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RESEARCH ARTICLE

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

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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.[1] In the United States, *Salmonella* is a major biological factor that causes bacterial foodborne infections.[1,2] In China, 70%-80% of food poisoning incidents are caused by *Salmonella*.[2]

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.[3] 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

TABLE 1 Primer sequences and lengths of 20 virulence genes of Salmonella

Location	Primer name	Primer sequence (5′-3′)	Length (bp)	Hybridization temperature and references
SPI-1	hilA-F	GACAGAGCTGGACCACAATAAGACA	312	55°C[8]
	hilA-R	GAGCGTAATTCATCGCCTAAAC		
SPI-1	sipB-F	GGACGCCGCCCGGGAAAAACTCTC	875	66.5°C[6]
	sipB-R	ACACTCCCGTCGCCGCCTTCACAA		
SPI-1	prgH-F	CTTCAGGYCAACTCCCTGATATAC	961	55°C[9]
	prgH-R	CCCTTGAGCCAGTCATCTTT		
SPI-2	ssrB-F	CTCATTCTTCGGGCACAGTTA	558	55°C[8]
	ssrB-R	CCTTATTACCCTGGCCTCATTT		
SPI-3	marT-F	CGTCGTCTCACAACAAACATTC	556	55°C[9]
	marT-R	CTGACAAATCAATGCCGTAACC		
SPI-3	mgtC-F	AAAGACAATGGCGTCAACGTATGG	500	65°C[7]
	mgtC-R	TTCTTTATAGCCCTGTTCCTGAGC		
SPI-4	siiD-F	GTCAGGGCGTTATCACTACTAAA	826	55°C[9]
	siiD-R	TTCACATCGGCCAGCATAG		
SPI-5	sopB-F	TCACTAAAAACCCAGGAGGCTTTT	1000	65°C[7]
	sopB-R	CGCCATCTTTATTGCGGATTTTTA		
SPI-6	pagN-F	TTCCAGCTTCCAGTACGTTTAG	440	55°C[8]
	pagN-R	GCCTTTGTGTCTGCATCATAAG		
SPI-7	vexA-F vexA-R	AAACTAAGCGCTCCCGATAC CAGTCGCGCAGTGAAATAATG	504	55°C[8]
SPI-8	nlpI-F nlpI-R	AGTCTTGGTTTGAGGGCATTAG TTCTTTCGCCTGCTTCTCATTA	333	55°C[8]
SPI-9	bapA-F	TAAGCGTCGGACTTGGAATG	543	55°C[8]
	bapA-R	CGTTCTTCAGCGTGTAGGTATAG		
SPI-11	pagC-F	CGCCTTTTCCGTGGGGTATGC	454	66.5°C[6]
	pagC-R	GAAGCCGTTTATTTTTGTAGAGGAGATGTT		
SPI-12	oafA-F	CGAGTGACTGGAACCAAAGA	510	55°C[9]
	oafA-R	CAAGCATAGAGCCAGAGTAGAG		
SPI-19	icmF-F	GCGTAGTCCAGATGAGACATTAG	724	55°C[8]
	icmF-R	GCGGCCAGATAGACGATATTT		
Plasmid	spvB-F	CTATCAGCCCCGCACGGAGAGCAGTTTTTA	717	66.5°C[6]
	spvB-R	GGAGGAGGCGGTGGCGGTGGCATCATA		
Plasmid	pefA-F	GCGCCGCTCAGCCGAACCAG	157	66.5°C[6]
	pefA-R	GCAGCAGAAGCCCAGGAAACAGTG		
Plasmid	spvR-F	CCGCTGAGCAGGGTTATTT	723	55°C[8]
	spvR-R	CTTGGTCGGGTAATACAAGGAG		
Genome	cdtB-F cdtB-R	ACAACTGTCGCATCTCGCCCCGTCATT CAATTTGCGTGGGTTCTGTAGGTGCGAGT	268	66.5°C[6]
Genome	tolC-F	GCAGACGCTGATCCTCAATAC	623	55°C[8]
	tolC-R	TTGCGCCGACGAAGTTATAC		

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.[4] Some data also show that the antibiotic resistance of an isolate is related to its virulence.[5]

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, cefotaxime/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,[6-9] 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 ddH_2O (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* Saintpaul (four isolates, 6.56%), *S* Derby (three isolates, 4.92%), and *S* Braenderup (three isolates, 4.92%). There

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/sulbactam (55.74%), and trimethoprim-sulfamethoxazole (39.34%). One isolate with antibiotic resistance (1.64%) was found for each of the following: cefoperazone/sulbactam, 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%). Cefoperazone/sulbactam had the lowest rate of antibiotic resistance (1.64%). Ceftizoxime 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*, *siiD*, *sopB*, *pagN*, *nlpI*, *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*, *siiD*, *sopB*, *pagN*, *nlpI*, *bapA*, *oafA*, *tolC*, and *cdtB* were detected in *S* Paratyphi B. In *S* Typhimurium, the detection rates of *hilA*, *sipB*, *marT*, *mgtC*, *sopB*, *pagN*, *nlpI*, *bapA*, *oafA*, and *tolC* were high, while the detection rate of *siiD* was low. In *S* Enteritidis, the detection rates of *icmF*, *spvB*, *spvR*, and *pefA* were high, while those of *siiD*, *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.

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

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

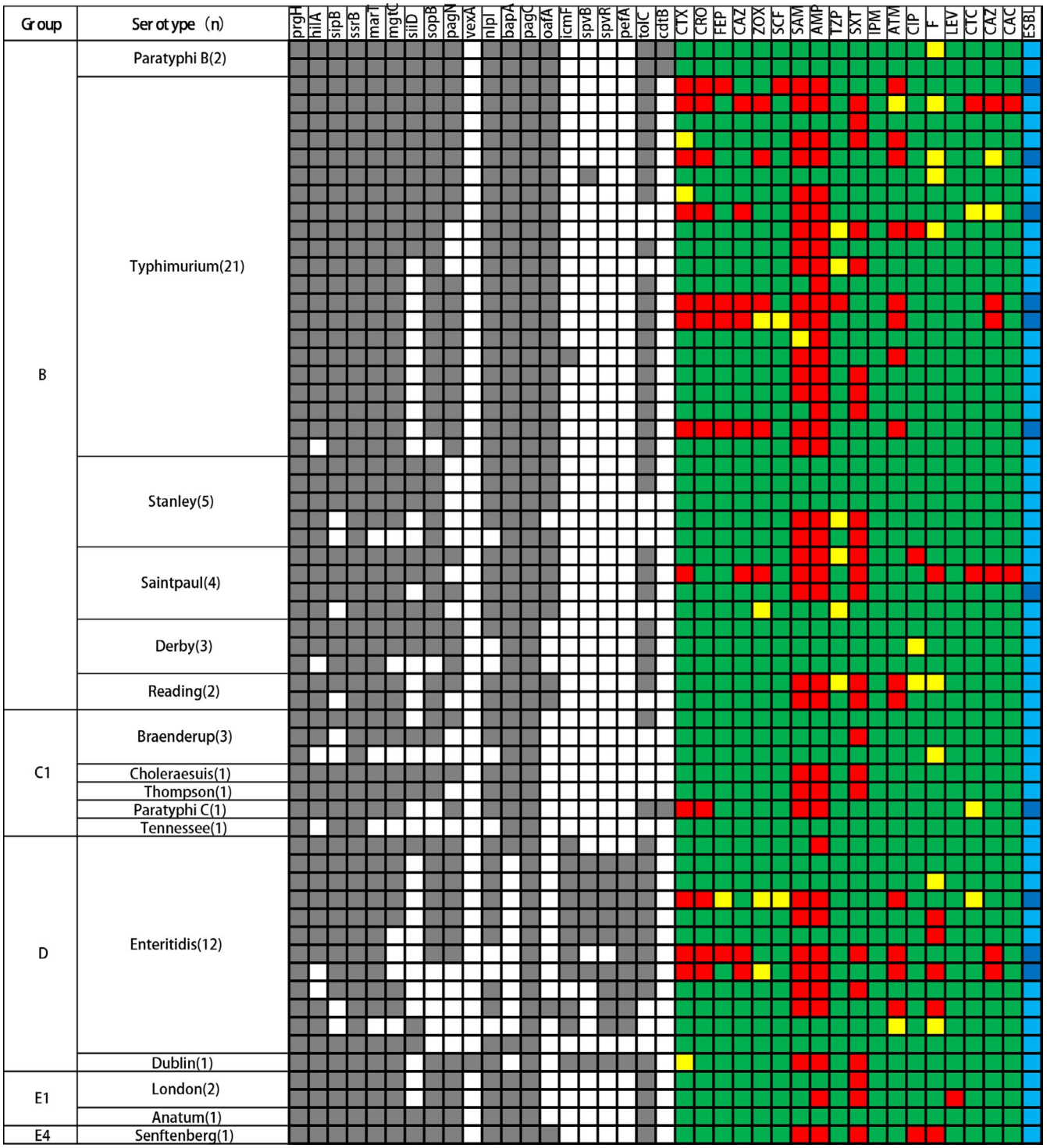

FIGURE 1 Note: Gray indicates that the detection of the virulence gene was positive; white indicates that the detection of the virulence gene was negative; green indicates that the isolate had antibiotic susceptibility; yellow indicates that the antibiotic susceptibility of the isolate was intermediate; 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

studied here, 15 (*hilA*, *sipB*, *prgH*, *ssrB*, *marT*, *mgtC*, *siiD*, *sopB*, *pagN*, *vexA*, *nlpI*, *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.[11] 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

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

[a]Criteria for cefoperazone/sulbactam were based on Enterobacteriaceae criteria for cefoperazone.

TABLE 4 Occurrence of virulence genes in Salmonella serotypes

Virulence gene	The total number of isolates (N = 61)	Typhimurium (N = 21)	Enteritidis (N = 12)	Stanley (N = 5)	Saintpaul (N = 4)	Others (N = 19)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
prgH	61 (100.00)	21 (100.00)	12 (100.00)	5 (100.00)	4 (100.00)	19 (100.00)
ssrB	61 (100.00)	21 (100.00)	12 (100.00)	5 (100.00)	4 (100.00)	19 (100.00)
pagC	61 (100.00)	21 (100.00)	12 (100.00)	5 (100.00)	4 (100.00)	19 (100.00)
marT	57 (93.44)	21 (100.00)	11 (91.67)	4 (80.00)	4 (100.00)	17 (89.47)
hilA	55 (90.16)	20 (95.24)	10 (83.33)	5 (100.00)	4 (100.00)	16 (84.21)
sipB	53 (86.89)	21 (100.00)	10 (83.33)	3 (60.00)	3 (75.00)	16 (84.21)
mgtC	53 (86.89)	21 (100.00)	8 (66.67)	4 (80.00)	4 (100.00)	16 (84.21)
nlpI	53 (86.89)	21 (100.00)	9 (75.00)	4 (80.00)	4 (100.00)	15 (78.95)
sopB	51 (83.61)	20 (95.24)	7 (58.33)	5 (100.00)	4 (100.00)	15 (78.95)
bapA	50 (81.97)	21 (100.00)	2 (16.67)	5 (100.00)	4 (100.00)	18 (94.74)
tolC	45 (73.77)	18 (85.71)	9 (75.00)	2 (40.00)	3 (75.00)	13 (68.42)
pagN	41 (67.21)	18 (85.71)	7 (58.33)	0 (0.00)	2 (50.00)	14 (73.68)
oafA	35 (57.38)	21 (100.00)	1 (8.33)	4 (80.00)	4 (100.00)	5 (26.32)
siiD	28 (45.90)	10 (47.62)	3 (25.00)	3 (60.00)	3 (75.00)	9 (47.37)
icmF	12 (19.67)	1 (4.76)	10 (83.33)	0 (0.00)	0 (0.00)	1 (5.26)
pefA	11 (18.03)	0 (0.00)	10 (83.33)	0 (0.00)	0 (0.00)	1 (5.26)
spvR	10 (16.39)	0 (0.00)	9 (75.00)	0 (0.00)	0 (0.00)	1 (5.26)
spvB	8 (13.11)	1 (4.76)	6 (50.00)	0 (0.00)	0 (0.00)	1 (5.26)
cdtB	3 (4.92)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	3 (15.79)
vexA	1 (1.64)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (5.26)

TABLE 5 Correlations between virulence genes and antimicrobial resistance[a]

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

[a]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.[12] 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 [6] 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.[18] 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*,[19] 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*.[19] 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.[20] 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.[21] 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.[22] 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.[23] 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.

C*dtB* was first discovered in *S* Typhi and *S* Paratyphi A and plays a role in cell apoptosis and necrosis.[24] 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.[25] 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.[27-30] 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 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.

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