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Salmonella isolated from the slaughterhouses and correlation with pork contamination in free market

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ABSTRACT

This study surveyed the distribution, antimicrobial susceptibility profiles and serotype of *Salmonella* isolated from three slaughterhouses, and performed molecular typing on these isolates, to understand the relationship between strains of *Salmonella* obtained from the pork production chain in Yangzhou, China. Samples from slaughtered pigs and the slaughtering environment were collected from three slaughterhouses in Yangzhou, Jiangsu province, from October 2012 to July 2013. The positive identification rates of *Salmonella* in slaughtered pigs and the environmental samples were 46.6% and 48.8%, respectively. The prevalence of *Salmonella* in slaughterhouses were affected by seasonal factors and reached the peak in summer. Among the *Salmonella* serovars identified, *S.* Derby was most prevalent in slaughterhouses, but other serovars like *S.* Typhimurium, *S.* Meleagridis and *S.* Anatum were also widespread. Antimicrobial susceptibility testing revealed that 32 and 131 different MDR patterns were found among the strains from the environment and slaughtered pig samples, respectively. Fifty-six isolates of *S.* Derby and 16 strains of *S.* Typhimurium were characterized by the technique of pulsed-field gel electrophoresis (PFGE) using the restriction enzyme *Xba* I. 35 and 11 PFGE patterns were generated among the selected isolates. Four isolates of *S.* Derby isolates with the same pattern (PF26) were isolated from cooling water, evisceration and carcass, suggesting that cross contamination occurred between the environment and the slaughtered pigs. Six *S.* Typhimurium in cluster 1 with the same ST type (ST19) came from different parts of the slaughtered pig, which could have occurred because of horizontal transmissions along the slaughtering process. The same PFGE patterns of *Salmonella* were found in both samples from carcasses in the slaughterhouse and in the Yangzhou pork market, proving that *Salmonella* had spread from the slaughterhouse to the pork market. In conclusion, our study demonstrate that serious cross contamination occurred in Yangzhou slaughterhouses and can contribute *Salmonella* contamination in pork sold in the local public market.

1. Introduction

Salmonella is recognized as a major human food-borne pathogen and can cause a heavy economic burden for health care systems worldwide. Animals used as food play a role as a primary reservoir of non-typhoid *Salmonella*, and most human *Salmonella* infections are a consequence of eating foods of animal origin contaminated with *Salmonella* (EFSA, 2011). Contaminated pork and pork products are major sources of human *Salmonella* infections in many countries (Bonardi et al., 2013; De Busser et al., 2011; Delhalle et al., 2009).

Research has shown that *Salmonella* transmission may occur throughout the pork production chain (Lo Fo Wong et al., 2004), and many opportunities exist within the pig slaughterhouse for the contamination of pork carcasses with *Salmonella*. Based on an EFSA report (EFSA, 2011), the prevalence of *Salmonella*-positive swine-breeding holdings and swine-production holdings in the European Union was 28.7% and 33.3%, respectively, indicated a serious contamination in pig farms. The prevalence of shedding pigs may increase from farm to abattoir, mainly because of the stress of transportation, consequently increasing interinfection of pigs

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during lairage. *Salmonella* is spread to the carcass surface mainly from the carrier pig during the evisceration and slaughter operations can influence the bacterial contamination of pork carcasses in many ways, therefore, proper slaughter operation and good hygienic practices in the slaughterhouse can have a positive effect on the elimination of contamination in carcasses (Bonardi et al., 2003; De Busser et al., 2011). Slaughterhouses vary in their capability of dealing with *Salmonella*-positive pigs. Proper cleaning and control measures can reduce the risk of carcass contamination in slaughterhouses. In this sense, identifying the serotype and genotype of *Salmonella* isolates throughout the pig production chain were necessary to determine the cross-contamination and explore the relationship of the strains isolated from the environment and the pig carcass.

Salmonella enterica serotypes Derby and Typhimurium were the two most commonly detected serotypes in carcasses and in both clinical and nonclinical veterinary swine samples (Foley, Lynne, & Nayak, 2008). *S.* Typhimurium was also the most frequently isolated serotype from all human invasive infections reported. Similar results were reported by EFSA, where the most frequently reported serovars in human salmonellosis were in agreement with those isolated in slaughter pigs (EFSA, 2011). These findings support the notion that pigs and pork contribute to *Salmonella* infection in humans, although it is acknowledged that foods from other animal species also play a role as a source of these infections in humans (Hugas et al., 2014).

China is the largest producer of pork with the fastest growth rate in the world and this growth will continue in 2014: swine production is expected to reach 723 million head, pork production is expected to increase by two percent to 54.7 million tons, and consumption expected to rise to 55 million tons (Schneider et al., 2014). However, few reports have been published on *Salmonella* contamination throughout the slaughter process in China. Therefore, the aim of this study was to investigate the prevalence and antimicrobial resistance patterns of *Salmonella* contamination along the slaughter line through the detection of *Salmonella* in slaughtered pigs and their slaughterhouse environmental samples. Furthermore, sero- and genotyping were performed to define clonal relationships between isolates, assessing the dispersion of recovered strains and their involvement in cross contamination. Some of the strains isolated from pork in our previous study were also involved in our analysis to reveal the correlation between these two studies.

2. Materials and methods

2.1. Slaughterhouse description and sample plan

The study was carried out from October 2012 to July 2013 in three slaughterhouses (A, B and C) in Yangzhou, which were distributed in different areas of Yangzhou and represented 80% of the annual number of pigs slaughtered in Yangzhou. Table 1 describes the situations of the three slaughterhouses. Each visit was performed on a Tuesday during the nine sampling visits: V1 (slaughterhouse A, December 2012; slaughterhouse B, November 2012; slaughterhouse C, October 2012), V2 (slaughterhouse A, April 2013; slaughterhouse B, March 2013; slaughterhouse C, February 2013), V3 (slaughterhouse A, July 2013; slaughterhouse B, June 2013; slaughterhouse C, May 2013) and sampling started with the first batch of pigs slaughtered that day.

2.2. Slaughterhouse samples

A total of 684 samples were collected from the three slaughterhouses, of which 522 were slaughtered pig samples and 162 were slaughterhouse environmental samples. The description of the number and type of samples are shown in Table 2.

2.2.1. Slaughtered pig samples

From each chosen slaughtered pig, samples were collected as follows:

Mesenteric lymph nodes (MLNs): At least 5 lymph nodes in the ileocaecal regions were cut out of the intestine packet with a sterile, disposable scalpel.

Carcass swabs: Carcass swabs were selected at three high-risk cross-contamination points along the slaughter line (submitting, evisceration, and after washing carcass). Carcasses were swabbed on the external and internal surfaces at four different points: cheek, ham (right and left), rib cage and neck-upper-shoulder. Sampling was done using a sterile moistened (0.1% peptone water) cotton ball to swab a square of 15 × 15 cm within each point (approx. 1350 cm^2 in total). The five cotton balls collected from each carcass were pooled and put into a sterile stomacher bag.

2.2.2. Environmental samples

During the slaughter process, environmental samples were also collected as follows:

Scalding and cooling water: During slaughtering activities, 10 ml of water samples were taken from the scalding and cooling tank (The temperature range were kept between 5 and 10 °C) once every hour. The water samples were collected using a sterile collection tube and the temperature was measured.

Lairage: At the beginning of the sampling, pens in the lairage were already filled with pigs to be slaughtered that day. For every visit, five pens were sampled using the overshoes method which was carried out by walking around in an "8"-shaped track in the pen using one pair of disposable, liquid absorbing overshoes (Cobbaut, Houf, Douidah, Van Hende, & De Zutter, 2008).

Floor: The slaughterhouses were divided into five parts, according to the overall layout of the slaughterhouses and each part covered a 1 × 1 m^2 area. Sampling was done using four sterile moistened (0.1% peptone water) cotton balls to swab each part. The four cotton balls collected from each part were pooled and put into a sterile stomacher bag.

Visceral processing countertop and waste water: At the end of slaughtering, four areas of the countertop were sampled according to its size and each part covered a 1 × 1 m^2 area. The sample method was the same as with the slaughterhouse floor. During slaughtering activities, water samples were taken from the sewerage tank in the visceral processing room or the drain in the countertop (slaughterhouse B without the visceral processing room).

Table 1
Description of the three slaughterhouses included in the study.

	Capacity of slaughterhouse (pigs/night)	Usage times of equipment (years)	Floor type of Lairage	Cleaning of slaughterhouse	Additional information
A	500	4	Solid	Daily	Visceral processing room
B	600	10	Solid	2 to 3 days	
C	400	8	Solid	Weekly	Visceral processing room