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Serovar diversity and antimicrobial resistance of non-typhoidal *Salmonella enterica* recovered from retail chicken carcasses for sale in different regions of China

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ABSTRACT

Non-typhoidal *Salmonella enterica* (NTS), the etiological agents in foodborne salmonellosis, is a major public health concern. This study describes the serovar diversity and antimicrobial resistance phenotypes identified in NTS isolates from retail whole chicken carcasses across six provinces of China. From food samples tested, a total of 2210 *Salmonella* isolates were recovered and these were serotyped by conventional and molecular serotyping methods and tested for their susceptibility to a panel of antimicrobial compounds. Sixteen serogroups and 52 serovars were identified, with serogroups B, D1 and C1 common among Enteritidis, Indiana and Infantis isolates. The serovar distribution varied both geographically and seasonally. Most (80.18%) of these isolates were found to be resistant to at least one antimicrobial compound and 54.6% were multi-drug resistant (MDR). Resistance to nalidixic acid (NAL) was common (70.6%) among the 11 tested compounds and no isolate was found to be resistant to carbapenems. There were 119 antimicrobial resistance profiles identified in the study collection. Two-hundred eighty-four isolates, including 99 *Salmonella enterica* serovar Indiana (*S.* Indiana), were resistant to seven or eight classes of antimicrobial compound. One-hundred eighty-three *S.* Indiana isolates were found to be co-resistant to ciprofloxacin and cefotaxime and 179 of these were confirmed as extended-spectrum β-lactamase producers. These data begin to describe the serovar diversity and antimicrobial resistance of NTS isolates recovered from retail chicken carcasses in parts of China. The findings highlight the emergence of ciprofloxacin and cefotaxime co-resistant *S.* Indiana, a feature displaying serious antimicrobial resistance but not commonly reported in human infections of *Salmonella* until recently. The food safety implications of these findings are discussed.

1. Introduction

Non-typhoidal *Salmonella enterica* (NTS) is a leading cause of human gastroenteritis giving rise to human and animal disease with concomitant economic losses world-wide. Among 4093 foodborne outbreaks reported globally between 1988 and 2007, 46.9% of these were attributed to NTS (Greig & Ravel, 2009). NTS infections cause approximately 1.2 million illnesses per year, with 23,000 hospitalizations, 450 deaths, and an estimated 365 million dollars in associated medical costs in the United States (Fàbrega & Vila, 2013; Mcsorley, 2014; Scallan et al., 2011). Although a

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decreasing trend in confirmed cases of human salmonellosis has been observed in the European Union (EU) in recent years, nonetheless, NTS remains the second most common causative agent in foodborne outbreaks (EFSA and ECDC., 2015). *Salmonella* ranks as the fourth most common aetiological agent of foodborne pathogens in China in 2012 (Kuang et al., 2015), and was responsible for 74 outbreaks of food-related infections (accounting for 7.4% of all reported foodborne outbreaks and 13.2% of verified cases). These bacteria rank as the second most important bacterial causative agent since 2013 (data not published), and continues to pose a significant public health challenge worldwide.

NTS is transmitted following the consumption of contaminated foods or by direct contact with colonized/infected animals. Food sources including eggs, dairy products, vegetables and processed foods have been associated with salmonellosis. Notably, poultry was recognized as an important source of this pathogen for humans (Chai, Cole, Nisler, & Mahon, 2017). Intensive poultry production and slaughtering practices may contribute to the spread of NTS among birds and raw poultry meat. Contamination can occur at several stages including production, slaughter, processing, handling, and storage (Dookeran, Gail, Akingbala, Tameru, & Lammerding, 2012). Particularly, in modern slaughterhouses the rapid line speeds during processing maintains the birds in close proximity throughout, increasing the risk of bacterial transfer between carcasses (Lupo et al., 2007). According to the Surveillance for Foodborne Disease Outbreaks of the United States, 11.2% of the outbreaks caused by *Salmonella* with a confirmed serotype were associated with the consumption of poultry meat in the period of 1998–2008, and *Salmonella* and poultry was considered to be the most commonly responsible pathogen-commodity pairs for outbreaks caused by bacteria (Gould et al., 2013). NTS is also the most frequently notified bacterial hazard, with serovar Typhimurium notifications relating to poultry *via* Rapid Alert System for Food and Feed (RASFF) being reported in the European Union (EU, 2011). Thus, foods of animal origin, especially poultry meat, are recognized as important vehicles for the transmission of *Salmonella* to humans due to cross-contamination or inadequate cooking.

Antimicrobial agents are widely used in animal and human medicine, not only to treat disease, but also, in some jurisdictions, as growth promoting substances. Over the last few decades, there has been growing public health concern about the emergence of bacteria, in particular NTS that have become resistant to antimicrobial agents. *Salmonella* serovars resistant to three or more classes of antimicrobial agents (referred to here as multidrug-resistant, MDR) (Magiorakos et al., 2012) have been isolated, thereby increasing the risk of treatment failure when use of these agents is indicated (Aminov, 2010, Bai, Zhao et al., 2016; Bai, Hurley et al., 2016). The extent to which resistant bacteria evolve appears to vary and is thought to be influenced by antimicrobial use in both animals and humans, along with geographical factors that remain to be elucidated (Gibbons et al., 2016). Evidence suggests that antimicrobial use in food-producing animals contributes to resistance among foodborne NTS, and acquisition of antimicrobial resistant (AMR) *Salmonella* isolates may be linked to international travel (Crump et al., 2011). People infected with antimicrobial resistant strains are more likely to suffer an adverse health event such as prolonged illness, increased severity of illness, hospitalization or death when compared with those infected with susceptible isolates (Cook et al., 2009). Therefore, on-going surveillance is a necessary step towards monitoring the emergence of MDR isolates of NTS.

The purpose of this study was to characterize 2210 NTS isolates recovered from 1438 retail whole chicken carcasses across six provinces of China. Serovars were identified, and the resistance profiles determined. These findings are discussed in the context of improving food safety controls across the country and limiting the potential for dissemination of MDR NTS in the future.

2. Materials and methods

2.1. Sample collection, Salmonella isolation and identification

From April 2011 through March 2012, a total of 1438 samples including freshly slaughtered, chilled and frozen retail whole chicken carcasses were collected from supermarkets and farmer's markets in Beijing (238), Guangdong province (240), Jilin province (240), Jiangsu province (240), Shaanxi province (240), and Inner Mongolia Autonomous (240), 20 samples for each month per sampling site. Each sample was transported on ice to local laboratories within 2 h after collection for isolation and identification of *Salmonella* using previously published protocols (Wang et al., 2013, 2014; Zhu et al., 2014). Briefly, each sample was immediately aseptically removed from the package and placed in a 3,500®stomacher bag (Seward, UK) followed by the addition of 500-mL buffered peptone water (BPW; Becton-Dickinson, Beijing, China) per kilogram of carcass. The bag was manually massaged for 3–5 min, triplicate 10 mL BPW chicken rinses were taken directly from each stomacher bag and placed into three empty sterile test tubes, from which a 1 mL sample of this rinse was transferred in triplicate into 9 mL BPW tubes followed by 1:10 dilution in triplicate tubes of BPW. All tubes were pre-enriched in a shaking incubator at 100 rpm for 22–24 h at 37 °C. From each tube, 0.5 mL and 0.1 mL volumes of the pre-enrichment mixture were transferred to 10 mL tetrathionate broth (TT, Becton-Dickinson) and Rappaport-Vassiliadis broth (RV, Becton-Dickinson) and incubated at 42 ± 1 °C with shaking at 100 rpm for 22–24 h. Following selective enrichment, a loopful of this broth culture was streaked on xylose lysine tergitol 4 (XLT4, Becton-Dickinson) agar in duplicate and incubated at 37 °C for 24 h to recover isolated colonies. After this step, one presumptive *Salmonella* colony from each XLT4 plate was inoculated onto a triple sugar iron slant (TSI, Becton-Dickinson) and incubated at 35 ± 1 °C for 24 h. Isolates with typical *Salmonella* phenotypes were confirmed by both amplification of the *inv*A gene by polymerase chain reaction (PCR) (Rahn et al., 1992) and the API 20E test (Cat No. 20100, BioMe'rieux, France). In total, 2210 NTS strains (Beijing, 455; Jilin, 726; Guangdong, 502; Jiangsu, 260; Shaanxi, 186; Inner Mongolia Autonomous, 81) were recovered for further study.

2.2. Salmonella serovar identification

For all confirmed *Salmonella* isolates (as described above), their serovars were determined by either classical slide agglutination with commercialized *Salmonella* antisera (State Serum Institute, Denmark) following the Kauffmann–White–Le Minor scheme (WKLM scheme) (Grimont & Weill, 2007), or by molecular serotyping using the xMAP® *Salmonella* Serotyping Assay Kit (SSA, Cat No. AGSSA4502, Luminex, U.S.A) following the recommended manufacturer's protocols (Dunbar, Ritchie, Hoffmeyer, Rana, & Zhang, 2015). Briefly, for Luminex serotyping, *Salmonella* isolates were inoculated on brain heart agar (BHA) [Cat No. CM918, Land Bridge, Beijing, China] and incubated at 37 °C for 18–24 h. DNA extraction was performed with an InstaGene matrix (Cat No. 732-6030, Bio-Rad Laboratories, CA, U.S.A). Three separate multiplex-PCR and hybridization reactions were performed for each sample to detect the expression of serotype-specific antigens using microsphere technology. The Qiagen HotStar™ (Cat No. 203443, QIAGEN, Valencia, U.S.A) and xMAP SSA Kits were used to prepare three separate PCR master mixes.