Prevalence and Characterization of Monophasic Salmonella Serovar 1,4,[5],12:i:- of Food Origin in China

Xiaojuan Yang1,2, Qingping Wu1,2*, Jumei Zhang1,2, Jiahui Huang1,2, Weipeng Guo1,2, Shuzhen Cai1,2
1 Guangdong Institute of Microbiology, State Key Laboratory of Applied Microbiology Southern China, Guangzhou, China, 2 Guangdong Provincial Key Laboratory of Microbial Culture Collection and Application, Guangdong Open Laboratory of Applied Microbiology, Guangzhou, China

* wuqp203@163.com

Abstract

Salmonella enterica subsp. enterica serovar 1,4,[5],12:i:- is a monophasic variant of Salmonella Typhimurium, which has recently been recognized as an emerging cause of infection worldwide. This bacterium has also ranked among the four most frequent serovars causing human salmonellosis in China. However, there are no reports on its contamination in Chinese food. Serotyping, polymerase chain reaction, antibiotic resistance, virulotyping, and multilocus sequence typing (MLST) assays were used to investigate the prevalence of this serological variant in food products in China, and to determine phenotypic and genotypic difference of monophasic isolates and Salmonella Typhimurium isolated over the same period. Salmonella 1,4,[5],12:i:- was prevalent in various food sources, including beef, pork, chicken, and pigeon. The study also confirmed the high prevalence (53.8%) of resistance to ampicillin, streptomycin, sulfonamides, and tetracycline in Salmonella 1,4,[5],12:i:-, which was higher than that in Salmonella Typhimurium. Moreover, Salmonella 1,4,[5],12:i:- isolates in our study were different from Salmonella Typhimurium isolates by the absence of three plasmid-borne genes (spvC, pefA, and rck) and the presence of gipA in all isolates. All Salmonella 1,4,[5],12:i:- isolates demonstrated MLST pattern ST34. Genomic deletions within the fljBA operon and surrounding genes were only found in Salmonella 1,4,[5],12:i:- isolates, with all isolates containing a deletion of fljB. However, hin and iroB were identified in all Salmonella 1,4,[5],12:i:- isolates. Three different deletion profiles were observed and two of them were different from the reported Salmonella 1,4,[5],12:i:- clones from Spain, America, and Italy, which provided some new evidence on the independent evolution of the multiple successful monophasic clones from Salmonella Typhimurium ancestors. This study is the first report of Salmonella 1,4,[5],12:i:- in food products from China. The data are more comprehensive and representative, providing valuable information for epidemiological studies, risk management, and public health strategies.
Introduction

*Salmonella* are often acquired from contaminated food, and are important causes of gastroenteritis and bacteremia, posing a worldwide threat to public health [1]. There are estimated to be 94 million cases of gastroenteritis globally per year, with 155,000 deaths attributed to *Salmonella* [2,3].

Since 2009, *Salmonella enterica* subsp. *enterica* serovar 1,4,[5],12:i:- has ranked among the four most frequent serovars causing human salmonellosis in China [2]. It is also one of the most common serovars isolated from humans and foods in several other countries [4,5]. This atypical serovar, lacking the phase 2 flagellar antigen [6,7], has become increasingly important since the mid-1990s worldwide. Currently, it is designated as a monophasic variant of *Salmonella* Typhimurium, with antigenic and genotypic similarities [6,8–10].

The mechanisms for the evolution of *Salmonella* 1,4,[5],12:i:- remain unknown. Several genes, including *fljB*, *fljA*, and *hin*, located within the *fljBA* operon, are involved in expression of phase 2 flagellar antigen. *fljB* encodes the phase 2 flagellar antigen, while *fljA* (encoding a negative regulator of *fliC*, coding for the phase 1 flagellar antigen) and *hin* (encoding a DNA invertase) are responsible for flagellar phase variation, a regulatory mechanism involving switching between the production of FliC or FljB [5]. Thus, mutations, deletions, or insertions of *fljB* itself, along with disorder in the regulatory mechanism of phase variation, could result in failure to express the phase 2 flagellar antigen [7,10–11]. Interestingly, various *fljB* deletion profiles, as well as deletions within other genes of the *fljBA* operon and surrounding genes (*iroB* and STM2757), have been detected [10,12–14].

Moreover, the public health impact of *Salmonella* 1,4,[5],12:i:- infection is increased by the high rate of antimicrobial resistance associated with this serovar. In particular, an epidemic multidrug-resistant (MDR) clonal lineage of *Salmonella* 1,4,[5],12:i:-, designated the European clone, which is resistant to ampicillin, streptomycin, sulfonamides, and tetracycline (R-type ASSuT) [15], has emerged in Europe and has been implicated in several outbreaks [16–18]. Some *Salmonella* 1,4,[5],12:i:- isolates from Spain, America, and Canada with additional resistance to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline (R-type ACSSuT) [6,19,20] have also been detected.

Several previous studies have examined *Salmonella* 1,4,[5],12:i:- from human cases of food poisoning in China [2,21]; however, systematic data for the prevalence of *Salmonella* 1,4,[5],12:i:- in the food chain is lacking. Therefore, the objective of this study was to characterize *Salmonella* 1,4,[5],12:i:- isolates collected from food samples across China from 2011–2014, and compare these isolates with *Salmonella* Typhimurium isolated in the same period to better understand the prevalence, phenotypic and genetic diversity, and susceptibility to antimicrobials of this serovar in China. Such data will provide a scientific basis for the development of food safety regulations.

Materials and Methods

Bacterial strains

From July 2011 to May 2014, 2800 food samples were collected from retail markets; these included meat and meat products (639), aquatic products (554), quick-frozen food (316), edible mushrooms (270), vegetables (272), dairy products (186), fruit (10), and ready-to-eat food (553). The sampling sites were distributed throughout the 24 provincial capitals of China (S1 Fig). In total, 524 *Salmonella* isolates were obtained and identified to serovar level according to the Kauffmann-White scheme [22]. For monophasic *Salmonella* serovar 1,4,[5],12:i:-, the absence of phase 2 flagellar antigen was verified using the flagellar phase reversal method as
described by Chiou et al [23]. Strains were definitively assigned to *Salmonella Typhimurium* or *Salmonella 1,4,[5],12:i:-* using the duplex-PCR assay developed by Tennant et al [24] and recommended by the European Food Safety Authority (EFSA) Panel on Biological Hazards [4]. The malic acid dehydrogenase gene (*mdh*), which is specific to *Salmonella Typhimurium* and *Salmonella 1,4,[5],12:i:-*, was also detected using primers previously described [8]. Ratios of *Salmonella 1,4,[5],12:i:-* occurrence were calculated against all serotyped *Salmonella* or *Salmonella Typhimurium*Typhimurium isolated from the same batch of food samples in the same period [11].

**Antimicrobial susceptibility testing**

Antimicrobial susceptibility was evaluated using the Kirby–Bauer disk diffusion method with 20 antimicrobial agents, in accordance with the Clinical Laboratory Standards Institute guidelines [25]. The antimicrobials used were ampicillin, amoxicillin-clavulanic acid, cephalothin, cefazolin, cefoxitin, ceftriaxone, cefotaxime, ceftazidine, cefoperazone, cefepime, chloramphenicol, tetracycline, nalidixic acid, ciprofloxacin, amikacin, gentamicin, streptomycin, kanamycin, trimethoprim-sulfamethoxazole, and sulfonamides (Oxoid, Basingstoke, UK).

**Detection of virulence-related genes**

Seven genes with reported contributions to virulence were selected. Four targets (*gipA, sodC1, sopE1, spsH1*) were located on prophages, while three (*spvC, pefA, rck*) were located on a virulence plasmid. Amplification was performed using primers and conditions described previously [26,27]. *Salmonella Typhimurium* ATCC14028 and *Salmonella Typhimurium* CMCC50115 were used as positive controls.

**Multilocus sequence typing (MLST)**

*Salmonella* isolates were characterized by MLST using previously reported primers specific for seven housekeeping genes: *aroC, dnaN, hemD, hisD, purE, sucA*, and *thrA*. The sequence type (ST) of each isolate was assigned according to the MLST database (http://mlst.ucc.ie/mlst).

**PCR-based characterization of gene deletion profiles of *Salmonella* isolates**

For all *Salmonella Typhimurium* and *Salmonella 1,4,[5],12:i:-* isolates, the presence of *fljA, fljB*, and *hin*, as well as *iroB*, which flanks the *fljBA* operon, and the closely located STM2757 and STM2758 genes, were analyzed using gene-specific primers and PCR conditions as previously described by Soyer et al [10] and García et al [12]. *fljA, fljB, hin*, and *iroB* are located at the 3′ end of cluster V, while STM2757 and STM2758 flank the 5′ end. This region spans 16 genes (from STM2758 to *iroB*) that were previously reported to be present in *Salmonella Typhimurium* LT2, but absent in a selection of Spanish *Salmonella 1,4,[5],12:i:-* isolates [28].

**Results**

As shown in [S1 Table](#) and [S2 Fig](#), serotyping, including flagellar phase reversal, identified 13 *Salmonella* isolates lacking phase 2 flagellar antigen. These *Salmonella 1,4,[5],12:i:-* isolates originated from beef (*n* = 6), pork (*n* = 5), chicken (*n* = 1), and pigeon (*n* = 1). All isolates produced a single 1,000-bp amplicon corresponding to a fragment of the IS200 element, typical of *Salmonella 1,4,[5],12:i:-*. The control group of 58 *Salmonella Typhimurium* isolates were also confirmed, with 55 isolates producing two amplicons (1,000-bp and 1,389-bp), which is specific for *Salmonella Typhimurium*, and the remaining three being positive for the 1,389-bp phase 2 flagellar gene but negative for the 1,000-bp IS200 fragment. Positive detection of *mdh*...
confirms the presence of *Salmonella* Typhimurium or its monophasic variant *Salmonella* 1,4, [5],12:i:-. In the current study, *mdh* was detected in all *Salmonella* 1,4, [5],12:i:- and *Salmonella* Typhimurium isolates. The overall *Salmonella* 1,4, [5],12:i:- occurrence ratio reached 2.5% (13/524), whereas *Salmonella* 1,4, [5],12:i:-/Salmonella Typhimurium ratio was 22.4% (13/58) in the period of 2011 to 2014.

All 71 of the *Salmonella* isolates examined in this study showed some level of antibiotic resistance. In total, 76.9% of *Salmonella* 1,4, [5],12:i:- and 58.6% of *Salmonella* Typhimurium isolates were resistant to three or more antimicrobials (Table 1). Among these antibiotics, high rates of resistance were observed for tetracycline, sulfonamides, ampicillin, nalidixic acid, streptomycin, kanamycin, trimethoprim-sulfamethoxazole, and chloramphenicol in both *Salmonella* 1,4, [5],12:i:- and *Salmonella* Typhimurium isolates (S2 and S3 Tables). The main antimicrobial resistance patterns of the 13 *Salmonella* 1,4, [5],12:i:- and 58 *Salmonella* Typhimurium isolates are shown in Table 1.

The 13 *Salmonella* 1,4, [5],12:i:- isolates showed highly similar virulence gene profiles. All isolates contained *gipA* (encoding a Peyer’s patch-specific virulence factor) and *sodC1* (putative Cu/Zn superoxide dismutase), carried by the Gifsy-1 and Gifsy-2 lambdoid prophages, respectively, while *sspH1* (encoding a *Salmonella* type III effector protein), located in Gifsy-3, and three genes associated with the *Salmonella* virulence plasmid (*spvC*, *pefA*, and *rck*) were absent. However, three *Salmonella* 1,4, [5],12:i:- isolates shared a different virulence gene profile, and were positive for *sopE1* (Salmonella type III effector protein). Overall, the prevalence of each of the virulence genes was similar between the *Salmonella* 1,4, [5],12:i:- and *Salmonella* Typhimurium isolates, except for the absence of the three plasmid genes and presence of *gipA* in all of the *Salmonella* 1,4, [5],12:i:- isolates (Tables 2 and 3).

Among the 13 *Salmonella* 1,4, [5],12:i:- and 58 *Salmonella* Typhimurium isolates, five distinct STs were identified based on a seven-gene MLST scheme (Table 4). The number of alleles for the seven housekeeping genes ranged from 2–554. *hisD554* and ST1922 were identified as a novel allele type and a novel MLST pattern, respectively. Two STs (ST19 and ST34) were predominant, and together accounted for 94.4% of isolates tested. ST19 was the most common *Salmonella* Typhimurium ST, accounting for 44 of 58 isolates. All the *Salmonella* 1,4, [5],12:i:- isolates were assigned to ST34, a single-locus variant of ST19 (with *dnaN19* instead of *dnaN7*); 10 *Salmonella* Typhimurium isolates were also designated ST34. One *Salmonella* Typhimurium isolate was identified as ST36, with two further isolates assigned to ST1544. These three isolates also differed from other *Salmonella* Typhimurium isolates based on duplex-PCR results (positive for the 1,389-bp phase 2 flagellar gene, but negative for the 1,000-bp IS200 fragment).

Table 1. Antimicrobial resistance patterns of *Salmonella* 1,4, [5],12:i:- and *Salmonella* Typhimurium isolates.

| R-types                  | *Salmonella* 1,4, [5],12:i:-, n (%) | *Salmonella* Typhimurium, n (%) |
|-------------------------|------------------------------------|-------------------------------|
| ASSuT± others           | 7 (53.8%)                          | 13 (22.4%)                    |
| ACSSuT± others          | 3 (23.1%)                          | 7 (12.1%)                     |
| Pansusceptible          | 0 (0.0%)                           | 0 (0.0%)                      |
| ≥ 1 Antimicrobial       | 13 (100.0%)                        | 56 (96.6%)                    |
| ≥ 3 Antimicrobial       | 10 (76.9%)                         | 34 (58.6%)                    |
| Total                   | 13 (100.0%)                        | 58 (100.0%)                   |

aASSuT resistance phenotype (ampicillin-streptomycin-sulfonamides-tetracycline);
bACSSuT resistance phenotype (ampicillin-chloramphenicol-streptomycin-sulfonamides-tetracycline).

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Genomic deletions within the fljBA operon were only found in *Salmonella* 1,4,[5],12:i:- isolates. The 13 *Salmonella* 1,4,[5],12:i:- isolates showed three different deletion profiles for cluster V, namely ΔfljAB-I, ΔfljAB-II, and ΔfljAB-III. ΔfljAB-I and ΔfljAB-II were the most common deletion profiles. ΔfljA was absent and hin was present in all *Salmonella* 1,4,[5],12:i:- isolates. However, fljA was present in only one isolate (deletion profile ΔfljAB-III). The deletion profiles ΔfljAB-I and ΔfljAB-II were marked by the presence and absence of STM2757 and STM2758, respectively (Table 5 and S3 Fig).

### Discussion

This study confirmed the presence of *Salmonella* 1,4,[5],12:i:- in food products in China. The *Salmonella* isolates examined in this study were collected from a nationwide food investigation covering most provincial capitals of China. Thus, the results were fairly comprehensive and representative of China as a whole. The occurrence of *Salmonella* 1,4,[5],12:i:- was higher in our study compared with other countries [11,29].

Previous studies have suggested that the vast majority of these monophasic *Salmonella* strains are from pigs and pork products [30,31], followed by isolates obtained from poultry and cattle. Isolation from other sources was believed to be rare [9,32]. In our study, although *Salmonella* 1,4,[5],12:i:- isolates were obtained from various sources, beef and pork products provided 84.6% of all tested isolates, proving to be the most important reservoirs in China.

The prevalence of MDR isolates observed in our surveillance of *Salmonella* Typhimurium (58.6%) and *Salmonella* 1,4,[5],12:i:- (76.9%) is similar to results from previous studies conducted in humans in China [2,21], and to rates of resistance reported in other countries [11,33]. The current study confirmed the prevalence of R-type ASSuT *Salmonella* Typhimurium isolates, with an even higher prevalence of this phenotype amongst *Salmonella* 1,4,[5],12:

### Table 2. Virulotyping: most commonly detected haplotypes.

| Serotypes            | Haplotypes                              | Number of strains |
|----------------------|-----------------------------------------|-------------------|
| *Salmonella* 1,4,[5],12:i:- | gipA; sodC1*                           | n = 10 (ST34)     |
|                      | gipA; sodC1; sopE1                      | n = 3 (ST34)      |
| *Salmonella* Typhimurium | gipA; sodC1; spvC; pefA; rck            | n = 33 (ST19); n = 2 (ST1544); n = 1 (ST1922) |
|                      | gipA; sodC1*                           | n = 10 (ST34); n = 2 (ST19) |
|                      | sodC1*                                  | n = 7 (ST19)      |
|                      | sodC1; spvC; pefA; rck                  | n = 2 (ST19)      |
|                      | sodC1; sopE1                           | n = 1 (ST36)      |

*Identical haplotypes.

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### Table 3. Prevalence (%) of the virulence genes by serotype.

| Serotypes            | gipA | sodC1 | sopE1 | sspH1 | spvC | pefA | rck |
|----------------------|------|-------|-------|-------|------|------|-----|
| *Salmonella* 1,4,[5],12:i:- | 100.0% | 100.0% | 23.1% | 0.0%  | 0.0% | 0.0% | 0.0% |
| *Salmonella* Typhimurium | 82.8% | 100.0% | 1.7%  | 0.0%  | 65.5%| 65.5%| 65.5%|

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i:- isolates. In addition, several isolates displayed an R-type ACSSuT phenotype (Table 1). R-type ASSuT was also the most common profile among Salmonella 1,4,[5],12:i:- strains isolated in Germany from pigs, pork products, and humans [30], as well as among strains isolated from humans and foodstuffs in England, France, Germany, Italy, Spain, Switzerland, and the Netherlands [1,34]. The emergence and spread of the R-type ASSuT Salmonella 1,4,[5],12:i:- clonal lineage deserves further attention as it has been responsible for several foodborne Salmonella outbreaks, such as in Luxembourg in 2006 [18] and in Italy in 2010 [16], which can be associated with severe diseases, even deaths.

Most of the virulence genes with reported contributions to pathogenicity are located in Salmonella pathogenicity islands (SPIs), fimbrial clusters, plasmids and prophages. SPIs and fimbrial clusters are highly conserved regions, while prophages and plasmids are variable within the Salmonella genome. Some differences were observed in the genes located on prophages (gipA, sodC1, sopE1, sspH1) and plasmids (spvC, pefA, rck) when comparing virulence gene profiles from Salmonella Typhimurium and Salmonella 1,4,[5],12:i:- in previous studies [12,26]. In our study, three plasmid-borne virulence genes (spvC, pefA, and rck) were highly prevalent amongst Salmonella Typhimurium isolates, while none of the Salmonella 1,4,[5],12:i:- isolates contained these genes. This is consistent with the findings of Capuano et al [26]. The absence of these plasmid-borne virulence genes has been reported previously in R-type ASSuT isolates. In addition, several isolates displayed an R-type ACSSuT phenotype (Table 1). R-type ASSuT was also the most common profile among Salmonella 1,4,[5],12:i:- strains isolated in Germany from pigs, pork products, and humans [30], as well as among strains isolated from humans and foodstuffs in England, France, Germany, Italy, Spain, Switzerland, and the Netherlands [1,34]. The emergence and spread of the R-type ASSuT Salmonella 1,4,[5],12:i:- clonal lineage deserves further attention as it has been responsible for several foodborne Salmonella outbreaks, such as in Luxembourg in 2006 [18] and in Italy in 2010 [16], which can be associated with severe diseases, even deaths.

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### Table 4. Allelic profiles and MLST types of the 71 Salmonella isolates.

| Serotypes (no. of isolates) | Allelic types | MLST patterns |
|-----------------------------|---------------|---------------|
|                             | aroC          | dnaN          | hemD | hisD | purE | sucA | thrA |               |
| Salmonella Typhimurium (44) | 10            | 7             | 12   | 9    | 5    | 9    | 2    | ST19          |
| Salmonella Typhimurium (10); Salmonella 1,4,[5],12:i:- (13) | 10            | 19            | 12   | 9    | 5    | 9    | 2    | ST34          |
| Salmonella Typhimurium (1)  | 10            | 7             | 12   | 554* | 5    | 9    | 2    | ST1922*²     |
| Salmonella Typhimurium (2)  | 10            | 7             | 12   | 230  | 5    | 9    | 2    | ST1544        |
| Salmonella Typhimurium (1)  | 18            | 14            | 12   | 9    | 5    | 18   | 21   | ST36          |

²Asterisks denote novel alleles and novel MLST patterns.

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### Table 5. Gene deletion profiles and types for the 13 Salmonella 1,4,[5],12:i:- isolates.

| Isolates | Target genes | Detection types |
|----------|--------------|-----------------|
|          | hin fljB fljA iroB STM2758 STM2757 |
| 2011−33  | + - - + + + | ΔfljAB-I       |
| 2011−34  | + - - + + + | ΔfljAB-I       |
| 2012−35  | + - - + + - | ΔfljAB-II      |
| 2012−36  | + - - + + + | ΔfljAB-I       |
| 2012−37  | + - - + + - | ΔfljAB-II      |
| 2013−45  | + - - + - - | ΔfljAB-II      |
| 2013−50  | + - - + - - | ΔfljAB-I       |
| 2013−51  | + - - + + + | ΔfljAB-II      |
| 2013−52  | + - - + + + | ΔfljAB-I       |
| 2014−54  | + - - + - - | ΔfljAB-II      |
| 2013−57  | + - - + - - | ΔfljAB-II      |
| 2013−58  | + - - + - - | ΔfljAB-II      |
| 2013−62  | + - - + - - | ΔfljAB-II      |

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Salmonella 1,4,[5],12:i:- strains of the European clone. In contrast, these genes have been identified in some Spanish clone Salmonella 1,4,[5],12:i:- strains, which are characterized by plasmid-mediated resistance to up to seven antimicrobial drugs, including ampicillin, chloramphenicol, gentamicin, streptomycin, sulfonamides, tetracyclines, and trimethoprim (R-type ACGSSuTtp) [12]. In addition, Capuano et al [26] also detected gipA in all Salmonella 1,4,[5],12:i:- isolates of food origin, but in only 76.9% of Salmonella Typhimurium isolates. Another aspect to consider is that the detection of the target virulence genes showed no relationship to the serotype (Salmonella Typhimurium or Salmonella 1,4,[5],12:i:-), while some correlation with the MLST pattern was observed. All ST34 Salmonella Typhimurium isolates, and a large number (76.9%) of the ST34 Salmonella 1,4,[5],12:i:- isolates, exhibited a virulence gene profile of “gipA; sodC1”.

All Salmonella 1,4,[5],12:i:- isolates in the present study were assigned to ST34, sharing identical STs with Salmonella Typhimurium isolates, which further confirmed that Salmonella 1,4,[5],12:i:- and Salmonella Typhimurium were genetically closely related. Furthermore, these data suggested that Salmonella Typhimurium strains of ST34 might be ancestral candidates for serotype 1,4,[5],12:i:- from China. ST34 has previously been associated with R-type ASSuT Salmonella 1,4,[5],12:i:- strains belonging to the European clone [15,35]. In contrast, R-type ACGSSuTtp Salmonella 1,4,[5],12:i:- strains of the Spanish clone have been assigned to ST19 [12,35]. In the current study, Salmonella 1,4,[5],12:i:- isolates represented only one ST, while Salmonella Typhimurium isolates belonged to five different STs. This indicated considerably lower sequence diversity amongst Salmonella 1,4,[5],12:i:- than Salmonella Typhimurium. This observation has also been reported by Guerra et al [36] and Soyer et al [10].

All Salmonella 1,4,[5],12:i:- isolates examined in the current study exhibited deletions in the fljBA operon and flanking genes, although variations were identified. In previous studies, various deletion profiles affecting the fljBA operon and surrounding genes have been observed in Salmonella 1,4,[5],12:i:- strains. Most Salmonella 1,4,[5],12:i:- isolates from Spain appear to be characterized by deletions of fljA, fljB, hin, and iroB and conservation of STM2757 [12,13,28], whereas some of the American Salmonella 1,4,[5],12:i:- isolates seem to contain deletions that eliminate fljA, fljB, and STM2757 but maintain hin and iroB [7,10]. The deletion profile of Salmonella 1,4,[5],12:i:- strains from Italy is characterized by the absence of the fljA, fljB, and hin genes and the presence of STM2757, as in Spanish strains, but a conserved iroB, as with the American strains [14]. Among the isolates described in this work, hin and iroB were always conserved. The ΔfljAB-II deletion profile was identical to the American Salmonella 1,4,[5],12:i:- strains, whereas the ΔfljAB-I and ΔfljAB-III profiles were newly described for Salmonella 1,4,[5],12:i:- strains. This observed variation supported the independent evolution of multiple successful monophasic clones from Salmonella Typhimurium ancestors [5]. Sequencing of the entire fljBA operon and flanking genes in future studies could give better insight into the genetic background of such variants.

Conclusions

This study is the first report of Salmonella 1,4,[5],12:i:- in food products from China. The main sources of Salmonella 1,4,[5],12:i:- in China appear to be pork and beef. The examined Salmonella 1,4,[5],12:i:- strains were mainly ST34, confirming the domination of the tetra-resistant ASSuT clone. The observed genomic deletion of the fljBA operon and its flanking genes in Salmonella 1,4,[5],12:i:- isolates revealed that multiple clones of this serovar are circulating in China, and several new clones were identified. Further studies are needed to assess these Salmonella 1,4,[5],12:i:- clones in more detail to obtain a better picture of the genetic background and relationship with Salmonella Typhimurium clones circulating in China.
Supporting Information

S1 Fig. Map of China illustrating provinces and cities included in the study. (TIF)

S2 Fig. Identification of 13 monophasic *Salmonella 1,4,[5],12:i:-* and 58 biphasic *Salmonella Typhimurium* isolates by duplex-PCR. The isolates produced a single 1,000-bp amplicon corresponding to a fragment of the IS200 element were assigned to *Salmonella 1,4,[5],12:i:-* (lanes 35–39, 46, 51–53, 55, 58, 59, 62). The isolates produced two amplicons (1,000-bp and 1,389-bp) (lanes 2–23, 26–28, 30–34, 40–45, 47, 50, 54, 56, 57, 60, 63, 64, 66–70, 73–74, 76–79) or a single 1,389-bp amplicon (lanes 29, 61, 75) were assigned to *Salmonella Typhimurium*. M, DL2,000 DNA Marker. Lanes 1, 25, 49, 65, 72, *Salmonella Typhimurium* ATCC14028 reference strain; lanes 24, 48, 71, 80, water-only control. (TIF)

S3 Fig. Genomic deletion analysis of cluster V by PCR using two reference strains and 13 *Salmonella 1,4,[5],12:i:-* isolates. M, DL2,000 DNA Marker. C, water-only control. Lane 1, *Salmonella Typhimurium* ATCC14028 reference strain; lane 2, *Salmonella Typhimurium* CMCC50115 reference strain; lane 3, isolate 2011–33; lane 4, isolate 2011–34; lane 5, isolate 2012–35; lane 6, isolate 2012–36; lane 7, isolate 2012–37; lane 8, isolate 2013–45; lane 9, isolate 2013–50; lane 10, isolate 2013–51; lane 11, isolate 2013–52; lane 12, isolate 2014–54; lane 13, isolate 2013–57; lane 14, isolate 2013–58; lane 15, isolate 2013–62. a) *fljB*, DNA fragments of the expected sizes for *fljB* (lanes 1 and 2), no amplicon (lanes 3–15); b) *hin*, DNA fragments of the expected sizes for *hin* (lanes 1–15); c) *fljA*, DNA fragments of the expected sizes for *fljB* (lanes 1, 2, and 10), no amplicon (lanes 3–9, 11–15); d) *iroB*, DNA fragments of the expected sizes for *iroB* (lanes 1–15); e) STM2757, DNA fragments of the expected sizes for STM2757 (lanes 1–4, 6, 7, 9–10), no amplicon (lanes 5, 8, 12–15); f) STM2758, DNA fragments of the expected sizes for STM2758 (lanes 1–4, 6, 7, 9–10), no amplicon (lanes 5, 8, 12–15). (TIF)

S1 Table. Results of serotyping, antimicrobial resistance, virulotyping and MLST analysis of *Salmonella* isolates in this study. aFor antimicrobial abbreviation, ampicillin (AMP), amoxicillin-clavulanic acid (AMC), cephalothin (KF), cefazolin (KZ), cefoxitin (FOX), ceftriaxone (CRO), cefotaxime (CTX), ceftazidime (CAZ), cefoperazone (CFP), cefepime (FEP), chloramphenicol (C), tetracycline (TE), nalidixic acid (NA), ciprofloxacin (CIP), amikacin (AK), gentamicin (CN), streptomycin (S), kanamycin (K), trimethoprim-sulfamethoxazole (SXT), and sulfonamides (Su). Names of antimicrobials with capital letters mean resistance; Names of antimicrobials with lowercase letters mean intermediate resistance; [teta], a tetra-resistant pattern including resistance to ampicillin, streptomycin, sulfonamide, and tetracycline (ASSuT R-type); [penta], a penta-resistant pattern including resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline (ACSSuT R-type). bVP1, virulence gene profile: *gipA; sodC1; spvC; pefA; rck.* (DOC)

S2 Table. Antimicrobial resistance profiles of *Salmonella 1,4,[5],12:i:-* isolates examined in this study. (DOC)

S3 Table. Antimicrobial resistance profiles of *Salmonella Typhimurium* isolates examined in this study. (DOC)
Author Contributions
Conceived and designed the experiments: XJY QPW JMZ. Performed the experiments: XJY JHH. Analyzed the data: XJY JHH. Contributed reagents/materials/analysis tools: XJY JMZ. Performed the experiments: XJY.

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