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Antimicrobial resistance and resistance genes in *Salmonella* strains isolated from broiler chickens along the slaughtering process in China

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ABSTRACT

A total of 189 *Salmonella* isolates were recovered from 627 samples which were collected from cecal contents of broilers, chicken carcasses, chicken meat after cutting step and frozen broiler chicken products along the slaughtering process at a slaughterhouse in Sichuan province of China. The *Salmonella* isolates were subjected to antimicrobial susceptibility testing to 10 categories of antimicrobial agents using the Kirby–Bauer disk diffusion method. Those antibiotics-resistant isolates were further investigated for the occurrence of resistance genes, the presence of class 1 integron as well as the associated gene cassettes, and the mutations within the *gyrA* and *parC* genes. Consequently, the prevalence of *Salmonella* was 30.14% (47.96% for cecal content, 18.78% for chicken carcasses, 31.33% for cutting meat and 14.00% for frozen meat, respectively). The predominant serotypes were *S.* Typhimurium (15.34%) and *S.* Enteritidis (69.84%). High resistance rates to the following drugs were observed: nalidixic acid (99.5%), ampicillin (87.8%), tetracycline (51.9%), ciprofloxacin (48.7%), trimethoprim/sulfamethoxazole (48.1%), and spectinomycin (34.4%). Antimicrobial resistance profiling showed that 60.8% of isolates were multidrug resistant (MDR), and MDR strains increased from 44.7% to 78.6% along the slaughtering line. 94.6% (n = 157) of beta-lactam-resistant isolates harbored at least one resistance gene of bla_{TEM} or $bla_{CTX\text{-}M}$. The relatively low prevalence of aminoglycoside resistance genes (*aac(3)-II*, *aac(3)-IV*, and *ant(2″)-I*) was found in 49 (66.2%) of antibiotic-resistant isolates. The tetracycline resistance genes (*tet*(A), *tet*(B), *tet*(C), and *tet*(G) and sulfonamide resistance genes (*sul1*, *sul2*, and *sul3*) were identified in 84 (85.7%) and 89 (97.8%) antibiotic-resistant isolates respectively. *floR* was identified in 44 (97.8%) florfenicol-resistant isolates. Class 1 integron was detected in 37.4% (n = 43) of the MDR isolates. Two different gene cassettes, $bla_{OXA\text{-}30}$-*aadA1* (19 isolates) and $bla_{OXA\text{-}30}$-*aadA1/drfA1-orfC* (2 isolates), were identified in class 1 integron-positive isolates. 97.9% (184/188) of quinolone-resistant isolates had at least one mutation within *gyrA* or *parC*. Overall, antimicrobial resistance showed an increasing trend along the slaughtering process. The results showed that broiler chicken products in the slaughterhouse were contaminated with MDR *Salmonella*, which might originate from food producing animals to some extent, and cross-contamination during slaughter, and facilitate the dissemination of the resistance genes to consumers along the production chain, which suggests importance of controlling *Salmonella* during slaughter for public health, underlying strict hygiene method and HACCP management to reduce cross-contamination.

1. Introduction

Salmonella is one of the most common foodborne pathogens, causing outbreaks of foodborne disease worldwide (Newell et al., 2010). Foodborne *Salmonella* infection typically causes a range of clinical syndromes in humans including typhoid fever, diarrhoeal disease, and may have a dramatically more severe systemic disease in the immunocompromised people (Gordon, 2008). Animal food products especially eggs and poultry meats, have been the most common vehicles of the *Salmonella* infections (Greig and Ravel, 2009). With the

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increasing consumption of poultry meat globally, bacterial pathogen *Salmonella*, as an important factor for affecting the safety of poultry and raw meat, will continue to receive growing attention (Henchion et al., 2014; Sofos, 2008).

Recently, there has been an increasing trend of antimicrobial resistance on a worldwide scale, especially for multidrug-resistant (MDR) *Salmonella* strains from food animals (Hur et al., 2012). In China, the usage of antimicrobial agents is greater than in most other countries; in a 2007 survey, nearly half of the 210, 000 tons of antibiotics produced in China, were used in livestock as therapeutic drugs and feed additives (Hvistendahl, 2012). Abuse of antimicrobial agents in food animal production is regarded as one of the important reasons for emergence of antimicrobial resistance in *Salmonella*; this resistance can be transmitted to the human population through the animal foodstuffs, which poses a serious threat to public health. Multidrug-resistant phenotypes in *Salmonella* of animal origin have been increasingly observed in China (Lai et al., 2014; Yang et al., 2013).

Various factors from farm to fork along the food chain heavily influence the microbiological safety of food (Newell et al., 2010). Successful prevention of foodborne salmonellosis originating from animal production comprise three lines of defence against *Salmonella*: a) controlling *Salmonella* in the food-producing animal at farms (pre-harvest control); b) improving hygiene during the slaughter and further processing of the meat (harvest control); c) the final food preparation by underlying good hygiene practices (post-harvest control) (Forshell and Wierup, 2006). Therefore, slaughter is the most appropriate stage of food chain for the evaluation on the carriage of zoonotic agents by farm animals, the level of *Salmonella* infection in animal carcasses and subsequently meat products in the finishing poultry population, and the proportions of self- and cross-contamination during slaughter and processing (Bonardi et al., 2013).

Most studies about the prevalence and antimicrobial resistance of *Salmonella* in either animals (Ahmed and Shimamoto, 2012; Lai et al., 2014; Mainali et al., 2014; Pan et al., 2010) at chicken farms or in retail poultry meats of marketplace (Chen et al., 2004; Kim et al., 2012; Yan et al., 2010; Yang et al., 2013; Yang et al., 2010; Yang et al., 2011), or in partial processing stages in the slaughtering line (Akbarmehr, 2012; Bai et al., 2015; Olsen et al., 2003; Rivera-Pérez et al., 2014; Ziech et al., 2016) have been separately performed. Additionally, several surveys have been carried out at the molecular level to monitor the distribution of resistance genes in *Salmonella* from broiler chickens and chicken meat (Asai et al., 2006; Gong et al., 2014; Li et al., 2013). To date, some research on prevalence of *Salmonella* from animals to chicken meat products along the slaughtering process (Choi et al., 2014; Cui et al., 2016; Li et al., 2013; Van der Fels-Klerx et al., 2008), and the potential role of the food production chain in the dissemination of antimicrobial resistance and resistance genes of *Salmonella* (Cui et al., 2016; Li et al., 2013) have been presented. However, the present studies have reported β-lactamase genes and class 1 integron of *Salmonella* isolates from the broiler chicken supply chain in China, while other kinds of resistance genes in *Salmonella* along the slaughtering process were still almost not reported. In addition, the poultry sector in China has experienced vigorous growth over the past two decades. Chicken production is the predominant subsector, accounting for 70% of poultry meat production. The poultry sector is no longer dominated by hundreds of millions of smallholders. Instead, the number of large producers in poultry and broiler chickens production increased substantially (Bingsheng and Yijun, 2007). Therefore, taking into account the important role of large-scale slaughterhouse and poultry farm, and the high consumption of chicken meat in China, more comprehensive investigations at the molecular level to monitor the distribution and dissemination of antimicrobial resistance during slaughter were needed.

Therefore, in this study, four processing points along the slaughtering process were selected to monitor the prevalence, antimicrobial resistance, and resistance gene dissemination of *Salmonella*. In detail, *Salmonella* isolates were collected at the four processing points in a broiler slaughter and processing chain during a three-year period of 2012–2014, in Sichuan province of China. We investigated the prevalence, serotypes, antimicrobial resistance of *Salmonella* isolates to ten categories of common antimicrobial agents, the presence of several kinds of antimicrobial resistance genes associated with β-lactams, aminoglycosides, tetracycline, florfenicol, and sulfonamide, and class 1 integron, *gyrA* and *parC* mutations of quinolone-resistant isolates. On the basis of these results, we analyzed the correlation between the antibiotics-resistant phenotypes, serotypes, and resistance genes of *Salmonella* isolated from different stages of the poultry meat production chain, and identified possible routes of *Salmonella* transmission.

2. Material and methods

2.1. Sample collection

From March 2012 to October 2014, a total of 627 samples were collected from four processing points (cecal content of broiler chicken, $n = 196$; chicken carcasses, $n = 181$; chicken meat after cutting step, $n = 150$; frozen chicken meat products, $n = 100$) during slaughter and processing at a local broiler chicken slaughtering plant in Sichuan province of China where about 30,000,000 broiler chickens are processed each year. All these samples were stored on ice during transportation to our laboratory, and analyzed within 3 h.

The main slaughter process in this slaughterhouse and four sampling points were given in Fig. S1 in Supplementary materials. Fresh broiler chicken cecal contents (point 1), representing samples of broiler at the farm level, were obtained after evisceration, and collected in sterile plastic stomacher bags in accordance with previously described method (Allen et al., 2007). Chicken carcasses (point 2) were sampled after evisceration and before chilling by using a whole-carcass swab method (McEvoy et al., 2005). Each swab was placed inside a sterile stomacher bag and pre-moistened immediately before use with 25 mL of buffered peptone water (BPW). The swabs after sampling were returned to its original bag, and transported on ice to lab. For sampling points 3 (chicken meat after cutting step) and 4 (frozen chicken meat products), 25 g of each sample was incised, and collected in a sterile plastic stomacher bag according to the methods described in the ISO 17604:2003 standard (International Organization for Standardization, Geneva, Switzerland). For sampling point 4, chicken meat products in this slaughterhouse were packaged after quick-freezing, and then the packaged quicken-frozen chicken meat products were directly collected without being stored by using our sampling method.

2.2. Salmonella isolation and serotyping

All of the samples were subjected to *Salmonella* isolation in accordance with national food safety standard of China-Food microbiological inspection: *Salmonella* (GB 4789. 4-2010), with some modifications for samples obtained at point 2. For samples obtained at point 2, they were stomached, incubated at 37 °C for 18 h (bacteria pre-enrichment), and then subjected to centrifugation (12,000 rpm, 4 °C, 10 min). Finally, the BPW supernatant was removed and discarded, and about 4 mL of the pre-enrichment culture containing bacterial precipitate was obtained. Then, aliquots of 1.0 mL were transferred into 10 mL of tetrathionate broth (TTB) and selenite cysteine (SC) broth, respectively. The subsequent procedure was carried out in accordance with GB 4789.4-2010.

Suspected *Salmonella* colonies from each sample were further identified on the basis of biochemical characterization and specific genes of *Salmonella* using duplex PCR assays (Cohen et al., 1993; Rahn et al., 1992). A single confirmed *Salmonella* isolate from each positive sample was serotyped by slide agglutination test for O and H antigens using commercially available antiserum (Tianrun Bio-Pharmaceutical, Ningbo, China) following manufacturer's instructions.