Serotypes, antibiotic resistance, and virulence genes of *Salmonella* in children with diarrhea

Meina Yue | Xiaoyu Li | Di Liu | Xue Hu

Hangzhou Children’s Hospital, Hangzhou, China

Correspondence
Xiaoyu Li, Hangzhou Children’s Hospital, Hangzhou, China.
Email: jsr9618@163.com

**Abstract**

**Background:** *Salmonella* is an important foodborne pathogen that causes acute diarrhea in humans worldwide. This study analyzed the relationships of serotypes and antibiotic resistance with virulence genes of *Salmonella* isolated from children with salmonellosis.

**Methods:** Serological typing was performed using the slide-agglutination method. The Kirby-Bauer disk diffusion method was used to test antibiotic susceptibility. Twenty virulence genes were detected by PCR.

**Results:** *Salmonella Typhimurium* (21 isolates, 34.43%) and *S Enteritidis* (12 isolates, 19.67%) were the predominant species among the 61 isolates. Ampicillin resistance was most common (63.93%), and among the cephalosporins, resistance was most often found to cefotaxime, a third-generation cephalosporin (19.67%). Among the 20 virulence genes, *prgH*, *ssrB*, and *pagC* were detected in all *Salmonella* isolates. In *S Typhimurium*, the detection rates of *hilA*, *sipB*, *marT*, *mgtC*, *sopB*, *pagN*, *nlpI*, *bapA*, *oafA*, and *tolC* were high. In *S Enteritidis*, the detection rates of *icmF*, *spvB*, *spvR*, and *pefA* were high. Nitrofurantoin resistance was negatively correlated with the virulence gene *bapA* ($P = .005$) and was positively correlated with *icmF*, *spvB*, *spvR*, and *pefA* ($P = .012$, $.008$, $.002$, and $.005$, respectively). The $P$ values between all other virulence genes and antibiotic resistance were >.05.

**Conclusion:** *Salmonella Typhimurium* and *S Enteritidis* were the main serotypes in children with diarrhea in Hangzhou, China. *Salmonella* exhibited a high level of resistance to common antibiotics, and a high rate of bacteria carrying virulence genes was observed. However, no significant correlation was found between virulence genes and resistance to common antibiotics.

**Keywords**

antibiotic resistance, children, *Salmonella*, serotype, virulence genes
Salmonella is a genus of Gram-negative bacteria of the family Enterobacteriaceae and is an important foodborne pathogen that causes acute diarrhea in humans worldwide. It is estimated that approximately 93.8 million people are infected with Salmonella every year worldwide, resulting in nearly 155,000 deaths. In the United States, Salmonella is a major biological factor that causes bacterial foodborne infections. In China, 70%-80% of food poisoning incidents are caused by Salmonella.

Salmonella is widely found in nature and has numerous serotypes. To date, more than 2500 serotypes have been identified, of which more than 20 can cause zoonoses, and the harmful species include S Typhimurium, S Enteritidis, and S Choleraesuis. The pathogenicity of Salmonella is mainly related to the virulence factors that it carries, including Salmonella pathogenicity islands (SPIs), virulence plasmids, pili, and enterotoxins. Research on virulence genes has become an

| Location | Primer name | Primer sequence (5′-3′) | Length (bp) | Hybridization temperature and references |
|----------|-------------|-------------------------|-------------|------------------------------------------|
| SPI-1    | hilA-F      | GACAGAGCTGGACCACAATAAGACA | 312         | 55°C\(^8\)                              |
|          | hilA-R      | GAGCGTATTCTATGCGCTAAC    |             |                                          |
| SPI-1    | sipB-F      | GGACGCCCGCCGGAGAAATTCTCT | 875         | 66.5°C\(^6\)                            |
|          | sipB-R      | AACCTCCCGTGCGCCGCTTCAACAA |             |                                          |
| SPI-1    | prgH-F      | CTTCCAGGYCAAATCTCCGTGATAC | 961         | 55°C\(^5\)                              |
|          | prgH-R      | CCGTTGAGCCGCTATCTT       |             |                                          |
| SPI-2    | ssrB-F      | CTCTTATTCTGGGCAGCTTTA    | 558         | 55°C\(^5\)                              |
|          | ssrB-R      | CTTATTCATCGTGGCTTCACTTT  |             |                                          |
| SPI-3    | marT-F      | CTTGCGTCTCAACAAAAACGTT  | 556         | 55°C\(^5\)                              |
|          | marT-R      | CTGACAATCAATGCGCTAAC     |             |                                          |
| SPI-3    | mgtC-F      | AAAGACTAGGCGCCATGATAG    | 500         | 65°C\(^7\)                              |
|          | mgtC-R      | TTTTTTTATGCCCTGGTCTCGAC  |             |                                          |
| SPI-4    | siiD-F      | GTACGAGCGCTTTACTACAAA    | 826         | 55°C\(^8\)                              |
|          | siiD-R      | TTGACATCGCGCGCATAG       |             |                                          |
| SPI-5    | sopB-F      | TCACTAAAAACCCAGAGCGTTTT  | 1000        | 65°C\(^7\)                              |
|          | sopB-R      | CGCCATCTTTATTCGGAGATTTTA |             |                                          |
| SPI-6    | pagN-F      | TTTCCAGCTTCAGGTAGTTAG    | 440         | 55°C\(^8\)                              |
|          | pagN-R      | GCCCTTTTGGTCATGAAAG      |             |                                          |
| SPI-7    | vexA-F      | AAACCAAGCCTCCCGATAC      | 504         | 55°C\(^8\)                              |
|          | vexA-R      | CAGTCCGCCAGTGAAATAAG     |             |                                          |
| SPI-8    | nplI-F      | AGTCCTGGTTGAGGCGATTAG    | 333         | 55°C\(^8\)                              |
|          | nplR-R      | TCTTCTCTGCTTTTCTACATT    |             |                                          |
| SPI-9    | bapA-F      | TAAAGCCTCGGACTGGAATG     | 543         | 55°C\(^8\)                              |
|          | bapA-R      | CGTCTCTCGCGGTAGGTATAG    |             |                                          |
| SPI-11   | pagC-F      | CGCTTTTGGGGGATAGGC       | 454         | 66.5°C\(^6\)                            |
|          | pagC-R      | GAAGCGGCTTATTTTGGAGGATGTT |           |                                          |
| SPI-12   | oafA-F      | CGAGTGACTGGAACAAAGA      | 510         | 55°C\(^8\)                              |
|          | oafA-R      | CAAGCATAGAGCGAGATAG     |             |                                          |
| SPI-19   | icmF-F      | GCCGTAGTCAGATGACCATAG    | 724         | 55°C\(^8\)                              |
|          | icmR-R      | GCGGCCAGATAGGATATTT      |             |                                          |
| Plasmid  | spvB-F      | CTATCGACCCCGGACGGAGCGCATTTTTTA | 717 | 66.5°C\(^6\)                              |
|          | spvB-R      | GGAGGGCGCGGTGGCGCAGCATATA |             |                                          |
| Plasmid  | pefA-F      | GCCGCCGTCAGCCGCAAACG     | 157         | 66.5°C\(^6\)                            |
|          | pefA-R      | GCACGAGAAGGCCGCAGAAGA    |             |                                          |
| Plasmid  | spvR-R      | CCGCTGAGCGGTTATTTT       | 723         | 55°C\(^8\)                              |
|          | spvR-R      | CTTGGCGGTAATACAGAGAG     |             |                                          |
| Genome   | cdtB-F      | ACAACTGTGCATCTCGCCCGGTCATT | 268        | 66.5°C\(^6\)                            |
|          | cdtB-R      | CAATTTTCGCTGGTTCTGTAGGTGCAGT |           |                                          |
| Genome   | tolC-F      | GCAGACCAGCTAGCTCAATAC    | 623         | 55°C\(^8\)                              |
|          | tolC-R      | TTGCCGCGACGACTTTT       |             |                                          |
important means to understand the pathogenicity of Salmonella. The same virulence genes have different effects on the pathogenicity of Salmonella from various sources, and the virulence genes carried by different serotypes of Salmonella also differ. Some data also show that the antibiotic resistance of an isolate is related to its virulence.

This study analyzed the relationships of serotypes and antibiotic resistance with virulence genes of Salmonella isolated from children with salmonellosis. The results provide scientific evidence to help understand the pathogenicity of salmonellosis in children and its treatments.

1 | MATERIALS AND METHODS

1.1 | Source of isolates

From 2013 to 2015, 61 Salmonella isolates were collected from the feces of children with acute diarrhea (daily defecation ≥3 times, altered fecal characteristics: loose stool, watery stool, and blood, mucus, or pus in the stool, duration ≤14 days) in the gastroenterology outpatient clinic of Hangzhou Children’s Hospital.

1.2 | Main reagents and equipment

The following equipment and reagents were used: Salmonella-Shigella (SS) agar plates; chromogenic plates for Salmonella screening (Shanghai Comagal Microbial Technology Co., Ltd.); a Vitek2 Compact Microbial Detection System (BioMérieux); DNA Engine polymerase chain reaction (PCR) amplifier; a Mini-Protean 3 electrophoresis system; Gel Doc XR + gel imaging system (Bio-Rad); primers (synthesized by Sangon Biotech Co., Ltd.); and 2 × Taq Master Mix and 1000 bp DNA marker (TaKaRa).

1.3 | Isolation, culture, and identification

Fresh fecal samples were inoculated on SS plates at 35°C for 18-24 hours. Suspicious colonies were cultured in CHROMagar selective culture medium and KIA at 37°C for 18-24 hours, and a Vitek 2 compact system (BioMérieux) was used to identify Salmonella isolates. The O and H antigens of Salmonella were identified according to the GB/T4789.4-2010 guidelines (Food Microbiological Examination: Salmonella), and the serotype was determined according to Kauffmann-White scheme. Normal saline was used as a control.

1.4 | Antibiotic susceptibility test

The Kirby-Bauer disk diffusion method was used to test antibiotic susceptibility. According to the standards provided by the American Clinical Laboratory Standards Institute (CLSI) document M100 in 2018 and the use of antibiotics in China, a total of 15 antibiotics were selected: cefotaxime, ceftriaxone, cefepime, ceftazidime, ceftizoxime, cefoperazone/sulbactam, ampicillin/sulbactam, ampicillin, piperacillin/tazobactam, trimethoprim-sulfamethoxazole, imipenem, aztreonam, ciprofloxacin, levofloxacin, and nitrofurantoin. For the extended-spectrum β-lactamase (ESBL) test, cefotaxime, ceftazidime/clavulanic acid, ceftazidime, and ceftazidime/clavulanic acid were selected. The specifications of the susceptibility paper, the interpretation of antibiotic susceptibility (resistant, intermediate, and susceptible), and the determination of ESBL test results followed the CLSI (2018) criteria. Escherichia coli ATCC25922 was used for quality control.

1.5 | Determination of virulence genes

The genomic DNA of each isolate was extracted by thermal lysis. Using the virulence gene-related primers reported in the literature, 20 virulence genes were detected by PCR (Table 1). A PCR mix (25 μl) was used, including 2 × Taq Master Mix (12.5 μl), upstream and downstream primers (10 μmol/L, 1.0 μL for each primer), template DNA (2.0 μl), and ddH2O (8.5 μL). PCR parameters: 94°C for 5 minutes; 30 cycles of 94°C for 45 seconds, 55-66.5°C for 45 seconds (Table 1), and 72°C for 1 minutes; and 72°C for 10 minutes. The amplified products were analyzed with 1.0% agarose gel electrophoresis, and the results were observed using the Gel Doc XR + gel imaging system. The results were confirmed by replicate experiments.

1.6 | Sequencing validation

Two to three positive PCR products of each virulence gene were randomly selected and sent to Sangon Biotech for gene sequencing. The sequencing results were confirmed using the National Center for Biotechnology Information/Basic Local Alignment Search Tool.

1.7 | Statistical analysis

The results of serological typing, virulence gene identification, and antibiotic susceptibility testing were entered into Excel for data analysis. SPSS 20.0 software was used for the statistical analysis. Fisher’s exact test was used for correlation analysis. A value of P < .05 was considered significant.

2 | RESULTS

2.1 | Isolate distribution characteristics

After biochemical identification and serological typing, 61 Salmonella isolates from children with acute diarrhea were collected. Salmonella Typhimurium (21 isolates, 34.43%) and S Enteritidis (12 isolates, 19.67%) were the predominant species, followed by S Stanley (five isolates, 8.20%), S Saint Paul (four isolates, 6.56%), S Derby (three isolates, 4.92%), and S Braenderup (three isolates, 4.92%).
were two isolates each of S Paratyphi B, S London, and S Reading, accounting for 9.84% (sum), and one isolate each of S Choleraesuis, S Anatum, S Tennessee, S Thompson, S Senftenberg, S Dublin, and S Paratyphi C, accounting for 11.48% (sum) (Table 2 and Figure 1).

2.2 | Antibiotic susceptibility of Salmonella isolates

The highest antibiotic resistance of Salmonella was found against ampicillin (63.93%), followed by ampicillin/sublactam (55.74%), and trimethoprim-sulfamethoxazole (39.34%). One isolate with antibiotic resistance (1.64%) was found for each of the following: cefoperazone/sublactam, piperacillin/tazobactam, and levofloxacin. No imipenem-resistant isolate was found. Among the cephalosporins, cefotaxime, a third-generation cephalosporin, had the highest rate of antibiotic resistance (19.67%). Cefotaxime and cefepime (a fourth-generation cephalosporin) had similar antibiotic resistance rates (8.20%). Eleven isolates were detected in ESBL test, resulting in a positive rate of 18.03% (Table 3 and Figure 1).

2.3 | Detection rate and distribution of virulence genes in various serotypes

The PCR results for the virulence genes of 61 Salmonella isolates from children with acute diarrhea are listed in Table 4. The detection rates of prgH, ssrB, and pagC were 100%. The detection rates of hilA, sipB, marT, mgtC, ssrB, sopB, pagN, nlpl, bapA, oafA, and tolC in Salmonella were high (45.90%-93.44%). The detection rates of icmF, spvB, spvR, and pefA were between 13.11% and 19.68%. The detection rate of cdtB was relatively low (4.92%), and vecA was only detected in one isolate of S Dublin.

Of the Salmonella isolates, 90.16% carried at least 10 virulence genes, and one isolate of S Dublin had 16 virulence genes. Of the virulence genes, prgH, ssrB, and pagC were detected in all serotypes: hilA, sipB, marT, mgtC, ssrB, sopB, pagN, nlpl, bapA, oafA, tolC, and cdtB were detected in S Paratyphi B. In S Typhimurium, the detection rates of hilA, sipB, marT, mgtC, ssrB, pagN, nlpl, bapA, oafA, and tolC were high, while the detection rate of ssrB was low. In S Enteritidis, the detection rates of icmF, spvB, spvR, and pefA were high, while those of ssrB, bapA, and oafA were low. In S Stanley, the detection rate of pagN was low, while those of hilA, sopB, and bapA were high. cdtB was detected in both S Paratyphi B and S Paratyphi C (Table 4 and Figure 1).

2.4 | Correlations between antibiotic resistance and virulence genes

Correlation analysis of resistance to 15 antibiotics and 20 virulence genes showed that nitrofurantoin resistance was negatively correlated with bapA (P = .005) and positively correlated with icmF, spvB, spvR, and pefA (P = .012, 0.008, 0.002, and 0.005, respectively) (Table 5). The P values between all other virulence genes and antibiotic resistance were greater than 0.05, indicating no significant correlations.

3 | DISCUSSION

Foodborne diseases caused by Salmonella have become a serious public health problem with a high economic burden in many countries and regions worldwide. In addition, the antimicrobial resistance of bacteria has become increasingly severe, which has attracted increasing attention. Salmonella is mainly found in the intestine of animals, has a wide variety of serotypes, and is pathogenic to humans and other animals. Among the serotypes that infect humans, S Typhimurium and S Enteritidis are the most common.10 The results of this study showed that S Typhimurium and S Enteritidis were the predominant species, accounting for 34.43% and 19.67% of isolates, respectively, while other species were sporadic Salmonella serotypes. The resistance of Salmonella to common antibiotics was high; the resistance rate to ampicillin was 63.93%, and among the cephalosporins, the resistance rate to cefotaxime, a third-generation cephalosporin, was 19.67%.

After Salmonella infects a human, it can encode a series of virulence factors through virulence genes on its chromosomes or genetic components carried by virulence plasmids, and its pathogenicity is closely related to these virulence factors. Salmonella contains many virulence factors, including pili, outer-membrane proteins, lipopolysaccharides, enterotoxin, a capsule, SPIs, and virulence plasmids. Some virulence factors are encoded by genes on chromosomes, and some are present in plasmids. Of the 20 Salmonella virulence genes

### Table 2: Distribution characteristics of serotypes of 61 Salmonella isolates

| Name of the isolate | Number (isolate) | Proportion (%) |
|---------------------|------------------|----------------|
| S Typhimurium       | 21               | 34.43          |
| S Enteritidis       | 12               | 19.67          |
| S Stanley           | 5                | 8.20           |
| S Saintpaul         | 4                | 6.56           |
| S Derby             | 3                | 4.92           |
| S Braenderup        | 3                | 4.92           |
| S Paratyphi B       | 2                | 3.28           |
| S London            | 2                | 3.28           |
| S Reading           | 2                | 3.28           |
| S Choleraesuis      | 1                | 1.64           |
| S Anatum            | 1                | 1.64           |
| S Tennessee         | 1                | 1.64           |
| S Thompson          | 1                | 1.64           |
| S Senftenberg       | 1                | 1.64           |
| S Dublin            | 1                | 1.64           |
| S Paratyphi C       | 1                | 1.64           |
studied here, 15 (hilA, sipB, prgH, ssrB, marT, mgtC, siiD, sopB, pagN, vexA, nplI, bapA, pagC, oafA, and icmF) are located on SPIs; spvB, spvR, and pefA are carried by plasmids, and tolC and cdtB are located in other parts of the *Salmonella* genome. The data in this study showed that all virulence genes were detected, but the distribution of virulence genes differed among serotypes. The virulence genes located on the SPIs (except vexA) exhibited high detection rates in all *Salmonella* isolates, suggesting that these virulence genes are widely distributed in each *Salmonella* isolate. Since the pathogenicity of *Salmonella* requires the interaction of many genes scattered

| Group | Serotype (n) |
|-------|-------------|
| B     | Paratyphi B(2) |
|       | Typhimurium(21) |
|       | Stanley(5) |
|       | Saintpaul(4) |
|       | Derby(3) |
|       | Reading(2) |
| C1    | Braenderup(3) |
|       | Choleraesuis(1) |
|       | Thompson(1) |
|       | Paratyphi C(1) |
|       | Tennessee(1) |
| D     | Enteritidis(12) |
|       | Dublin(1) |
| E1    | London(2) |
|       | Anatum(1) |
| E4    | Senftenberg(1) |

**FIGURE 1** Note: Gray indicates that the detection of the virulence gene was positive; white indicates that the detection of the virulence gene was negative; green indicates that the isolate had antibiotic susceptibility; yellow indicates that the antibiotic susceptibility of the isolate was intermediate; red indicates that the isolate was antibiotic-resistant; and light blue indicates that the ESBL test was negative, while dark blue indicates that the ESBL test was positive.
### TABLE 3  Distribution of antibiotic susceptibility of 61 Salmonella isolates

| Antimicrobial Agent         | Resistant | Intermediate | Susceptible |
|----------------------------|-----------|--------------|-------------|
|                            | Number of isolates | Rate (%) | Number of isolates | Rate (%) | Number of isolates | Rate (%) |
| Ampicillin                 | 39        | 63.93        | 0           | 0.00      | 22          | 36.07     |
| Ampicillin/sulbactam       | 34        | 55.74        | 1           | 1.64      | 26          | 42.62     |
| Trimethoprim-Sulfamethoxazole | 24   | 39.34        | 0           | 0.00      | 37          | 60.66     |
| Aztreonam                  | 14        | 22.95        | 2           | 3.28      | 45          | 73.77     |
| Cefotaxime                 | 12        | 19.67        | 3           | 4.92      | 46          | 75.41     |
| Ceftriaxone                | 11        | 18.03        | 0           | 0.00      | 50          | 81.97     |
| Ceftazidime                | 8         | 13.11        | 0           | 0.00      | 53          | 86.89     |
| Nitrofurantoin             | 6         | 9.84         | 9           | 14.75     | 46          | 75.41     |
| Cefepime                   | 5         | 8.20         | 1           | 1.64      | 55          | 90.16     |
| Ceftizoxime                | 5         | 8.20         | 4           | 6.56      | 52          | 85.25     |
| Ciprofloxacin              | 3         | 4.92         | 2           | 3.28      | 56          | 91.80     |
| Cefoperazone/sulbactam a   | 1         | 1.64         | 2           | 3.28      | 58          | 95.08     |
| Piperacillin/tazobactam    | 1         | 1.64         | 6           | 9.84      | 54          | 88.52     |
| Levofloxacin               | 1         | 1.64         | 0           | 0.00      | 60          | 98.36     |
| Imipenem                   | 0         | 0.00         | 0           | 0.00      | 61          | 100.00    |

*Criteria for cefoperazone/sulbactam were based on Enterobacteriaceae criteria for cefoperazone.*

### TABLE 4  Occurrence of virulence genes in Salmonella serotypes

| Virulence gene | Typhimurium (N = 21) | Enteritidis (N = 12) | Stanley (N = 5) | Saintpaul (N = 4) | Others (N = 19) |
|---------------|----------------------|----------------------|-----------------|-------------------|-----------------|
| prgH          | 21 (100.00)          | 12 (100.00)          | 5 (100.00)      | 4 (100.00)        | 19 (100.00)     |
| ssrB          | 21 (100.00)          | 12 (100.00)          | 5 (100.00)      | 4 (100.00)        | 19 (100.00)     |
| pagC          | 21 (100.00)          | 12 (100.00)          | 5 (100.00)      | 4 (100.00)        | 19 (100.00)     |
| marT          | 21 (100.00)          | 11 (91.67)           | 4 (80.00)       | 4 (100.00)        | 17 (89.47)      |
| hilA          | 20 (95.24)           | 10 (83.33)           | 5 (100.00)      | 4 (100.00)        | 16 (84.21)      |
| sipB          | 21 (100.00)          | 10 (83.33)           | 3 (60.00)       | 3 (75.00)         | 16 (84.21)      |
| mgtC          | 21 (100.00)          | 8 (66.67)            | 4 (80.00)       | 4 (100.00)        | 16 (84.21)      |
| nlpI          | 21 (100.00)          | 9 (75.00)            | 4 (80.00)       | 4 (100.00)        | 15 (78.95)      |
| sopB          | 20 (95.24)           | 7 (58.33)            | 5 (100.00)      | 4 (100.00)        | 15 (78.95)      |
| bapA          | 21 (100.00)          | 2 (16.67)            | 5 (100.00)      | 4 (100.00)        | 18 (94.74)      |
| tolC          | 18 (85.71)           | 9 (75.00)            | 2 (40.00)       | 3 (75.00)         | 13 (68.42)      |
| pagN          | 18 (85.71)           | 7 (58.33)            | 0 (0.00)        | 2 (50.00)         | 14 (73.68)      |
| oafA          | 21 (100.00)          | 1 (3.33)             | 4 (80.00)       | 4 (100.00)        | 5 (26.32)       |
| siiD          | 20 (95.24)           | 3 (15.79)            | 4 (80.00)       | 4 (100.00)        | 5 (26.32)       |
| icmF          | 1 (4.76)             | 7 (32.14)            | 0 (0.00)        | 0 (0.00)          | 1 (5.26)        |
| pesF          | 0 (0.00)             | 10 (83.33)           | 0 (0.00)        | 0 (0.00)          | 1 (5.26)        |
| spvR          | 1 (4.76)             | 1 (4.76)             | 0 (0.00)        | 0 (0.00)          | 1 (5.26)        |
| spvB          | 1 (4.76)             | 9 (75.00)            | 0 (0.00)        | 0 (0.00)          | 1 (5.26)        |
| cdtB          | 1 (4.76)             | 6 (50.00)            | 0 (0.00)        | 0 (0.00)          | 1 (5.26)        |
| vexA          | 0 (0.00)             | 0 (0.00)             | 0 (0.00)        | 0 (0.00)          | 1 (5.26)        |
TABLE 5 Correlations between virulence genes and antimicrobial resistance

| Virulence gene × antimicrobial agent | P value of Fisher’s exact test |
|-------------------------------------|-------------------------------|
| bapA × Nitrofurantoin               | .005                          |
| icmF × Nitrofurantoin              | .012                          |
| spvB × Nitrofurantoin              | .008                          |
| spvR × Nitrofurantoin              | .002                          |
| pefA × Nitrofurantoin              | .005                          |

*Table 5 only lists the correlated virulence genes and antimicrobial agents.*

throughout its genome, a wide distribution of virulence genes is necessary for the virulence of Salmonella. The plasmid virulence genes spvB, spvR, and pefA were mostly detected in S Enteritidis, and the virulence gene cdTB was detected in both S Paratyphi B and S Paratyphi C.

The virulence factors on the chromosomes of Salmonella are mainly on SPIs, which encode and express most virulence factors and help Salmonella to infect, reproduce, and spread in a complex host environment. The genes hil, sip, and prg in SPI-1 encode regulators, secrete effector proteins of T3SS, participate in the colonization and invasion of intestinal epithelial cells by Salmonella, and can cause macrophage necrosis and inflammatory responses. In this study, prgH was detected in all isolates, which is consistent with the findings of Xiong et al. and Yang, and hilA and sipB were detected in most isolates. SPI-2 is a virulence factor that plays a major role in the pathogenesis of systemic diseases and is present in all Salmonella species except S Bongori. The detection rate of the ssrB gene in SPI-2 in this study was 100%. Carrying SPI-1 + SPI-2 is positively correlated with the pathogenicity of Salmonella, indicating that the Salmonella serotypes detected in this study had strong virulence.

Salmonella pathogenicity islands-3 can be used as a virulence marker for the detection of Salmonella. The virulence genes mgtC and marT, in SPI-3, were detected in 86.89% and 93.44% of the isolates in this study, respectively, and can be used as virulence markers for Salmonella screening. pagC is another good virulence marker for the detection of Salmonella. One study (1995) showed that pagC might be the best choice for a probe or PCR target in future detection protocols. Another study demonstrated that in food production, pagC can be used as a biomarker for the detection of Salmonella that is in the viable but not culturable state. This study showed that the detection rate of pagC was 100%; therefore, it may be an ideal virulence marker for the detection of Salmonella.

Salmonella pathogenicity islands-9 is associated with the formation of biological membranes. In Salmonella, deletion of the virulence gene BapA can result in an inability to generate biological membranes, whereas overexpression of BapA enhances the formation of biological membranes. The formation of the bacterial membrane is a gradual process. First, adsorption onto the surface of an object is the key to membrane formation, and the adhesion structure of Salmonella includes the pili and the bapA protein.

Therefore, it is speculative whether the presence of both the virulence gene encoding pili and the bapA gene could provide favorable conditions for the formation of bacterial membranes. This study examined pefA, a virulence gene encoding pili, and bapA, a gene related to membrane formation, and found that the detection of bapA or pefA was complementary to the other, that is, isolates with pefA were negative for bapA, and vice versa. Whether this phenomenon is related to the formation of bacterial membranes is still unknown. Since no specific relevant experiments have been performed in this regard, we aim to conduct in-depth research on this phenomenon in the future.

CdtB was first discovered in S Typhi and S Paratyphi A and plays a role in cell apoptosis and necrosis. Later, cdtB was found in some nontyphoid Salmonella serotypes, such as S Aberdeen, S Javiana, S Schwarzengrund, and S Goldcoast, but the effects were different from those of S Typhi. In our study, cdtB was detected in both S Paratyphi B and S Paratyphi C; however, we did not examine the role of the cdtB gene in these serotypes. In future work, the source of cdtB and its role will be further studied.

Virulence and antibiotic resistance are two important characteristics of Salmonella, and their relationship is complex. Domestic and international scholars have conducted much research on the relationship between the virulence and antibiotic resistance, with different conclusions. In this study, among the 15 antibiotics and 20 virulence genes tested, only nitrofurantoin resistance was negatively correlated with bapA and positively correlated with icmF, spvB, spvR, and pefA, and spvB; spvR and pefA were present in the virulence plasmids. It is possible that the resistance genes to nitrofurantoin and the virulence genes are carried by the same plasmid, but this speculation needs to be tested. This relationship deserves clinical attention, and further research is needed in this field, especially regarding the distribution of virulence genes in common serotypes that cause human infection and their relationships to antibiotic resistance. Such research will allow a better understanding of the pathogenic characteristics and evolutionary process of Salmonella as well as determination of how Salmonella spreads between hosts. In clinical practice, a personalized treatment plan can be developed after the characteristics of virulence genes and antibiotic resistance genes in various serotypes are fully understood, which will be key to the prevention, control, and treatment of salmonellosis.

ORCID
Meina Yue https://orcid.org/0000-0001-6659-9042

REFERENCES
1. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal Salmonella gastroenteritis. Clin Infect Dis. 2010;50(6):882-889.
2. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States—major pathogens. Emerg Infect Dis. 2011;17(1):7-15.
3. Angulo FJ, Melbak K. Human health consequences of antimicrobial drug-resistant Salmonella and other foodborne pathogens. Clin Infect Dis. 2005;41(11):1613-1620.
4. Cheng Q, Pang RL, Wang RC, et al. Comparative study on pathogenicity of Salmonella isolates from different sources of laboratory mice and the detection of their virulence genes. *Chin J Zoonoses*. 2013;29(5):460-465.

5. Sun L, Qiu P, Liu XL, et al. Research progress on bacterial resistance influencing virulence. *Anim Hus Vet Med*. 2016;48(11):120-123.

6. Nolan HP, Tang M, Zhang HP, et al. The preliminary study about distribution features of Salmonella virulence genes in Nantong city. *J Qiqihar Med Coll*. 2009;30(19):2355-2357.

7. Chen F, Cheng DR, Wang LF, et al. Study on virulence island marker gene and analysis of mgtC gene sequence of Salmonella from avian. *Jiangsu Agr Sci*. 2010;06:53-56.

8. Shen B, Wang ZF, Hu XJ, et al. Detection analysis on virulence genes of *Salmonella* typhimurium pathogenicity Island of Zhoushan. *Inter J Epidemiol Infect Dis*. 2015;42(5):343-346.

9. Hu XJ, Shen B, Yang SJ, et al. PCR detection of iconic genes in 19 *Salmonella* pathogenicity Islands. *Prog Vet Med*. 2016;37(5):20-25.

10. https://www.who.int/en/news-room/fact-sheets/detail/salmonella. Accessed May 17, 2020.

11. Han Y, Liang W, Li B, et al. Pathogenic-related virulence factors in *Salmonella*. *Chin J Antibiot*. 2013;38(05):402-407.

12. Golovina AG. *Salmonella* pathogenicity islands: big virulence in small packages. *Microbes Infect*. 2000;2(2):145-156.

13. Haraga A, Ohlson MB, Miller SI. *Salmonella*e interoperl play with host cells. *Nat Rev Microbiol*. 2008;6(1):53-66.

14. Coombes BK, Brown NF, Valdez Y, et al. Expression and secretion of *Salmonella* pathogenicity Island-2 virulence genes in response to acidification exhibit differential requirements of a functional type III secretion apparatus and SsaL. *J Biol Chem*. 2004;279(48):49804-49815.

15. Hensel M. *Salmonella* pathogenicity island 2. *Mol Microbiol*. 2000;36(5):1015-1023.

16. Wang JZ, Dong R, Wang LQ, et al. Isolation, Identification and pathogenicity Island gene detection of *Salmonella* in commercial eggs. *Food Sci*. 2012;33(16):154-158.

17. Nolan LK, Giddings CW, Brown J. The distribution of invA, pagC and spvC genes among *Salmonella* isolates from animals. *Vet Res Commun*. 1995;19(3):167-177.

18. Xu J, Suiza K, Okuno K, Takaya A, Yamamoto T, Isogai E. Membrane vesicle protein PagC as a novel biomarker for detecting pathogenic *Salmonella* in the viable but not culturable state. *J Vet Med Sci*. 2018;80(1):133-137.

19. Latasa C, Roux A, Toledo-Arama A, et al. BapA, a large secreted protein required for biofilm formation and host colonization of *Salmonella* enterica serovar. *Mol Microbiol*. 2006;58(5):1322-1339.

20. Wagner C, Hensel M. Adhesive mechanisms of *Salmonella* enterica. *Oxyg Transp Tissue XXXIII*. 2011;715:17-34.

21. Williams K, Gokulan K, Shelman D, et al. Cytotoxic mechanism of cytolethal distending toxin in non-typhoidal *Salmonella* Serovar (*Salmonella* Javiana) during macrophage infection. *DNA Cell Biol*. 2015;34(2):113-124.

22. Chu C, Chiu CH. Evolution of the virulence plasmids of non-typhoid *Salmonella* and its association with antimicrobial resistance. *Microbes Infect*. 2006;8(7):1931-1936.

23. Klijipers AFA, Bonacic Marinovic AA, Wijnands LM, et al. Phenotypic Prediction: Linking in vitro Virulence to the Genomics of 59 *Salmonella* enterica Strains. *Front Microbiol*. 2018;9:3182.

24. Morosini ML, Ayala JA, Baquero F, et al. Biological cost of AmpC production for *Salmonella* enterica serotype. *Antimicrob Agents Chemother*. 2000;44(11):3137.

25. Liu WH, Guo AZ, Xu VYD, et al. Studies on the resistance and outer membrane protein of S. typhimurium. *Chin J Microecology*. 2007;01:48-49+53.

26. Fábrega A, Madurga S, Giralt E, et al. Mechanism of action of and resistance to quinolones. *Microb Biotechnol*. 2009;2(1):40-61.

27. Osman KM, Hassan WM, Mohamed RA. The consequences of a sudden demographic change on the seroprevalence pattern, virulence genes, identification and characterisation of integron-mediated antibiotic resistance in the *Salmonella* enterica isolated from clinically diarrhoeic humans in Egypt. *Eur J Clin Microbiol Infect Dis*. 2014;33(8):1323-1337.

**How to cite this article:** Yue M, Li X, Liu D, Hu X. Serotypes, antibiotic resistance, and virulence genes of *Salmonella* in children with diarrhea. *J Clin Lab Anal*. 2020;34:e23525. https://doi.org/10.1002/jcla.23525