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Phenotypic characteristics and genotypic correlation between *Salmonella* isolates from a slaughterhouse and retail markets in Yangzhou, China

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ABSTRACT

An epidemiological investigation of *Salmonella* spp. in pig and pork samples from one slaughterhouse and its downstream retail markets in Yangzhou, Jiangsu Province, China, was conducted from October 2013 to March 2014. A total of 71.8% (155/216) and 70.9% (78/110), respectively, of the slaughterhouse and retail market samples were recovered positive for *Salmonella*. All *Salmonella* isolates were characterized using serotyping, antimicrobial resistance detection, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE). Seven serotypes were shared by isolates from the two sources, with the most common serotypes being *Salmonella* Derby, Typhimurium, and Uganda. Antimicrobial sensitivity testing revealed that the highest antimicrobial resistance rate was against tetracycline (49.7% and 37.2% in isolates from the slaughterhouse and retail market, respectively) with many multidrug-resistant (MDR) isolates in both sources. MLST analysis showed that eight sequence type (ST) patterns were shared, and ST40 occupied an absolute superiority among isolates from both sources. PFGE permitted the resolution of *Xba*I macrorestriction fragments of the selected 31 *Salmonella* Derby and 19 *Salmonella* Typhimurium into 30 and 10 distinct pulsotypes, displaying the high similarity between the isolates from the two sources. Our findings indicated that *Salmonella* isolates from a slaughterhouse and its downstream retail markets were phenotypically and genetically homologous. Additionally, *Salmonella* may propagate along the slaughter line and pork production chain from the slaughterhouse to retail markets.

1. Introduction

Salmonella, one of the most important pathogens studied every year in the world, can cause severe foodborne disease in humans and animals, impacting health and productivity (Majowicz et al., 2010). In China, approximately 70 to 80% of foodborne pathogenic outbreaks are caused by *Salmonella* (Wang et al., 2007). The majority of human *Salmonella* infections are associated with the ingestion of contaminated foods, such as pork, poultry, beef, egg, milk, cheese, and vegetables (Zhao et al., 2008).

Pigs have been recognized as one important reservoir for *Salmonella* (Li et al., 2013). *Salmonella* can be transferred to humans via pork along the food chain (Hauser et al., 2011). Therefore, as one of the countries with the largest pork production and consumption around the world, great attention should be paid to the prevalence and control of *Salmonella* with respect to pig slaughtering, pork manufacturing, and retail in China.

In recent years, many studies reported the prevalence and characterization of *Salmonella* along the pork production chain; the contamination of *Salmonella* was 10 to 40% in slaughterhouses, and the major serotypes were *Salmonella* Typhimurium and Derby (Algino et al., 2009; Arguello et al., 2012; Bonardi et al., 2013; Duggan et al., 2010). In retail markets, the prevalence of *Salmonella* isolates from pork samples was 1 to 40% (Li et al., 2014; Mihaiu et al., 2014; Miranda et al., 2009; Thai et al., 2012), and the serovars were diverse.

Though much attention has been focused on *Salmonella* in pig slaughterhouses and retail markets, intensive and simultaneous research regarding the prevalence, serotypes, antimicrobial resistance, multilocus sequence types (STs), and pulsed-field gel electrophoresis (PFGE) profiles of *Salmonella* in both sources is limited, especially in China. Therefore, the objective of this study was to analyze the distribution, antimicrobial susceptibility profiles, and molecular characteristics of *Salmonella* spp. collected from one pig slaughterhouse and its downstream pork retail markets in Yangzhou, Jiangsu Province, China, to determine the clonal relationships between isolates and the possible route of exposure.

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2. Material and methods

2.1. Sample collection

From October 2013 to March 2014, 326 samples were collected from one slaughterhouse, which processed about 600 pigs per workday, and its downstream retail markets in Yangzhou, Jiangsu Province, China. The sampling was separated into two parts.

i) From October 2013 to March 2014, 216 samples were collected at ten different stages including lairage, submitting, scalding water, cooling water, evisceration, visceral processing countertop, waste water, carcass dressing, mesenteric lymph nodes (MLNs), and floor along the slaughter line. Samples were randomly taken during three visits to the slaughterhouse every 2 months. All samples were collected using sterile sponges that were pre-moistened with buffered peptone water (BPW; Difco, BD, Sparks, MD, USA) as described previously (Bonardi et al., 2013). To prevent cross-contamination, gloves were worn during sampling and changed after each sample. Holding pens were sampled using the overshoe method. Carcasses were swabbed in each high-risk contamination area of 100 cm^2 (heel, belly, hip, notum, and jowl). Waste water, scalding and cooling water were sampled using sterile tagged collection tubes once per hour, and the temperature was measured. Ground specimens (visceral processing countertop and floor of each stage) were sampled by swabbing an area of 50 cm × 50 cm within each location. MLNs were collected and processed as previously reported (Anonymous, 2006). Samples were immediately stored in a cooled container after collection and sent to the laboratory for analysis.

ii) During the days after the slaughterhouse sampling, the retail samples were obtained. One hundred ten pork samples were collected from two downstream retail markets. Sampling was performed as described previously (Li et al., 2014). However, it was not possible to obtain both pork types (chop and piece) because of their availability.

2.2. Isolation and identification of Salmonella

For swab samples from the slaughterhouse, the pre-enrichment step was performed by suspending each sample in 50 mL BPW, and incubating samples at 37 °C for 16 to 18 h. Then, 0.1 mL of the BPW suspensions was subcultured in 10 mL subpackaged Rappaport–Vassiliadis (RV) enrichment broth (Difco, BD, Sparks, MD, USA) at 42 °C for 24 h. One loopful of each RV broth culture was then streaked onto xylose lysine tergitol 4 (Difco, BD, Sparks, MD, USA) agar plates, which were incubated at 37 °C for 24 to 48 h. One presumptive *Salmonella* colony per plate was picked and biochemically confirmed using an API-20E test kit (bioMérieux, Marcy l'Etoile, France). All strains were serotyped according to the Kauffmann–White scheme by slide agglutination with O and H antigen-specific sera (Tianrun Bio-Pharmaceutical, Ningbo, China). For pork samples from retail markets, each sample (25 ± 0.5 g) was aseptically weighed and transferred into 225 mL BPW and incubated at 37 °C for 18 h. The pre-enrichment and following isolation and identification were performed as described above.

2.3. Antimicrobial susceptibility testing

The Kirby–Bauer disk diffusion method was used to determine the isolates' antimicrobial susceptibility (Li et al., 2014). A total of 14 antimicrobial agents were applied: ampicillin (AMP, 10 μg), cefazolin (CFZ, 30 μg), ceftriaxone (CRO, 30 μg), cefotaxime (CTX, 30 μg), gentamicin (GEN, 10 μg), kanamycin (KAN, 20 μg), streptomycin (STR, 10 μg), nalidixic acid (NAL, 30 μg), ciprofloxacin (CIP, 5 μg), ofloxacin (OFX, 5 μg), norfloxacin (NOR, 5 μg), chloramphenicol (CHL, 30 μg), tetracycline (TET, 30 μg), and trimethoprim-sulfamethoxazole (SXT, 1.25–23.75 μg). Results were interpreted according to the established Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2013).

2.4. Multilocus sequence typing (MLST)

Confirmed isolates were grown aerobically in LB broth with shaking overnight at 37 °C. Genomic DNA was extracted with a TIANamp Bacteria DNA Kit (Tiangen, Beijing, China) in strict accordance with the manufacturer's protocol. MLST was carried out as described online (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/documents/primersEnterica_html). All polymerase chain reaction products were purified and sequenced by Nanjing GenScript Biotech Co. (Nanjing, China), and the alleles and STs were assigned according to the MLST scheme at http://mlst.warwick.ac.uk/mlst/dbs/Senterica. A minimum spanning tree was generated using BioNumerics software, version 6.5 (Applied Maths, Kortrijk, Belgium) to analyze the distribution of STs in the slaughterhouse and retail markets.

2.5. PFGE

PFGE was performed according to the protocol by the Centers for Disease Control and Prevention (CDC) (Ribot et al., 2006) with some modifications. In brief, *Salmonella* isolates were streaked onto LB plates and incubated overnight at 37 °C. The pathogen concentration was modulated with bacterial suspensions until a McFarland turbidity of 4.0–4.5 was attained. DNA was digested with 50 U *Xba*I (Takara, Dalian, China) at 37 °C for 3 h. The digested DNA was separated by electrophoresis in 0.5 × TBE buffer at 14 °C for 20 h using a CHEF Mapper electrophoresis system (Bio-Rad, Hercules, CA, USA). The pulse time was ramped from 2.16 to 63.8 s. In addition, a control strain of *Salmonella* Braenderup (H9812), which served as a molecular weight standard, was processed with each batch of isolates. The gels were stained with ethidium bromide, and DNA patterns were visualized on a UV transilluminator (Bio-Rad, Hercules, CA, USA). Dendrograms were created by BioNumerics software version 6.5 (Applied Maths, Kortrijk, Belgium) using the unweighted pair group method with arithmetic means. The band-matching settings with optimization of 0.5% and position tolerance of 1.5% were applied.

2.6. Data analysis

Statistical comparison of prevalence and individual resistance to the 14 antimicrobial agents of *Salmonella* isolates in the slaughterhouse and retail markets were analyzed with the X^2 test, which was performed using the Statistical Package for the Social Sciences (version 15.0, SPSS, Chicago, IL, USA) with $P < 0.05$ considered as statistically significant.

3. Results

3.1. Salmonella prevalence and serotypes

A total of 155 (71.8%) and 78 (70.9%) isolates, respectively, were retrieved and scored as positive for *Salmonella* among the samples from the slaughterhouse and retail markets. Table 1 shows the detailed prevalence of *Salmonella* per visit in the slaughterhouse and retail markets,

Table 1
Prevalence of *Salmonella* isolated from slaughterhouse and retail markets.

Sample source	No. of times	No. of samples	No. of samples (%)positive for *Salmonella*	Total (%)
Slaughterhouse	Visit 1	72	46 (63.9)	155 (71.8)
	Visit 2	72	52 (72.2)	
	Visit 3	72	57 (79.2)	
Retail markets	Visit 1	33	15 (45.5)	78 (70.9)
	Visit 2	35	26 (74.3)	
	Visit 3	42	37 (88.1)	
Total		326	233 (71.5)	233 (71.5)