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Short communication

Prevalence and antimicrobial resistance of *Salmonella* isolated from an integrated broiler chicken supply chain in Qingdao, China

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ABSTRACT

The present study analyzed the prevalence and antimicrobial resistance of *Salmonella* along an integrated broiler chicken supply chain. A total of 172 *Salmonella* isolates were recovered from 1148 samples collected from four sample sources (breeder farms, broiler farms, abattoir, and retail markets), representing nine production stages. These *Salmonella* isolates were examined for antimicrobial susceptibility to 12 different antimicrobial agents using a disk diffusion assay. Among them, 168 were identified as six different serotypes of *Salmonella enterica*. The predominant serotype was *S.* Enteritidis (n = 116), followed by *S.* Infantis (n = 18), *S.* Gueuletapee (n = 16), *S.* Derby (n = 12), *S.* Meleagridis (n = 4), and *S.* London (n = 2). The remaining four isolates were serogroup-untypeable. A majority of the 172 isolates (96.51%) was resistant to one or more antibiotics and 61.05% of the *Salmonella* isolates showed a multidrug resistance phenotype. Statistical analysis indicated the one risk product stage for *Salmonella* contamination occurred in the sample source at the abattoir, specifically the stage of Carcasses after chilling. The majority of *S.* Enteritidis isolates shared the same pulsed-field gel electrophoresis (PFGE) cluster, suggesting that the *S.* Enteritidis strain might spread along the broiler chicken supply chain. The prevalence and antimicrobial resistance of *Salmonella* in different production stages suggest the importance of controlling *Salmonella* in the broiler chicken supply chain for public health, underlying the need for improved measures of reducing carcass contamination in abattoirs and the appropriate use of antimicrobials in broiler flocks.

1. Introduction

Salmonellosis, caused by *Salmonella enterica*, is one of the most frequently reported foodborne illnesses worldwide (Scallan et al., 2011; Shao, Shi, Wei, & Ma, 2011). Oral transmission is one of the most common routes of *Salmonella* infection; humans are infected through the ingestion of contaminated animal-derived food, indicating that *Salmonella* from animals can be transmitted to humans via the food chain (Fearnley, Raupach, Lagala, & Cameron, 2011).

The increase of antibiotic resistance in *Salmonella* has become a worldwide problem in recent decades (White, Zhao, Simjee, Wagner, & McDermott, 2002). Food contamination with multidrug-resistant (MDR) bacteria poses a major threat to public health, as there is an abundance of evidence showing that antibiotic-resistant bacteria of animal origin can be transmitted to humans (Khemtong & Chuanchuen, 2008).

Chicken is one of the most widely consumed animal meats in the world; however, it is also recognized as an important reservoir of *Salmonella* (Adu-Gyamfi, Torgby-Tetteh, & Appiah, 2012; Thai & Yamaguchi, 2012). In addition, previous research has indicated the

Abbreviations: PFGE, Pulsed-field gel electrophoresis; MDR, multidrug resistant; DOX, doxycycline; GEN, gentamicin; CHL, chloramphenicol; NAD, nalidixic acid; CIP, ciprofloxacin; AMP, ampicillin; CAZ, ceftazidime; CFZ, cefazolin; AMC, amoxicillin/clavulanic acid; MEM, meropenem; SXT, trimethoprim/sulfamethoxazole; PB, polymyxinB; HACCP, hazard analysis and critical control point.

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importance of considering slaughter and other stages in the meat supply chain for preventing *Salmonella* contamination (Arguello, Alvarez-Ordonez, Carvajal, Rubio, & Prieto, 2013; Marin, Balasch, Vega, & Lainez, 2011; Schmidt et al., 2012). During the various stages of chicken slaughter and processing, all potentially edible tissues are at risk of contamination from sources both within and outside the animal, including the environment, equipment, and operators. Thus, due to the high consumption of chicken meat and increased occurrence and invasiveness of MDR *Salmonella*, the prevalence and antibacterial resistance of *Salmonella* spp. in the broiler chicken supply chain need to be monitored.

In order to collect information on *Salmonella* cross-contamination along the broiler chicken supply chain in Qingdao City, China, the prevalence of *Salmonella* spp. in nine distinct stages of the chicken supply chain and antimicrobial resistance were investigated. Pulsed-field gel electrophoresis (PFGE) was performed to study genetic relatedness of the dominant serotype *S.* Enteritidis isolates from different production stages. The study describes the characteristics of *Salmonella* in the broiler chicken supply chain, which contributes to the understanding of antimicrobial resistance in this supply chain and may aid the creation of strategies to prevent *Salmonella* contamination.

2. Materials and methods

2.1. Sample collection

A total of 1148 samples (detailed in Table 1) were collected during August and September, 2013, from a vertically-integrated commercial broiler chicken supply chain in Qingdao City, China, in which more than 90,000,000 broiler chickens are reared, slaughtered, and sold per year. In this study, we selected related breeder and broiler farms that constitute a vertically integrated broiler supply chain; all animals were part of the same cohort through this chain. Samples were collected from four types of sources (three breeder farms (approximately 8000 birds/flock), two broiler farms (approximately 15,000 birds/flock), one abattoir, and three retail markets), including nine production stages (Breeder, 5-day-old broiler, 20-day-old broiler, Adult broiler: 45 days old, sampled after slaughter but prior to evisceration, Pre-cleaning carcasses: carcasses sampled after evisceration but prior to cleaning, Post-cleaning carcasses: carcasses sampled after evisceration and cleaning, Carcasses after chilling, Segmented chicken: leg or breast fillet of butchered carcass, and Retail chicken: whole carcass packaged and sold to consumers, purchased the same day or day after delivery to the market.

One sample was collected from each animal or meat product as appropriate. At farms, rectal swabs were collected from randomly selected individual animals at three of the stages (Breeder, 5-day-old broiler, and 20-day-old broiler). Broiler cecal samples, representing samples of the Adult broiler stage, were collected from random animals at the abattoir. We chose the swab sampling method because previous reference noted that whole-carcass sampling by swabbing is necessary for optimum *Salmonella* recovery (McEvoy, Nde, Sherwood, & Logue, 2005). Additionally, *Salmonella* contamination at abbatoirs often occurs on the surface of the carcass. We used large swabs moistened with buffered peptone water and swabbed the entire surface of the carcass. Whole carcasses or meat from the next four stages (Pre-cleaning carcasses, Post-cleaning carcasses, Carcasses after chilling, and Segmented chicken) were sampled in the chicken processing chain, using cotton swabs across the surface of the meat. Carcasses from the Retail chicken stage were collected from three markets, and were swabbed in the same manner. Sampling was timed to follow folks through rearing and processing, and sampling on farms included numerous locations to ensure a representative sample. All samples obtained were immediately transported to the laboratory in an insulated ice chest containing ice packs. Microbial analysis was performed immediately upon arrival at the laboratory.

2.2. Isolation and serotyping of Salmonella

Pre-enrichment of the sample for *Salmonella* was performed according to a method described previously (Li et al., 2013), with modifications. Briefly, cotton swab samples or 5 g of fecal matter were placed into sterile 5 mL plastic tubes containing 2 mL of buffered peptone water and incubated at 37 °C for 6–12 h. A 0.2-mL aliquot of these pre-enriched cultures were then inoculated into 2 mL of selenite cysteine broth, which was incubated at 37 °C for 24 h. Selenite cysteine broth cultures were then streaked onto CHROMagar *Salmonella* plates (CHROMagar, Paris, France) and incubated at 37 °C for 24 h. Isolates with a typical phenotype (mauve colony) were confirmed by PCR using a previously described method (Rahn et al., 1992). *Salmonella* isolates were serotyped by slide agglutination for O and H antigens using commercially available antiserum (Tianrun Bio-Pharmaceutical, Ningbo, China), according to manufacturer's instructions.

Table 1
Prevalence of *Salmonella* isolated from nine stages of the broiler chicken supply chain.

Source of isolates	Stages of isolates	Total no. of samples	No. of *Salmonella*-positive isolates (%)	Serotypes (no. Of isolates)
Breeder farms	Breeder	150	2 (1.33)	*S.* Infantis (2)
Broiler farms	5-day-old broiler	100	8 (8.00) ↑↑	*S.* Enteritidis (8)
	20-day-old broiler	100	13 (13.00)	*S.* Enteritidis (12); untypeable (1)
	Adult broiler	290	33 (11.38)	*S.* Enteritidis (14); *S.* Infatis (15); *S.* Meleagridis (4)
Abattoir	Pre-cleaning carcasses	100	13 (13.00)	*S.* Enteritidis (8); *S.* Derby (4); untypeable (1)
	Post-cleaning carcasses	103	15 (14.56)	*S.* Enteritidis (12); *S.* Gueuletapee (2); *S.* London (1)
	Carcasses after chilling	99	26 (26.26) ↑	*S.* Enteritidis (25); untypeable (1)
	Segmented chicken	80[a]	33 (41.25)	*S.* Enteritidis (18); *S.* Derby (8); *S.* Gueuletapee (5); *S.* London (1); non-serogroup (1)
Retail markets	Retail chicken	126	29 (23.02) ↓↓	*S.* Enteritidis (19); *S.* Infatis (1); *S.* Gueuletapee (9)
Total		1148	172 (14.98)	*S.* Enteritidis (116); *S.* Infatis (18); *S.* Gueuletapee (16); *S.* London (2); *S.* Meleagridis (4); *S.* Derby (12); untypeable (4)

"↑" indicates a significant increase ($p \leq 0.008$).
"↓↓" and "↑↑" indicate highly significant decrease and increase ($p \leq 0.002$).
[a] 40 samples were collected from raw chicken leg. 40 samples were collected from raw chicken breast fillet.