATGAATGTCATCACAAAATG	927	56	Kiratisin et al., 2008
	bla_{PER}-R	TCAATCCGGACTCACT			
bla_{GES}	bla_{GES}-F	ATGCGCTTCATTCACGCAC	864	57	Kiratisin et al., 2008
	bla_{GES}-R	CTATTTGTCCGTGCTCAGG			