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Prevalence and profile of *Salmonella* from samples along the production line in Chinese beef processing plants

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ABSTRACT

This work investigated the prevalence of *Salmonella*, the serotypes and antibiotic resistance of the isolated strains from four beef processing plants of China. The prevalence of *Salmonella* in hide ($n = 70$), feces ($n = 70$), pre-evisceration carcass ($n = 70$), post-evisceration carcass ($n = 70$), post-washing carcass ($n = 70$), chilled carcass ($n = 80$), and raw meat ($n = 80$) samples was 20.0%, 18.6%, 1.4%, 1.4%, 2.9%, 1.3%, and 1.3%, respectively. Among the four plants, there were significant differences in the prevalence of *Salmonella* on hides and in feces. During the processing, *Salmonella* was significantly reduced after hide removal. Seven serotypes of *Salmonella* were identified among the eighty-three isolates. *Salmonella* Agona was the dominant serotype ($p < 0.05$, 53.0%), followed by *Salmonella* Senftenberg (16.9%), *Salmonella* Meleagridis (10.8%), and *Salmonella* Derby (9.6%). None of the isolated strains were found to be resistant to sixteen commonly used antimicrobial agents. The results of this study indicate that *Salmonella* contamination is common in samples along the production line, with *S.* Agona as dominant serotype. Specific measures should be taken to prevent and/or treat *Salmonella* contamination in corresponding products in Chinese beef processing plants. Furthermore, the current research might provide baseline information of *Salmonella* prevalence profile in Chinese beef processing plant, which could be used for the future study.

1. Introduction

Salmonella is the most common food-borne pathogen in the world (Voetsch et al, 2004), and typhoid and paratyphoid fevers caused by *Salmonella* are the most common diseases in developing countries and can be fatal under poor sanitary conditions (Rhoades, Duffy, & Koutsoumanis, 2009). *Salmonella* is the leading pathogen found in China, causing approximately 70%–80% of foodborne diseases, most of which display an association with the consumption of contaminated meat products (Wang, Zheng, & Wang, 2007).

The muscle surface of the cattle carcass is sterile, and contamination with *Salmonella* occurs during in-plant and/or out-plant slaughtering processes (Rivera-Betancourt et al., 2004). The hide is the predominant reservoir of *Salmonella*, which is mainly transmitted from feces through animal–animal and/or animal-environment-animal contact. Because of mechanical tearing, *Salmonella* is easily transferred to the carcass during hide removal. Puncture of the bowel and rumen during evisceration can lead to distribution of the pathogen during processing. Environmental and internal cross-contamination may also contribute to the prevalence of foodborne pathogens (Galland, 1997). Because the slaughtering line provides a direct connection between the carcass and the pre-slaughter animal, it is easily contaminated by processes that distribute pathogens during slaughter. Moreover, without appropriate intervention methods or supervision, the slaughtering line becomes a medium, or even a reservoir, for the direct transmission of pathogens to the carcass, thereby affecting the safety of the resultant products.

Several studies in the United States, Northern Ireland, Australia, and Belgium have reported prevalence of *Salmonella* on cattle carcasses varying between 0.2% and 4.1% (Ghafir et al., 2005; Li, Sherwood, & Logue, 2004; Madden, Espie, Moran, McBride, & Scates, 2001; Vanderlinde, Shay, & Murray, 1998). China has developed the Hazard Analysis Critical Control Points plan to decrease the risk of contamination in processing plants. Adequate microbiological data are needed to assess the effectiveness of control programs. However, the historical mode of farming in China

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in which farmers show great respect for cattle prevented the development of the Chinese beef industry until 1978. Traditionally, the Chinese consume only well-done and not raw or lightly cooked beef. Although there is some published data on the prevalence of *Salmonella* in retail Chinese foods (Yan et al., 2010; Yang et al., 2013), there is no available information about the contamination profile in products along the processing line of Chinese beef plants.

Thus, this study was designed to address the following two aims relevant to beef safety in China: (i) estimation of the incidence of *Salmonella* in beef processing line, (ii) determination of the serotypes and the antimicrobial susceptibility profiles of the isolated *Salmonella* strains.

2. Materials and methods

2.1. Experimental design and sample collection

A total of 510 samples were collected from four beef processing plants (designated plants A through D) in summer (July–September, 2010). Three of these plants were located in different cities distributed geographically in the northeast, northwest, and southwest regions of a province in eastern China, and the fourth plant was located in a province in northeast China. All of the commercial plants included in this study were equipped with a production line capable of slaughtering 30–50 animals per hour. Following dressing, the carcasses were washed with cold water before chilling. Six points in the processing line were selected for sampling in this study: (i) hide – samples were collected before the hide removal but after the slaughtering of the animal; (ii) pre-evisceration carcasses – samples were taken after hide removal; (iii) post-evisceration carcasses – samples were obtained after evisceration; (iv) post-washing carcasses – samples were collected after spraying with water; (v) chilled carcasses – samples were taken from carcasses that had been stored in a chilling room for approximately 24 h; and (vi) meat – samples were collected from cut and deboned carcasses. For the first five points, samples were obtained via gauze swabbing. The sampling sties were chosen according to a previous study, but with a small change in the size of the gauze pads (Breum & Boel, 2010). Briefly, two standard 20 × 20 cm gauze pads moistened with 0.9% sodium chloride and 0.1% peptone diluents were swabbed at 13 points on the carcass, and the two swabs used for both sides of each carcass were pooled in a stomacher bag. The total area sampled on each side was approximately 2500 cm^2. For step (vi), a meat sample approximately of 25 g was randomly collected from beef cuts and placed in an ice storage box. In addition, fecal samples (approximately 10 g) were collected after de-legging. A sterile plastic bag was inverted over the sampler's hand to prevent contamination of the exposed inner surface of the bag and the bag was inserted inside the rectum to remove fecal material. All samples were transported to the laboratory in coolers containing ice packs.

2.2. Pre-enrichment of the samples

In the laboratory, sterile trypticase soy broth (TSB) (Beijing Land Bridge Technology Co., Ltd, Beijing, China) was added to the stomacher bags (BagPage® Interscience, France) containing the gauze pads. A total of 100 ml of TSB was added to the swab samples, while the feces (10 g) and meat (25 g) samples were pre-enriched in 90 or 225 ml of TSB, respectively. After being stomached for 1 min at the speed of 8 extrusions per second (BagMixer® 400 Interscience, France), all samples were processed following the method described by Barkocy-Gallagher et al. (2002). Briefly, the whole stomacher bags were incubated at 25 °C for 3 h and then at 42 °C for 6 h. Following incubation, 1 ml of the liquid was pipetted into a 1.5 ml tube and stored at 4 °C overnight for subsequent immunomagnetic separation. Prior to transfer, an additional enrichment step was performed specifically for the fecal samples, in which 10 ml of the pre-enrichment mixture was transferred to 90 ml of Tetrathionate Broth (TTB), followed by incubation at 37 °C for an additional 24 h. After this enrichment, 1 ml of the resulting liquid was pipetted into a 1.5 ml tube and stored as described previously.

2.3. Recovery and confirmation of Salmonella

Immunomagnetic separation (IMS) was carried out using immunomagnetic beads coated with an anti-*Salmonella* antibody (Dynabeads® anti-*Salmonella*, Dynal A.S., Oslo, Norway). The entire IMS procedure was conducted as per the manufacturer's instructions. After separation, a post-enrichment step was performed for all of the bead-*Salmonella* complexes: 50 μL of the IMS-bead complex recovered by enrichment was added to 10 ml of Rappaport–Vassiliadis R10 Broth (RV) (Beijing LandBridge Technology Co., Ltd, Beijing, China), followed by incubation at 37 °C for 18 h (Cudjoe & Krona, 1997). After incubation, a full loop of this selectively enriched sample was streaked onto Hektoen enteric (HE) agar (Beijing Land Bridge Technology Co., Ltd.) and CHROMagar *Salmonella* (CHROMagar Co., Ltd, Paris, France), then incubated for 24 h at 37 °C. A minimum of two colonies were isolated and streaked for further purification on MacConkey agar plates (24 h at 37 °C) and were confirmed as GB/T 4789.4 (Ministry of health of the Peoples' Republic of China, 2010) via biochemical and serological methods. The biochemically confirmed isolates were serotyped by Kauffman–White classification scheme in Fu Jian Center for Disease Control and Prevention, China. O and H antigens were characterized using slide agglutination with hyperimmune sera (S&A Company, Thailand) and the serotype was assigned following the manufacturer's instructions.

2.4. Detection of the invA gene

The *inv*A gene was detected using a polymerase chain reaction (PCR)-based method described previously (Rahn et al., 1992). The primers (*inv*A Forward: 5′-GTGAAATTATCGCCACGTTCGGGCAA-3′, *inv*A Reverse: 5′-TCATCGCACCGTCAAAGGAACC-3′) for these assays were commercially synthesized by TaKaRa Biotechnology Co. Ltd (Dalian, China). PCR was carried out in a total volume of 50 μL containing 4 μL of template DNA, 0.2 μL of the forward and reverse primers in total, 0.15 μL of Taq enzyme, 5 μL of PCR buffer, 2.5 μL of $MgCl_2$, 2 μL of dNTPs, and 36.15 μL of nuclease-free water. Positive (*Salmonella* Typhimurium) and negative control (sterile water) were conducted in the detection procedure.

PCR was performed in a DNA thermal cycler. After an initial denaturation step of 7 min at 72 °C, 35 cycles of amplification were performed. Each cycle consisted of the following steps: 1 min at 94 °C for denaturation, 30 s at 53 °C for primer annealing, and 1 min at 72 °C for extension, with a final extension at 72 °C for 7 min. The samples were analyzed by mixing 5 μL of the reaction mixture with gel loading buffer, followed by resolution via electrophoresis on 2% agarose gels together with the DL1000® DNA ladder (TaKaRa Biotechnology Co., Ltd.). Image documentation was carried out with a gel imaging system and viewed on a computer. The PCR products were sequenced by TaKaRa Biotechnology Co. Ltd (Dalian, China).

2.4.1. Antimicrobial susceptibility testing

A disk diffusion test was performed according to approved protocols (Clinical and Laboratory Standards Institute, 2006a) to determine the susceptibility of the *Salmonella* isolates to nalidixic acid, ciprofloxacin, tetracycline, streptomycin, gentamicin, chloramphenicol, amoxicillin/clavulanic acid, ampicillin, cephalotin,