Comparative characterization of nontyphoidal Salmonella isolated from humans and food animals in China, 2003–2011

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Abstract

Food animals are major reservoirs from which specific pathogenic Salmonella strains emerge periodically. Probing the identity and origin of such organisms is essential for formulation of highly-focused infection control measures and analysis of factors underlying dissemination of such strains. In this work, the genetic and phenotypic features of animal and human clinical isolates collected at different geographical localities in China during the period 2003–2011 were characterized and compared. Animal-specific serotypes were identified, with S. Enteritidis, S. Cremieu and S. Fyris being recovered almost exclusively from chicken, ducks and pigs respectively. Nevertheless, only four serotypes were commonly found to be transmitted among both animal and human clinical isolates: S. Enteritidis, S. Typhimurium, S. Derby and S. Indiana. Strains of the serotypes S. Enteritidis and S. Typhimurium not only accounted for up to 50% of
all human clinical isolates tested, but often shared identical genetic profiles with the animal isolates. Using a recently identified mobile efflux gene, oqxAB, as a genetic marker for assessing the efficiency of transmission between animal and human isolates, we demonstrated that a newly emerged genetic trait could be simultaneously detectable among both animal and human clinical isolates. Findings in this work show that transmission of Salmonellae between animal and human is highly efficient and serotype dependent.

Keywords: Infectious disease, Microbiology, Veterinary medicine

1. Introduction

Non-typhoidal Salmonella infections are important public health problems worldwide [1, 2, 3, 4]. In the past two decades, the rate of antimicrobial resistance and number of newly identified resistance determinants in Salmonellae have increased markedly [5, 6, 7, 8]. Most Salmonella infections are thought to be due to consumption of contaminated food, in particular meat, poultry and dairy products [9, 10]. Food animals are presumably the major reservoirs of new epidemic strains that often exhibit high level virulence and antimicrobial resistance phenotypes [11]. Typical examples include the S. Typhimurium DT104 strain in the UK [12, 13], S. Newport in the US [14, 15] and S. Enteritidis strains that exhibit close association with eggs and chicken [16, 17, 18, 19]. In response to the growing threat of outbreaks and drug resistance of foodborne pathogens, industrialized nations have implemented numerous intervention approaches designed to reduce the rate of colonization by Salmonellae and other foodborne pathogens in food animals and animal-derived products [20, 21]. To enhance the effectiveness of such measures, efforts have been made to identify factors that determine the pathways of transmission of Salmonellae from animals to humans, through assessment of the temporal and spatial distribution of Salmonella serotypes in various sources. Comprehensive surveillance programs conducted on both human and animal isolates in the European countries and the U.S. showed that the dominant serotypes that caused human clinical infections were not due to an overwhelming prevalence of these serotypes in animals [22, 23]. Hence, the determining factors that contribute to Salmonella clinical infections are still not well defined. To date, only a few studies have evaluated and compared the diversity of PFGE types of temporally and geographically matched animal and human clinical Salmonella isolates [24, 25]. Efforts to generate information on the serotype and PFGE diversity of clinical strains and zoonotic and foodborne pathogens, which is critical to source-tracking and identification of potentially host-restricted serotypes, have been limited by small sample size and undesirable research design.
In China, *Salmonella* surveillance programs have been confined to human clinical samples. The few previous studies which reported the prevalence of different *Salmonella* serotypes in animals and food products showed that *Salmonella* prevalence rate in China varies wildly between different regions [26, 27, 28, 29, 30]. Yet there is little information on major food-animal reservoirs of disease-causing *Salmonella* strains, and the corresponding modes of transmission. To fill this knowledge gap, we strived to compare the antimicrobial resistance and serotype distribution of major collections of *Salmonella* strains isolated from both animals and hospital patients in China during the period of 2003–2011, so as to depict the features of transmission of specific serotypes of *Salmonellae* from animals to human. Findings of this work shall help identify the major serotypes and specific strains involved in *Salmonella* infections of zoonotic origin in China.

2. Materials and methods

2.1. Isolation and identification of *Salmonella* strains

A total of 5926 samples including chicken liver and lung (540), chicken intestinal content (225), chicken meat (671), chicken faeces (1781), swine faeces (1906), pork (291), duck intestinal content (304), and cow faeces (208) collected from the provinces of Sichuan, Guangdong, Jiangxi, Henan, Jiangsu and Shandong in China during the period 2003–2011 were included in this study. Chicken samples were collected from one-day old chick in chicken hatcheries, chicken farms or slaughter houses, duck samples were from slaughter house, swine and cow samples were collected from healthy animals in farms and slaughter house. Samples were collected at least twice in each province every year since 2005.

Fresh samples were pre-enriched in buffered peptone water (BPW, Difco, Cockeysville, MD) and selenite cystine broth (SC, Oxiod), followed by streaking onto CHROMagar *Salmonella* agar (CHROMagar Company, Paris, France) and bismuth sulphite agar (Oxiod). Suspected *Salmonella* colonies were selected for biochemical study using the API 20 E (bioMérieux, Marcy l’ Etoile, France) micro-substrate system for genus identification. The suspected *Salmonella* pullorum strains were subjected to motility test on indole–lysine semisolid agar to determine their mobility.

Clinical *Salmonella* isolates were obtained from stool and blood samples of infected patients in hospitals. Sample collection was coordinated by the State Key Laboratory for Infectious Disease Prevention and Control, Chinese Centre for Disease Control and Prevention, National Institute for Communicable Disease Control and Prevention, Beijing, People’s Republic of China (ICDC). Eight participating cities and
provinces in mainland China including Guangdong, Guangxi, Henan, Fujian, Sichuan, Beijing, Shanghai and Chongqing were involved in *Salmonella* isolation. All isolates were recovered within the period 2005–2011. All experimental protocols were approved and performed in accordance to the approved guidelines of Chinese Centre for Disease Control and Prevention.

### 2.2. Serotyping

*Salmonella* serotyping was conducted by the slide agglutination approach, using *Salmonella* antisera (S & A Reagents Lab Ltd., Bangkok, Thailand) according to the Kaufman-White scheme.

### 2.3. PCR detection of *oqxAB*

All isolated *Salmonella* strains were subjected to PCR assay to test the presence of the *oqxA* and *oqxB* genes using the primers *oqxA*-F (5’-GACAGCCTGCACA-GAATG-3’), *oqxA*-R (5’-GCGTGGCTTTGAAGTGC-3’), and *oqxB*-F5’-TTCTCCCCGCCGGAAGTAC, *oqxB*-R (5’-CTCGGCCATTTTGGCGC GTA) for *oqxA* and *oqxB* respectively [8, 31]. Positive detection of either one or both genes was considered as *oqxAB* positive for the *Salmonella* isolates.

### 2.4. Antimicrobial susceptibility tests

Minimal inhibitory concentration (MIC) was tested by the microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [32]. The MIC interpretative criteria were adopted from CLSI. *Escherichia coli* ATCC 25922 was used as control strains in determining MIC values. The following antimicrobial agents were tested (concentration range in mg/L): ampicillin (2–128), Amoxicillin/Clavulanic Acid (1–64), tetracycline (2–128), cefazolin (4–128), ceftiofur (0.25–128), ceftriaxone (0.25–128), chloramphenicol (4–128), streptomycin (1–128), kanamycin (1–128), enrofloxacin (0.008–128), ciprofloxacin (0.008–128), olaquindox (0.25–128), gentamicin (2–128), and trimethoprim/sulfamethoxazole (2/38-8/152). All antimicrobial agents were obtained from the China Institute of Veterinary Drug Control (Beijing, China).

### 2.5. Pulsed-field Gel Electrophoresis (PFGE)

The chromosomal DNA of *S. Derby*, *S. Indiana*, *S. Typhimurium*, and *S. Enteritidis* strains were digested with restriction enzyme XbaI and subjected to PFGE according to the Pulse Net Standardized Laboratory Protocol (U.S. Centers for Disease Control and Prevention, Atlanta, GA) using the CHEF Mapper XA Pulsed Field Gel Electrophoresis System (Bio-Rad). Electrophoresis parameters: 6.0 V/cm, 120° angle, initial and final switch times of 5 and 40s, respectively; run time was 18.5 h at 14 °C.
Cluster analysis of PFGE types was performed according to the Dice coefficient method [33] using the InfoQuest FP Software/Version 4.5 (Bio-Rad).

3. Results

3.1. Relative prevalence and serotype profiles of *Salmonellae* in different animal sources

A total of 1133 *Salmonella* strains were recovered from 5926 animal samples collected from different provinces in China during the period 2003—2011. Table 1 provides a statistical summary of the rate of isolation of *Salmonellae* from different animal sources. *Salmonellae* were most frequently isolated from chicken samples (isolation rate of 26%), followed by duck (15%), pigs (10%) and cows (7%). The overall isolation rate was 19%. Twenty-five *Salmonella* serotypes were identified among the 1133 *Salmonella* isolates, the most prevalent being Enteritidis (415), followed by Pullorum (194), Indiana (132), Derby (120) and Typhimurium (56). Table 2 summarizes the most frequently isolated serotypes in each specimen type; specific serotype appears to be linked to specific animal source, for example, 98% of *S*. Enteritidis, 100% of *S*. Pullorum and 100% of *S*. Indiana strains identified in this work were recovered from chicken, 96% of *S*. Cremieu were recovered from duck, 87% of *S*. Derby and 100% of *S*. Fyris originated from pigs; likewise, *S*. Give could only be recovered from pigs and cows.

| Year | Number of isolates by Sources (Number of samples) | Total number (Number of samples) |
|------|--------------------------------------------------|----------------------------------|
|      | Chicken 20 (113) pigs 1 (8) ducks 0 (0) cows 0 (0) | 21 (121)                         |
| 2004 | 92 (436) 12 (130) 0 (0) 0 (0)                      | 104 (566)                        |
| 2005 | 89 (349) 0 (6) 0 (0) 0 (0)                         | 89 (355)                         |
| 2006 | 110 (429) 0 (5) 0 (0) 0 (0)                        | 110 (434)                        |
| 2007 | 18 (66) 30 (298) 5 (50) 14 (208)                   | 67 (622)                         |
| 2008 | 257 (930) 27 (276) 0 (0) 0 (0)                     | 284 (1206)                       |
| 2009 | 157 (563) 60 (586) 28 (198) 0 (0)                  | 245 (1347)                       |
| 2010 | 70 (210) 34 (330) 0 (0) 0 (0)                      | 104 (540)                        |
| 2011 | 30 (121) 66 (558) 13 (56) 0 (0)                    | 109 (735)                        |
| Total by sources | 843 (3217) 230 (2197) 46 (304) 14 (208) | 1133 (5926) |
| Isolation rate | 26.2% 10.5% 15.1% 6.7% | 19.1% |
3.2. *Salmonella* serotype prevalence among human clinical isolates

Upon determining the relative prevalence rates of various *Salmonella* serotypes among animal isolates, we sought to check the degree by which they matched the corresponding profiles of human clinical isolates. According to the data provided by CDC in China, the number of clinical *Salmonella* isolates recorded in specific study centers scattered among eight cities or provinces in China increased yearly from 360 in 2005 to 2163 in 2011 (no human isolate was collected before 2005). Similar to the situation in the US, the two most prevalent serotypes were S. Enteritidis and S. Typhimurium, which accounted for around 50% of all *Salmonella* infections (Table 3). S. Enteritidis was also the most prevalent among the animal isolates. Other serotypes ranking from the third to tenth place of prevalence in each year of the study period (2005–2011) are listed in Table 3. Comparing the data of animal and human clinical isolates, four serotypes were found in both groups: S. Enteritidis, S. Typhimurium, S. Derby and S. Indiana. Except for S. pullorum which is naturally a poultry-specific strain, there are serotypes which were found only among animal isolates but not human clinical isolates: S. Give, Fyris and Cremieu. On the contrary, several serotypes such as S. Senftenberg and S. Weltevreden, which were commonly observed among clinical isolates, were rarely recoverable in animals.

| Animal          | Location of isolation | Specimen type     | No. of samples | No. of *Salmonella* isolates recovered | Prevalent serotypes (of isolates) |
|-----------------|-----------------------|-------------------|----------------|---------------------------------------|-----------------------------------|
| Chicken         | Slaughter house       | Chicken liver and lung | 540            | 196                                   | Pollorum (170), Rough (18), II (4) |
|                 | Slaughter house       | Intestinal contents | 225            | 31                                    | Enteritidis (16), Indiana (15), Indiana (102), Enteritidis (54), Derby (10), S. Typhimurium (9) |
|                 | Slaughter house       | Chicken meats      | 671            | 182                                   |                                    |
| One-day old chick | Chick farm (hatchery) | Fecal samples     | 1781           | 434                                   | Enteritidis (315), Rough (80), Pollorum (14) |
| Pig             | Pig farm              | Fecal samples     | 1906           | 198                                   | Derby (90), S. Typhimurium (27), Enteritidis (25), Give (24), Fyris (20) |
| Pig             | Market                | Pork              | 291            | 32                                    | Derby (15), Typhimurium (11), Enteritidis (5) |
| Duck            | Slaughter house       | Intestinal contents | 304            | 46                                    | Cremieu (24), Pollorum (10), Typhimurium (9), Rough (4) |
| Cow             | Cow farm              | Fecal samples     | 208            | 14                                    | Give (10), Derby (4) |
| Total           |                       |                   | 5936           | 1133                                  | Enteritidis (415), Pollorum (194), Indiana (132) |

3.2. *Salmonella* serotype prevalence among human clinical isolates

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Table 3. The ten most frequently isolated Salmonella serotypes causing human infection in China in each year of the period 2005–2011.

| Order of prevalence | 2005 (n = 360) | 2006 (n = 441) | 2007 (n = 515) | 2008 (n = 641) | 2009 (n = 829) | 2010 (n = 1324) | 2011 (n = 2163) |
|---------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| 1                   | S. Enteritidis (23.0) | S. Enteritidis (18.4) | S. Enteritidis (30.4) | S. Enteritidis (32.4) | S. Typhimurium (28.0) | S. Typhimurium (31.0) | S. Enteritidis (30.4) |
| 2                   | S. Typhimurium (20.1) | S. Typhimurium (18.0) | S. Typhimurium (25.7) | S. Typhimurium (26.1) | S. Enteritidis (23.3) | S. Enteritidis (21.3) | S. Typhimurium (25.7) |
| 3                   | S. Derby (4.5) | S. Derby (3.4) | S. Derby (3.4) | S. Derby (3.0) | S. Senftenberg (5.1) | S. Derby (4.2) | S. Derby (3.4) |
| 4                   | S. Anatum (2.5) | S. Anatum (2.5) | S. Anatum (2.5) | S. Anatum (2.7) | S. Agona (3.8) | S. Agona (3.2) | S. Anatum (2.5) |
| 5                   | S. London (2.5) | S. London (2.5) | S. London (2.5) | S. London (2.7) | S. Derby (3.1) | S. Stanly (2.9) | S. London (2.5) |
| 6                   | S. Senftenberg (2.0) | S. Senftenberg (2.0) | S. Senftenberg (2.2) | S. Senftenberg (2.2) | S. Stanly (2.8) | S. Senftenberg (2.0) | S. Senftenberg (2.0) |
| 7                   | S. Weltevreden (1.8) | S. Weltevreden (1.8) | S. Weltevreden (1.8) | S. Infantis (2.2) | S. Weltevreden (2.2) | S. Meleagris (2.0) | S. Weltevreden (1.8) |
| 8                   | S. Istanbul (1.6) | S. Istanbul (1.6) | S. Istanbul (1.6) | S. Istanbul (1.7) | S. Thompson (1.8) | S. London (1.9) | S. Istanbul (1.6) |
| 9                   | S. Thompson (1.6) | S. Thompson (1.6) | S. Thompson (1.6) | S. Thompson (1.7) | S. Newport (1.7) | S. Thompson (1.9) | S. Thompson (1.6) |
| 10                  | S. Agona (1.5) | S. Agona (1.5) | S. Agona (1.5) | S. Agona (1.4) | S. Agona (1.4) | S. Newport (1.8) | S. Agona (1.5) |

3.3. Antimicrobial susceptibility of different serotypes of Salmonella isolated from animal and human

We next compared the antibiotic susceptibility profiles of animals and human clinical isolates to determine whether the vast majority of Salmonella strains causing human infections originated from food animals, in which case we expected that the two groups of isolates shared similar antibiotic susceptibility profiles. Our data, depicted in Tables 4 and 5, showed that striking similarities and discrepancies in antibiotic susceptibility profiles were both observable when serotypes commonly found in animal and human clinical isolates were compared. S. Enteritidis in both groups exhibited similar susceptibility profiles, with the exception that animal S. Enteritidis isolates displayed a higher resistance rate to trimethoprim/sulfamethoxazole. Interestingly, low rate of resistance to ciprofloxacin coupled with a high nalidixic acid resistance rate was observable in both animal and human isolates of S. Enteritidis. S. Typhimurium from both groups also exhibited very similar antimicrobial susceptibility profiles, with the resistance rate being slightly higher among the animal isolates. The most dramatic difference between animal and human Salmonella isolates was observed in S. Indiana, in which around 92% of animal S. Indiana isolates were
resistant to ceftriaxone, whereas none of the human isolates was resistant to cephalosporins. The rate of resistance to other antibiotics was also generally higher among animal *S. Indiana* strains (Tables 4 and 5).

### 3.4. Features of transmission of animal isolates to human

To further confirm whether a significant proportion of clinical *Salmonella* strains originated from animals, 40 animal isolates belonging to each of serotypes commonly observed in both groups (*S. Enteritidis*, *S. Typhimurium*, *S. Derby* and *S. Indiana*) were randomly selected and subjected to PFGE typing, and then compared to those of human clinical *Salmonella* strains in the China CDC database (Fig. 1). For *S. Enteritidis*, half of the 40 animal *S. Enteritidis* isolates were found to belong to the PFGE pattern CN0004, to which 167 out of the 1550 *S. Enteritidis* strains identified in the China CDC also belonged. Likewise, eight out of the 40 animal isolates belonged to CN0003, which was the most prevalent PFGE pattern in human isolates in the CDC database, covering as many as 444 out of the 1550 *S. Enteritidis* isolates in the database. Only 3 animal *S. Enteritidis* strains were found to belong to new PFGE patterns which could not be identified in the CDC database. Identical PFGE patterns were also observable in both animal and human clinical isolates for the serotypes of *S. Typhimurium* and *S. Indiana*, but not *S. Derby* (Fig. 1).

| Antibiotics                  | % of resistance in different serotypes of *Salmonella* |
|------------------------------|-------------------------------------------------------|
|                              | Enteritidis n = 415 | Pollorum n = 194 | Indiana n = 132 | Derby n = 120 | Typhimurium n = 56 | Give n = 34 | Cremieu n = 25 | Fyris n = 24 | Other serotypes n = 133 |
| Ampicillin                   | 35 | 24 | 97 | 15 | 82 | 94 | 0 | 89 | 44 |
| Amoxicillin/Clavulanic Acid  | 16 | 36 | 89 | 11 | 82 | 91 | 0 | 92 | 47 |
| Cefazolin                    | 4  | 13 | 95 | 9  | 9  | 9  | 0 | 15 | 19 |
| Ceftriaxone                  | 4  | 37 | 94 | 4  | 5  | 0  | 0 | 19 | 18 |
| Chloramphenicol              | 4  | 16 | 81 | 48 | 68 | 82 | 0 | 92 | 33 |
| Streptomycin                 | 1  | 58 | 92 | 29 | 64 | 82 | 0 | 89 | 33 |
| Gentamicin                   | 1  | 6  | 93 | 10 | 68 | 77 | 0 | 81 | 27 |
| Kanamycin                    | 3  | 7  | 95 | 8  | 77 | 85 | 0 | 89 | 44 |
| Tetracycline                 | 30 | 52 | 97 | 88 | 82 | 85 | 0 | 96 | 68 |
| Nalidixic Acid               | 99 | 86 | 99 | 3  | 82 | 82 | 0 | 96 | 64 |
| Ciprofloxacin                | 1  | 35 | 75 | 13 | 55 | 74 | 0 | 89 | 34 |
| Sulfisoxazole                | 45 | 97 | 100| 96 | 86 | 94 | 100| 27 | 72 |
| Trimethoprim/Sulfamethoxazole| 49 | 96 | 99 | 55 | 82 | 85 | 0 | 31 | 65 |
Table 5. Resistance rate of commonly identified serotypes of human clinical *Salmonella* isolates.

| Antibiotic                        | % of resistance in different serotypes of *Salmonella* |
|-----------------------------------|------------------------------------------------------|
|                                   | Enteritidis n = 483 | Typhimurium n = 710 | Derby n = 167 | Senftenberg n = 83 | Weltevreden n = 79 | Newport n = 64 | Agona n = 57 | Anatumn n = 47 | Indiana n = 25 | Other serotypes n = 38 |
| Ampicillin                        | 42 | 47 | 7 | 0 | 0 | 0 | 15 | 70 | 52 | 42 |
| Amoxicillin/Clavulanic Acid       | 9  | 26 | 0 | 0 | 0 | 0 | 5  | 6  | 0  | 0  |
| Cefazolin                         | 4  | 4  | 0 | 0 | 0 | 0 | 0  | 0  | 4  | 0  |
| Ceftriaxone                       | 4  | 4  | 0 | 0 | 0 | 0 | 0  | 0  | 4  | 0  |
| Chloramphenicol                   | 6  | 43 | 68| 0 | 0 | 0 | 10 | 70 | 52 | 11 |
| Streptomycin                      | 31 | 52 | 22| 0 | 0 | 0 | 12 | 66 | 48 | 8  |
| Gentamicin                        | 11 | 35 | 0 | 0 | 0 | 0 | 8  | 30 | 44 | 3  |
| Kanamycin                         | 15 | 44 | 10| 0 | 0 | 0 | 15 | 43 | 53 | 2  |
| Tetracycline                      | 21 | 49 | 89| 0 | 1 | 0 | 20 | 80 | 52 | 24 |
| Nalidixic Acid                    | 94 | 73 | 56| 13| 1 | 0 | 22 | 78 | 100| 26 |
| Ciprofloxacin                     | 0  | 20 | 0 | 0 | 0 | 0 | 0  | 10 | 72 | 0  |
| Sulfoisoxazole                    | 6  | 55 | 33| 14| 0 | 0 | 25 | 80 | 64 | 32 |
| Trimethoprim/Sulfamethoxazole     | 2  | 38 | 33| 0 | 0 | 0 | 10 | 40 | 52 | 26 |
3.5. Synchronous emergence of \textit{oqxAB} positive \textit{Salmonella} strains in animals and humans

The rate of transmission of a newly emerged \textit{Salmonella} strain among animal and humans has never been demonstrated. In a previous study, we have identified a plasmid mediated RND efflux pump, \textit{oqxAB}, which was found to emerge in \textit{Salmonella} spp.. We showed that the gene encoding this efflux pump (\textit{oqxAB}) began to emerge in clinical \textit{S. Typhimurium} strains in 2006 and that the \textit{oqxAB} positive rate steadily increased from 12\% in 2006 to 43\% in 2011 [31]. This gene is therefore considered a good marker for determining whether the majority of clinical \textit{Salmonella} strains that cause human infections originated from animals. Screening of the animal isolates showed that the overall prevalence rate of \textit{oqxAB} in \textit{Salmonella} animal isolates was about 18\%, with varying positive rate among different serotypes. Consistent with previous findings, no \textit{oqxAB} gene was detectable in the 157 \textit{Salmonella} strains isolated before 2006. \textit{S. Fyris} exhibited the highest \textit{oqxAB} positive rate, reaching 95\%. Approximately about 50\% of \textit{S. Typhimurium}, \textit{S. Indiana} and \textit{S. Derby} strains were positive for \textit{oqxAB}, whereas \textit{S. Enteritidis} and other serotypes exhibited a very low \textit{oqxAB} positive rate (0.9\%) (Table 6). The simultaneous detection of marker gene \textit{oqxAB} in both animal and human \textit{Salmonella} isolates constitutes

![Fig. 1. Different serotypes of \textit{Salmonella} isolates with same PFGE patterns were detected in both animal and human clinical isolates. Number in parentheses represents the total number of isolates in the database that have been used in comparison.](https://doi.org/10.1016/j.heliyon.2018.e00613)

| Year   | Sources          | \textit{oqxAB} positive rate (number of isolates screened) |
|--------|------------------|----------------------------------------------------------|
|        |                  | Overall | \textit{S. Typhimurium} | \textit{S. Enteritidis} | \textit{S. Derby} | \textit{S. Indiana} | \textit{S. London} |
| Before 2006 | Animal isolates | 0 (157) | 0 (20) | 0 (80) | 0 (35) | 0 (20) | 0 (2) |
|          | Human clinical isolates | 0 (210) | 0 (50) | 0 (100) | 0 (40) | 0 (10) | 0 (10) |
| 2006 onward | Animal isolates | 19\% (723) | 29\% (42) | 0.9\% (431) | 46\% (115) | 48\% (130) | 0 (5) |
|          | Human clinical isolates | 18\% (1143) | 27\% (660) | 1\% (383) | 29\% (70) | 20\% (10) | 0 (20) |

Table 6. The relative \textit{oqxAB} positive rate of five most prevalent serotypes of animal and human clinical \textit{Salmonella} isolates recovered before and after the year 2006.
evidence of efficient transmission of Salmonella among animals and human. Most interestingly, an earlier study in our laboratory has reported the expansion of an oqxAB-positive S. Typhimurium ST34 clone which belonged to the CN0006 PFGE pattern in both Hong Kong and China [31]. This exact PFGE pattern was also detectable in 14 out of the 40 S. Typhimurium animal isolates tested, which were all oqxAB positive, suggesting that this specific clone are among the few which can be transmitted most efficiently from animals to human.

4. Discussion

To date, more than 2700 serotypes of Salmonella have been identified, but the ability of each serotype to cause human infections is known to vary significantly [34]. Since Salmonella infection is considered a major zoonotic disease, assessment of the differential prevalence of serotypes of animal and human clinical isolates may help depict the relative virulence and transmission potential of major Salmonella serotypes and identify strains which exhibit the highest infection risk. Serotype-specific features of Salmonellae have been observed in animal, food and clinical isolates. In the US, CDC surveillance data showed that the most prevalent serotypes in clinical isolates were S. Enteritidis, S. Typhimurium, S. Newport and S. Javiana, whereas among isolates that cause animal diseases, the most prevalent serotypes were S. Typhimurium, S. Dublin, S. Cerro, S. Agona and S. Derby [22]. In animal isolates that rarely cause infections in animals, S. Kentucky, S. Enteritidis and S. Senftenberg were the common serotypes [22]. Such discrepancy, which was also observable in our study, is likely due to the wide range of infection potential exhibited by animal Salmonella strains, among which only a certain proportion could cause human infections. Alternatively, it could also be due to other factors, such as eating habits that prevent certain food-specific serotypes from causing human infections. These possibilities will be discussed below.

Data on the surveillance of Salmonella in pigs in 2000 and 2006 from USDA showed that 6% and 7.2% of the samples were positive to Salmonella in these two years respectively, with strains belonging to the serotypes S. Derby, S. Typhimurium var. 5- and S. Agona being the most common. Among such strains, S. Derby was commonly resistant to streptomycin, sulfisoxazole, and tetracycline [35]. A similar isolation rate (10.5%), profile of dominant serotypes, and resistance pattern were observed among Salmonella strains isolated from pigs in this study. At present, data of surveillance of Salmonella recovered from poultry and the corresponding food products were not available in USDA and FDA, although S. Enteritidis is considered as a major threat to human health [36]. In this study, S. Enteritidis was confirmed to be closely associated with the meat and GI tract of chicken, as well as with one-day old chick, presumably due to the contamination of eggs.

Due to a lack of comprehensive surveillance system in China, the relative prevalence of Salmonella serotypes in human and non-human sources is unknown. The present
work represents the first attempt to address this issue, with a hope of identifying the major serotypes/zoonotic strains responsible for causing human infections. The fact that the majority of *Salmonella* isolates recovered from one-day old chicks were *S. Enteritidis* supports the idea that *S. Enteritidis* is the major serotype of *Salmonellae* that can cause egg contamination, since organisms residing in one-day old chick are expected to be mainly derived from eggs. Notably, in both chicken and pigs, *Salmonellae* isolated from intestinal content and meat products exhibited similar serotypes, suggesting that contamination of chicken meat and pork by fecal particles of the respective animals is common.

Consistent with the data from NARMS (https://www.fda.gov/AnimalVeterinary/SafetyHealth/ANTimicrobialResistance/NationalAntimicrobialResistanceMonitoringSystem/ucm416741.htm), we observed a high rate of resistance to tetracycline, ampicillin, sulfamethoxazole, streptomycin and chloramphenicol in both animal and human *Salmonella* strains, with the animal isolates displaying a higher rate of resistance to the majority of the agents tested. Consistent with our findings, data from NARMS also showed that *S. Enteritidis* displayed much lower resistance to most of the antibiotics than *S. Typhimurium*. The most striking difference is that *S. Enteritidis* strains from various sources exhibited very high resistance to nalidixic acid, yet the rate among US strains was very low. The rate of resistance among human *Salmonella* isolates collected in different countries was similar to each other. However, the rate of resistance to ciprofloxacin was strikingly different between animal *S. Typhimurium* strains in the US isolates (~0.2%) and in China (~55%). On the other hand, the rate of resistance to cephalosporins among animal isolates, in particular *S. Typhimurium* in the US (~20%), was much higher than that of China (~5%).

In this work, human clinical isolates exhibited significantly different profiles of serotype and antimicrobial susceptibility when compared to the animal isolates, suggesting that the infection potential of different serotypes may vary significantly. For instance, several serotypes commonly found among human clinical isolates, such as *S. Senftenberg* and *S. Weltevreden*, were not recoverable in all animal specimens tested. Since these serotypes are prevalent in food samples in Western countries [37, 38], the possibility that some clinical strains of such serotypes in China originated from imported foods cannot be ruled out. In this work, animal isolates generally exhibited a higher rate of resistance to most antimicrobial compounds. We hypothesize that this phenotypic discrepancy between animal and human clinical isolates may be related to the difference in serotype patterns of animal and human clinical isolates, and may to some extent be due to the reduced physiological fitness of the more resistant animal isolates, which may have limited their abilities to infect humans among the same serotype of *Salmonella* [39, 40]. From another viewpoint, the high prevalence of antibiotic resistance in zoonotic *Salmonella* strains infers that organisms which end up causing infections in humans are likely to be both drug...
resistant and physiologically fit, presumably due to acquisition of compensatory mutations [41]. In this work, several common serotypes of animal isolates, such as S. Cremieu, S. Fyris and S. Give, were not found among human clinical isolates. We postulate that the low infection rate of these serotypes may be due to their low infection potential or different host specificity. On the other hand, human Salmonella isolates that do not share the same PFGE patterns as animal isolates may originate from other food sources that were not tested in this study. Nevertheless, S. Enteritidis and S. Typhimurium, which were prevalent in both animal and human clinical isolates, exhibited similar rate of resistance to the test antimicrobials. This finding might suggest that human infections caused by Salmonella strains of these two serotypes mainly originated from animals. This idea is further supported by the finding that several PFGE patterns of animal S. Enteritidis and S. Typhimurium clinical isolates constituted the most prevalent PFGE types among human clinical isolates.

Currently, the efficiency by which a newly emerged strain is transmitted among animal and humans is not known. By screening the oqxAB gene in both animal and human isolates, we confirm that this gene started to emerge in both groups from 2006 onwards, with similar prevalence rate being recorded among all the four serotypes, which were commonly found in both animal and human clinical isolates. These data indicated that animal isolates can be spontaneously transmitted from animals to humans or vice versa, bringing along the new genetic features that they have acquired. To summarize, findings in this work suggest that animal-borne Salmonella strains, which have the highest potential to cause human infections in China are those which reside in chicken, belong to the serotypes of Enteritidis or Typhimurium, and exhibit moderate to high rate of resistance to antibiotics.

**Declarations**

**Author contribution statement**

Congming Wu: Conceived and designed the experiments; Performed the experiments.

Meiying Yan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Lizhang Liu: Performed the experiments; Analyzed and interpreted the data.

Jing Lai: Performed the experiments; Analyzed and interpreted the data.

Edward Wai-chi Chan: Analyzed and interpreted the data; Wrote the paper.

Sheng Chen: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.
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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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