ORIGINAL RESEARCH

Molecular Characteristics of Salmonella Spp. Responsible for Bloodstream Infections in a Tertiary Hospital in Nanjing, China, 2019-2021

Miao-Miao Hua¹, Jia Li², Jie Zheng², Jing-Jing Wang², Chang Liu², Yan Zhang², Zhi-Feng Zhang², Xiao-Li Cao^{1,2}, Han Shen^{1,2}

¹Department of Laboratory Medicine, Nanjing Drum Tower Hospital Clinical College of Jiangsu University, Nanjing, People's Republic of China; ²Department of Laboratory Medicine, Nanjing University Medical School Affiliated Nanjing Drum Tower Hospital, Nanjing, People's Republic of China

Correspondence: Xiao-Li Cao; Han Shen, Email cao-xiao-li@163.com; shenhan10366@sina.com

Objective: To investigate the clinical and molecular characteristics of *Salmonella* spp. causing bloodstream infections (BSIs) in our hospital.

Methods: We studied 22 clinical *Salmonella* isolates from BSIs and 16 from non-BSIs, performing antimicrobial susceptibility testing (AST) and whole genome sequencing (WGS). The analysis included serovars, antibiotic resistance genes (ARGs), virulence factors (VFs), sequence types (STs), plasmid replicons, and genetic relationships. We also assessed pathogenicity of the isolates causing BSIs through growth, biofilm formation, and anti-serum killing assays.

Results: WGS analysis identified 13 *Salmonella* serovars, with four responsible for BSIs. *S. Enteritidis* was the most prevalent serovar, involved in 19 (50.0%) cases. BSIs were caused by 17*S*. Enteritidis, two *S*. Typhimurium, two *S*. Munster and one *S*. Diguel. Of the 38 isolates, 27 (71.1%) exhibited high resistance to ampicillin, and 24 (63.2%) to ampicillin/sulbactam. Thirty-six types of ARGs were identified, with *bla*TEM-1B (n = 25, 65.8%) being the most frequent. Ten plasmid replicons were found; the combination of IncFIB(S)-IncK1 was the most common in *S*. Enteritidis (94.7%). Fifteen STs were identified, among which ST11 was the most prevalent and clonally disseminated, primarily responsible for BSIs. A total of 333 different VFs were detected, 177 of which were common across all strains. No significant differences were observed between the BSI and non-BSI isolates in terms of resistance rates, ARGs, plasmid replicons, and VFs, except for seven VFs. No strong pathogenicity was observed in the BSI-causing isolates. **Conclusion:** BSIs were predominantly caused by clonally disseminated *S*. Enteritidis ST11, the majority of which carried multiple

ARGs, VFs and plasmid replicons. This study provides the first data on clonally disseminated *S*. Enteritidis ST11 causing BSIs, highlighting the urgent need for enhanced infection control measures.

Keywords: Salmonella enteritidis, bloodstream infections, serovars, antibiotic-resistance genes, virulence factors, sequence type

Introduction

Salmonella spp. are significant etiological agent of food-borne diseases and gastroenteritis in both humans and animals.¹ The genus *Salmonella* is divided into *Salmonella enterica* and *Salmonella bongori*,² with *S. enterica* further subdivided into typhoidal and non-typhoidal *S. enterica* (NTS). Currently, over two thousand NTS serovars have been identified, each with distinct host specificities.² Gastroenteritis is the primary clinical manifestation in healthy individuals, while bacteremia, which primarily affects the vulnerable or elderly,³ is comparatively rare. The World Health Organization reports that foodborne pathogens are responsible for 94 million cases of gastroenteritis annually, leading to significant global health burdens,³ with NTS being particularly impactful.

The increasing rates of antimicrobial resistance in *Salmonella*, together with the frequent emergence of multi-drug resistant (MDR) strains, are a matter of global concern.⁴ In particular, the increasing resistance of *Salmonella* spp. to clinically important antimicrobial agents, such as fluoroquinolones and third-generation cephalosporins, has become a global concern.⁵

2363

leading to clinical management difficulties and an increase in morbidity and mortality,⁴ due to the lack of clinical efficacy of first- and second-generation cephalosporins and aminoglycosides.

Salmonella pathogenicity is closely linked to numerous virulence factors (VFs) that facilitate adhesion, invasion, intracellular survival, fimbrial expression, systemic infection, toxin production, Mg²⁺ production, and iron uptake.⁶ Notably, *Salmonella* pathogenicity islands (SPIs) represent some of the most critical VFs.⁷ SPI-1 and SPI-2 play pivotal role in systemic infections. They encode type III secretion systems (T3SS) that form a channel in the host cell membrane, facilitating the internalization of bacterial effector proteins.⁸ Furthermore, SPI-2 is essential for bacterial survival within macrophages and for causing lethal bacteremia in mice.⁹

Furthermore, the formation of biofilm is an important VF that contributes to the ability to cause acute, latent, or chronic disease. The exact outcome of an infection is determined by the growth state of *Salmonella*, the immune status of infected host and the immune response elicited.¹⁰ In addition to biofilm formation, the MDR in *Salmonella* significantly enhances the survival ability of pathogens in the human intestinal tract under the selective pressure of antibiotic use,¹¹ which provides favorable conditions for the dissemination of such strains in healthcare centers.

Prior to 2019, *Salmonella* spp. was rarely detected in the clinical laboratories of our hospital. However, *Salmonella* spp. have been increasingly implicated in a variety of infections, with BSIs being the most common. *S.* Enteritidis was the predominant serovar, accompanied by some rare serovars according to the slide agglutination test. We were curious about the characterization of these strains and whether there was a clonal spread of *S*. Enteritidis, as well as the pathogenicity of the *S*. Enteritidis associated with BSIs.

In this study, we aimed to characterize *Salmonella* spp. causing BSIs in our hospital, including the distribution of VFs, antibiotic resistance genes (ARGs), mutations in target genes, plasmid replicons and genetic relationship, with the *Salmonella* spp. causing non-BSIs as a control group. Additionally, bacterial growth, biofilm formation, and anti-serum killing experiments were performed to evaluate the pathogenic ability of the strains causing BSIs.

Materials and Methods

Bacterial Isolates

In total, 22 consecutive and non-replicate *Salmonella* isolates that caused BSIs were collected from patients at our hospital between January 2019 and December 2021. Meanwhile, 16 *Salmonella* isolates that did not cause BSIs during the same period were used as a control group. These 16 *Salmonella* isolates were collected from stool (n = 11), urine (n = 3), secretions (n = 1), and abdominal fluid (n = 1). All strains were initially identified using a Vitek 2.0 matched GN panel (BioMerieux, France) and matrix-associated laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) (BioMerieux).

Clinical Data Collection

The clinical information of the 38 patients was collected from electronic medical records, including general information (agender, age, department, and length of hospitalization), specimen information (source and collection date), infection indicators (White blood cell count and percentage, C-reactive protein, procalcitonin, interleukin-6), underlying diseases, invasive procedures, and immune diseases. This study was conducted according to the tenets of the Declaration of Helsinki and approved by the Ethics Committee of Nanjing Drum Tower Hospital (2023–390). A BSI was defined when a patient presented with systemic signs of infection and positive blood cultures accompanied by abnormal infection indicators.¹²

Antimicrobial Susceptibility Testing (AST)

The antimicrobial susceptibility of the 38 clinical *Salmonella* isolates was evaluated for 13 antimicrobial agents, namely ampicillin (AMP), ampicillin/sulbactam (SAM), ceftriaxone (CRO), ceftazidime (CAZ), cefepime (FEP), imipenem (IPM), ertapenem (ETP), aztreonam (ATM), levofloxacin (LEV), piperacillin/tazobactam (TZP), sulfamethoxazole/ trimethoprim (SXT), cefoperazone/sulbactam (SCF), and ciprofloxacin (CIP) by microbroth dilution testing. *Escherichia coli* ATCC 25922 was used for quality control. Results were interpreted according to the Clinical Laboratory Standard Institute guidelines 2023.¹³

Fresh colonies were collected for genomic DNA analysis using a bacterial DNA extraction kit (Tiangen Biochemical Technology Co., Ltd., China) following the manufacturer's protocol. The extracted DNA was sent to Tiangen Biochemical Technology Co., Ltd. for purity check and WGS.

Genomic Analysis

The sequencing data was trimmed and assembled using CLC Workbench version 21.0.4 for genome submission to GenBank and further analysis. The distribution of ARGs and mutations in the target genes was further investigated using the same software. PlasmidFinder 2.1 (https://cge.food.dtu.dk/services/PlasmidFinder/) was used to analyze the presence of plasmid replicons, and SeqSero 1.2 (https://cge.food.dtu.dk/services/SeqSero/) was used for sequence type (ST) and serovar prediction.

Investigation of the Distribution of Virulence Factors

The 38 genomes were annotated by Prokka (Prokaryotic Genome Rapid annotation software) to obtain annotated GenBank files.¹⁴ VFDB database (<u>http://www.mgc.ac.cn/VFs/download.htm</u>) was used to compare with the nucleotide coding sequence files of all VFs extracted in batches from 38 genomes using BLASTN software to obtain the detailed distribution of VFs in all genomes.¹⁵ The thresholds "E-value = 1e-5, identity \geq 70, coverage \geq 90%, and match length \geq 30" were strictly set to minimize false positive results.

Construction of a Phylogenetic Tree

The phylogenetic tree was constructed as follows: First, the genomic DNA was annotated using Prokka,¹⁴ and the single nucleotide polymorphism (SNP) alignment sequences of 3505 core genes were obtained using Scoary software.¹⁶ A nucleotide substitution model was constructed using Jmodeltest.¹⁶ Then, a maximum likelihood phylogenetic tree was constructed using RaxML and 1000 bootstrap samples.¹⁷ Finally, the phylogenetic tree was imported into Itol software, branches with bootstrap values less than 50 were removed, and the final phylogenetic tree was obtained. Based on the phylogenetic tree, a heat map was constructed by combining serotypes, STs, ARGs and VFs to show the characteristics of the 38 isolates.

Growth and Biofilm Formation Assays

The ability of the 22 Salmonella isolates causing BSIs to grow and form biofilms was evaluated using the following Methods: a single bacterial colony was inoculated into 5 mL of LB liquid medium and subjected to shaking cultivation at 150 rpm at 35° C.

Overnight. The culture was then diluted 1/100 with saline and transferred to two 96-well plates (100 μ L/well). Saline was used as a blank control. For growth ability, OD620 values were measured at 1-hour intervals using a microplate reader (SpectraMax M5) over a period of 7 hours. For the biofilm formation assay, after the 1:100 diluted culture was statically cultured at 37°C for 48 hours, the biofilm was stained with 0.1% crystal violet, then 30% glacial acetic acid was used to dissolve the crystals, and the optical density at 550 nm was measured on a microplate reader (SpectraMax M5). The results were analyzed using GraphPad Prism 9.5.0 software (San Diego, California, USA). Each experiment was repeated three times.

Anti-Serum Killing Assay

Briefly, single colonies were suspended in 3mL of saline and adjusted to 0.5 McFarland standard, which was further diluted to 10^6 CFU/mL. Serum was mixed with the 10^6 CFU/mL dilution in a ratio of 1:3 (125ul: 375ul) and incubated in a constant temperature incubator at 37 °C for 3 hours. Colonies on the agar plates were counted at t = 0, 1, 2 and 3 hours. The survival rates of bacteria at different time points were calculated to evaluate the anti-serum ability. The results were analyzed using GraphPad Prism 9.5.0 software (San Diego, California, USA).

Results

Clinical Characterization

Out of the 38 patients, 17 were male and 21 were female, resulting in a male-to-female ratio of 1:1.24. The period with the highest incidence of *Salmonella* infection was from July to November (n = 33, 86.8%). Patients with BSIs had a significantly longer hospitalization period compared to those without BSI (P < 0.05). There were no significant differences in risk factors between patients with *Salmonella* BSIs and those without, except for the length of hospitalization.

Antimicrobial Resistance Phenotypes

High resistance to ampicillin (n = 27, 71.1%) and ampicillin/sulbactam (n = 24, 63.2%) were found, along with low-level resistance to ceftazidime (n = 3, 7.9%), ceftriaxone (n = 2, 5.3%), and cefepime (n = 1, 2.6%). No resistance to imipenem, ertapenem, cefoperazone/sulbactam, and piperacillin/tazobactam was observed (Figure 1A).

Out of the 38 isolates, 28 (73.7%) were resistant to at least two antimicrobials, and seven (18.4%) were MDR, defined as resistant to three or more antimicrobials, contributing to five distinct MDR profiles (Figure 1B). The most frequent MDR profiles observed were AMP-SAM-SXT and AMP-CAZ-ATM-SXT-CIP-LEV. Additionally, one isolate was resistant to



Figure I Phenotypic resistance profile of the Salmonella isolates. (A) The percentages of antibiotic resistance of the 38 Salmonella isolates. (B) The antibiotic resistance profiles of the 38 Salmonella isolates.

Abbreviations: AMP, ampicillin; SAM, ampicillin/sulbactam; SXT, sulfamethoxazole/trimethoprim; ATM, aztreonam; CIP, ciprofloxacin; LEV, levofloxacin; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; SCF, cefoperazone/sulbactam; ETP, ertapenem; TZP, piperacillin/tazobactam; IPM, imipenem.

seven antimicrobial agents (AMP-CRO-CAZ-FEP-ATM-SAM-SXT), while only two isolates were pan-susceptible. There were no statistically significant differences in resistance rates between BSI-causing and non-BSI-causing groups.

Serovars

Totally, 13 *Salmonella* Serovars were identified, with Enteritidis (n = 19, 50.0%) being the most common. The distribution of the remaining ones were shown in Figure 2A. Four serovars were responsible for BSIs, including 17*S*. Enteritidis, 2*S*. Typhimurium, 2*S*. Munster and 1*S*. Diguel (Figure 2B). In addition, 11 serovars caused non-BSIs (Figure 2C).

Antibiotic Resistance Genes

In total, 36 types of ARG were identified (Figure 3A). Among them, *bla*TEM-1B (n = 25, 65.8%), *aph(6)-Id* (n = 19, 50.0%), and *sul2* (n = 19, 50.0%) were the most prevalent. Notably, *bla*TEM-1B was detected in all 19S. Enteritidis isolates. Additional β -lactam resistance genes were also detected (Figure 3B). Multiple resistance genes to aminoglycosides, which are not clinically effective, were also detected (Figure 3C). Importantly, 17 of the 19S. Enteritidis strains contained *aph(6)-Id*, *aph(3'')-Ib*, and *sul2* (Table 1). Furthermore, multiple ARGs were found in three S. Kentucky, two



Figure 2 Serovar distribution of the Salmonella isolates. (A) Distribution of serovars causing Salmonella infections in our hospital. (B) Serovar distribution of 22 Salmonella isolates causing bloodstream infections. (C) Serovar distribution of 16 Salmonella isolates causing non-bloodstream infections.



Figure 3 Antimicrobial resistance genes identified in the 38 Salmonella isolates. (A) Distribution of the 36 antimicrobial resistance genes identified among the 38 Salmonella isolates. (B) β -lactam resistance genes identified in the 38 Salmonella isolates. (C) Aminoglycoside resistance genes identified in the 38 Salmonella isolates.

S. Typhimurium, one S. Muenster, and one S. Thompson isolates, resulting in MDR phenotypes (Table 1). We also found that 89.5% (n = 34) of Salmonella isolates had mutations in gyrA and/or parC, including D87Y (n = 19), D87N (n = 4), and S83F (n = 4) in gyrA, and T57S (n = 15), S80I (n = 3), and S80R (n = 1) in parC. Four isolates had double mutations in both gyrA (D87N and S83F) and parC (T57S and S80I/S80R), while the remaining strains had a single mutation in either gyrA (D87Y) or parC (T57S). No mutations were detected in gyrB or parE.

Sequence types

Fifteen *Salmonella enterica* STs were identified, with ST11 (n = 19, 50.0%) being the most common (Figure 4). The phylogenetic tree constructed from the 38 *Salmonella* isolates demonstrated specific genetic relationships among the same serovars (Figure 4). Nineteen isolates of *S*. Enteritidis ST11 shared a strong genetic relationship, indicating clonal dissemination and the potential for outbreaks of this particular clone within a hospital setting (Figure 4).

Plasmid Replicons

Twenty-six out of 38 isolates contained ten different plasmid replicons. The most prevalent plasmid replicons were IncFIB(S), IncFII(S), and IncX1, each found in 52.6% (n = 20) of cases (Table 1). Eight distinct replicon combinations were identified, with the most frequent plasmid profile being IncFIB(S)-IncFII(S)-IncX1 (n = 17, 44.7%), all of which were located in a single contig and were exclusive to *S*. Entertitidis.

Table I Serovar, Resistance Profile, Resistance Gene, and Plasmid Replicon(s) of the 38 Salmonella Isolates

Strain	Serovars ^b	Resistance Profile ^c	Resistance Gene	Plasmid Replicon(s)	
SE ^a 02	Enteritidis	AMP-SAM	blaTEM-IB, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)- IncXI	
SE04	Enteritidis	AMP-SAM	blaTEM-1B	IncFIB(S)-IncFII(S)-IncXI-ColpVC	
SE09	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncXI	
SEI2	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2, tet(A)	IncFIB(S)-IncFII(S)-IncX1	
SE13	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)-IncX1	
SE14	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2, tet(A)	IncFIB(S)-IncFII(S)-IncX1	
SE15	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2, tet(A)	IncFIB(S)-IncFII(S)-IncX1	
SE16	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)-IncX1	
SE17	Enteritidis	AMP-SAM	blaTEM-1B	IncFIB(S)-IncFII(S)-IncX1	
SE18	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)-IncX1	
SE19	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2, tet(A)	IncFIB(S)-IncFII(S)-IncX1	
SE20	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2, tet(A)	IncFIB(S)-IncFII(S)-IncX1	
SE23	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)-IncX1	
SE25	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2, tet(A)	IncFIB(S)-IncFII(S)-IncX1	
SE27	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2, tet(A)	IncFIB(S)-IncFII(S)-IncX1	
SE29	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)-IncX1	
SE3 I	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)-IncX1	
SE37	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)-IncX1	
SE36	Enteritidis	AMP-SAM	blaTEM-1B, aph(6)-ld, aph(3")-lb, sul2	IncFIB(S)-IncFII(S)-IncX1	
SE26	Rissen	AMP-SXT	blaTEM-1B, aadA2, ant(3")-la, qacE, dfrA12, sul1, sul3, cmlA1, tet(A)	-	
SELL	Typhimurium	_d	aac(6')-laa	IncFIB(S)-IncFII(S)	
SE28	Typhimurium	AMP-SAM-SXT	blaTEM-1B, aadA2, ant(3")-la, aac(6')-laa, dfrA12, sul2, sul3, cmlA1, floR	Col(BS512)-Incl2(Delta)	
SE30	Typhimurium	-	aac(6')-laa	IncFIB(S)- IncFII(S)	
SE35	Typhimurium	AMP-SAM-SXT	blaOXA-10, ant(3")-la, aph(6)-ld, aac(6')-laa, aph(3")-lb, dfrA14, sul2, cmlA1, floR, ARR-3, tet(A), tet(B), qnrS1	IncHI2-IncHI2A- IncQI	
SE24	Kentucky	AMP-SAM-CIP-LEV	blaTEM-1B, aadA7, aadA17, aph(3')-la, aac(3)-ld, aac(3)-lld, aac(6')-laa, rmtB, qacE, mph(A), dfrA14, sul1, lnu(F), ARR-3, tet(A)	-	
SE32	Kentucky	AMP-CAZ-ATM- SXT-CIP-LEV	blaTEM-IB, blaCTX-M-55, aadA7, aadAI7, aph(3')-la, aac(3)-ld, aac(3)-lld, aac(6')-laa, qacE, mph(A), floR, Inu)-F), ARR-3, tet(A), fosA3	-	
SE33	Kentucky	AMP-CAZ-ATM- SXT-CIP-LEV	blaTEM-IB, blaCTX-M-55, aadA7, aadA17, aph(3')-la, aac(3)-ld, aac(3)-lld, aac(6')-laa, qacE, mph(A), dfrA14, sul1, floR, lnu(F), ARR-3, tet(A), fosA3	-	
SEOI	Muenster	AMP-CRO-CAZ- FEP-ATM-SAM-SXT	blaTEM-IB, blaCTX-M-55, blaLAP-2, aadA22, aac(3)-IId, aph(6)-Id, dfrAI4, sul3, floR, Inu(F), ARR-3, tet(A), qnrSI	-	
SE03	Muenster	-	-	-	
SE34	Anatum	-	aac(6')-laa	-	
SE07	London	-	aac(6')-laa	-	
SE22	London	-	aac(6')-laa	-	
SE05	Give	-	-	-	
SE21	Senftenberg	-	-	Col(pHAD28)	
SE08	Cannstatt	-	aac(6')-laa	-	
SE38	Indiana	CIP-LEV	aph(3')-IIa, aac(6')-Iaa, mph(A)	IncXI	
SE06	Thompson	AMP-CRO-ATM- SAM	blaCTX-M-65, blaOXA-10, aadA1, aadA2, aadA22, ant(3")-la, aac(3)-lVa, aph(4)-la, dfrA14, cmIA1, floR-Inu(F), ARR-3, tet(A), qnrS1	IncHI2-IncHI2A	
SE10	Diguel	-	aac(6')-laa	-	

Notes: ^aSE: Salmonella Enterica. ^bSerovars confirmed by the SeqSero 1.2 based on WGS. ^d-: none.

Abbreviations: ^cAMP, ampicillin; SAM, ampicillin/sulbactam; SXT, sulfamethoxazole/trimethoprim; ATM, aztreonam; CIP, ciprofloxacin; LEV, levofloxacin; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; SCF, cefoperazone/sulbactam; TZP, piperacillin/tazobactam; IPM, imipenem.

Virulence Factors

Of the 38 *Salmonella* isolates, a total of 333 different VFs were identified across 10 categories (<u>Supplementary Table 1</u>). VFs related to adhesion, effector delivery systems, and motility accounted for 86.8% of the total. Each strain carried approximately 250–282 VFs, with 177 VFs present in all isolated *Salmonella* strains (Table 2). The remaining 156 VFs were present only in some isolates, with varying detection rates.

	Tree s	cale: 0	.1 🕨		I							mpetitive advantage tor
Major virulence factors												ystem ni 55
												ilivery s bial acti irval Metabh Metabh Metabh Metabh Metabh AP-2 AP-2 AP-2
	0				56		112					hherence fector de aume m mume m mume m mume blanc fblanc
							Nomenclature	Date	Specimen	Department	MLST	A H A H A H A H A H A H A H A H A H A H
•						SE30	of genus Enteritidis	2020	blood	Rheumatology & Immunology	ST11	
				_		SE16	Enteritidis	2020	stool	Oncology	ST11	
ļ			_	_		SE10	Enteritidis	2019	blood	Orthopedics	ST11	
			_	_		SE14	Enteritidis	2020	blood	Rheumatology & Immunology	ST11	
ļ				_		SE12	Enteritidis	2020	blood	Rheumatology & Immunology	ST11	
			_	_		SE12	Enteritidis	2021	blood	Intensive Care Unit (ICU)	ST11	
			_	_		SE13	Enteritidis	2019	blood	Vascular Surgery	ST11	
			_	_		SE15	Enteritidis	2019	blood	Intensive Care Unit (ICU)	ST11	
			_			SE04	Enteritidis	2021	blood	Rheumatology & Immunology	ST11	
			_	_		SE04	Enteritidis	2020	blood	Infectious Diseases	ST11	
			_	_		SEST	Enteritidis	2020	blood	Hematology	ST11	
				_		SE25	Enteritidis	2021	blood	Vascular Surgery	ST11	
				_		SE09	Enteritidis	2021	blood	Intensive Care Unit (ICU)	ST11	
I				_		SE17	Enteritidis	2019	blood	Rheumatology & Immunology	ST11	
I			_	_		SE30	Enteritidis	2020	blood	Traditional Chinese Medicine (TCM)	STIL	
I			_	_		SEIS	Enteritidis	2021	blood	Emergency Medicine	ST11	
I			_	_		SE27	Enteritidis	2021	blood	Vascular Surgery	STIL	
I			_	_		SE02	Enteritidis	2019	blood	Rheumatology & Immunology	STIL	
I			_	_		SE20	Enteritidis	2021	stool	Gastroenterology	STIL	
I			_	_		SE19	Anatum	2021	stool	Oncology	ST64	
				—		SE34	London	2021	stool	Gastroenterology	ST155	
			—	_		SE07	London	2019	stool	Henatonathic clinic	ST155	
l		╢_				SE22	Thompson	2021	stool	Geriatric Medicine	ST26	
				_		SEUG	Typhimurium	2019	blood	Rheumatology & Immunology	ST34	
		Ш		_		5E33	Typhimurium	2020	stool	Rheumatology & Immunology	ST 19	
		Ц∟	—	_		SE28	Typhimurium	2019	urine	Urology	ST 19	
				_		SESU	Typhimurium	2020	blood	Hematology	ST128	
						SEII	Indiana	2019	urine	Urology	ST17	
				_	_	SE38	Diguel	2021	blood	Henatonathic clinic	ST5494	
		Ш,		╡.		SEIU	Give	2021	abdominal fluid	General surgery dent	ST516	
		ור		Ч		SE05	Muenster	2021	blood	Henatopathic clinic	ST321	
				L	—[SEUS	Muenster	2020	blood	Emergency Medicine	ST321	
		۲	_		_	SEUI	Senftenberg	2019	stool	Hepatopathic clinic	ST14	
			Ъ			SE21	Rissen	2020	stool	Rheumatology & Immunology	ST469	
		l	<u>ا_</u>			SE00	Cannstatt	2021	secretion	Orthopedics	ST2390	
			Ц			SE08	Kentucky	2020	urine	Urology	ST198	
					_	SE22	Kentucky	2020	stool	Gastroenterology	ST198	
						SE32	Kentucky	2020	stool	Gastroenterology	ST198	
						0002						

Figure 4 The phylogenetic tree and heatmap of the 38 Salmonella isolates from human patients reconstructed based on the whole-genome sequencing-derived SNPs.

Functional Coto come and	Visulant Faster Shared by all the 20 lastetee	The Other VEs New Shound (r. = 154)
Number of Genes	Virulent Factor Shared by all the 38 isolates $(n = 177)$	The Other VFS Non-Shared (n = 156)
	(1 - 177)	
Adherence (n = 120)	STM2689*, csgA, csgB, csgC, csgD, csgE, csgF, csgG, fimA, fimC, fimD, fimF, fimH, fimI, fimW, fimY, fimZ, hcpA, misL, ratB, stbA, stbB, stbC, stbD, steC, sthA, sthB, sthC, sthD, sthE	SEAG_RS23305, SEAG_RS23320, SEAG_RS23325, SNSL254_RS24270, SNSL254_RS24285, SNSL254_RS24290, STM3026, STM4261, STM4571, STM4574, STM4575, bcfA, bcfB, bcfC, bcfD, bcfE, bcfF, bcfG, bcfH, clpE, ehaB, faeD, htpB, lpfA, lpfB, lpfC, lpfD, lpfE, nmpC, pefA, pefB, pefC, pefD, pegA, pegB, pegC, pegD, safA, safB, safC, safD, sefA, sefB, sefC, sefD, shdA, sinH, staA, staB, staC, staD, staE, staF, staG, stbE, stcA, stcB, stcC, stcD, stdA, stdB, stdC, steA, steB, steD, steE, steF, stfA, stfC, stfD, stfE, stfF, stfG, stiA, stiB, stiC, stiH, stjB, stjC, stkA, stkB, stkC, stkD, stkE, stcF, stfC, tcfD, tcfD, stfE, stfF, stfC,
Effector delivery system (n = 116) Motility (n = 53)	STM0266, apeE, clpV, hilA, hilC, hilD, iacP, iagB, invA, invB, invClsctN, invE, invG, invH, invl, invl, orgAlsctK, orgB/SctL, orgC, pipB, prgH, prgl, prgl, prgK, sicA, sicP, sifA, sipAlsspA, sipBlsspB, sipClsspC, sipD, sopA, sopBl sigD, sopD, sopD2, sopE2, spa0/sctQ, spaP, spaQ, spaR, spaS, spiClssaB, sprB, sptP, ssaC, ssaD, ssaG, ssaH, ssal, ssaJ, ssaK, ssaL, ssaM, ssaN, ssaP, ssaQ, ssaR, ssaS, ssaT, ssaU, ssaV, sscA, sscB, sseA, sseB, sseD, sseE, sseG, sseJ, ssrA, ssrB, steC cheA, cheB, cheM, cheR, cheW, cheY, cheZ, flgA, flgB, flgC, flgD, flgE, flgF, flgG, flgH, flgI, flgI, flgI, fliZ, fliF, fliG, fliH, fliI, fliI, fliK, fliL, fliM, fliN, fliO, fliF, fliQ, fliR, fliG, fliH, fliI, fliI, fliK, fliL, fliM, fliN, fliO, fliP, fliQ, fliR, fliG, fliH, fliI, fliI, fliK, fliL, fliM, fliN, fliO, fliP, fliQ, fliR,	SKE, SKG, SKG, UJA, UJB, UJC, UJD SG_RS05215, SG_RS05220, STM0267, STM0268, STM0269, STM0270, STM0271, STM0272, STM0273, STM0274, STM0276, STM0278, STM0279, STM0280, STM0281, STM0282, STM0283, STM0284, STM0285, STM0286, STM0287, STM0289, STM0290, avrA, gogB, pipB2, sifB, slrP, sopE, spvC, spvD, ssaE, ssaO, ssaX, sseC, sseF, ssel/srfH, sseK1, sseK2, sseL, sspH2, steA, tae4, tlde1 fliC, fljA, fljB
Nutritional/Metabolic factor (n = 16)	יווג, יווז, יווד, יווב, יווג, יחטבא, יחטנא entE, entF, fepA, fepD, fes, iroB, iroC, iroD, iroE, iroN, metC	entA, entB, entS, fepG, mgtB
Immune modulation (n = 10)	gndA, IbxC, ugd	galF, gtrA, gtrB, rck, rfbH, rfbK1. tcpC
Regulation $(n = 5)$	fur, phoP, phoQ, rcsB, rpoS	
Exotoxin (n = 5)		cdtB, hlyE, pltA, pltB, spvB
Antimicrobial activity/Competitive	acrA, acrB, mig-14	mig-5
advantage (n = 4)		
Invasion (n = 3)	STM0306, ibeC, ompA	
Invasion Stress survival (n = 1)		sodCl

Table 2 The Functional Category of 333 Virulent Factors in the 38 Salmonella Isolates

Notes: ^a-: none. STM2689*: a pseudogene.

Notably, we identified a significant number of T3SS-related VFs, including the *prg, hil, org, inv*, and *spa* operons encoded by SPI-1 and the *ssa, ssr, ssc*, and *sse* operons encoded by SPI-2. These T3SS-associated VFs showed extremely high prevalence rates, with *sseC, sseF, sseI/srfH, sseK1, sseK2, sseL*, and *sspH2* occurring at rates ranging from 63.2% to 97.4%. Additionally, the remaining VFs of SPI-1 and SPI-2, with the exception of *ssaX*, were prevalent in all 38 isolates.

Furthermore, we found that the same *Salmonella* serovars shared identical or similar VF profiles (Figure 4), and some VFs were present only in specific serovars. The comparison of VFs between BSI-causing and non-BSI-causing strains showed that the distribution of 7 VFs was significantly higher among the BSI-causing strains (P<0.01), These included effector delivery system genes (*ssaO* and *ssel/srfH*), immune modulation genes (*rck* and *rf1*), exotoxin (*spvB*), antimicrobial activity/competitive advantage (*mig-5*), and stress survival (*sodCI*).



Figure 5 Growth curves of 22 Salmonella isolates causing BSIs.



Figure 6 Anti-serum killing ability of randomly selected Salmonella isolates from that causing BSI.

Pathogenic Ability

The growth curve showed that the 22 *Salmonella* strains causing BSIs had comparable growth abilities, except for two strains that exhibited rapid growth ability (Figure 5). However, they did not show strong resistance to anti-serum killing (Figure 6). Additionally, 16 (72.7%) isolates were found to be weak biofilm producers, and the other 6 isolates were non-biofilm producers (Figure 7A). Of note, significant differences in biofilm formation were observed between ST11 and non-ST11 *Salmonella* isolates (Figure 7B). The biofilm formation of *S*. Entertidis was significantly higher than that of *S*. Typhimurium and *S*. Muenster (Figure 7C).

Discussion

Salmonella infections are a global public health problem due to the pathogen's high pathogenicity. BSIs caused by this pathogen occur mainly in children in low-income countries, such as those in Africa, often resulting in prolonged hospitalization and high mortality. Therefore, data on the epidemiology of BSIs caused by Salmonella at the regional level or in resource-limited settings are crucial. In this study, we provided data on the molecular and clinical characteristics of Salmonella isolates causing BSIs to better design new prevention methods and to identify the need for optimized therapeutic strategies.

We found that S. Enteritidis was the most common serovar, followed by S. Typhimurium, which is in line with previous research,¹⁸ indicating that S. Enteritidis and S. Typhimurium are the primary pathogens responsible for



Figure 7 Biofilm formation. (A) Biofilm formation of 22 Salmonella isolates causing BSI. (B) Comparison of biofilm formation ability between STII and non-STII Salmonella isolates. (C) Comparison of biofilm formation ability among distinct servoras. **** P<0.0001.

Salmonella infections in humans. Notably, this is the first time that *S*. Diguel has been implicated in human infections. *Salmonella* infections were found to occur predominantly in elderly patients with severe disease during autumn, which is consistent with previous studies.^{19–21} Additionally, in our hospital, the *Salmonella* isolates causing BSIs were mainly found in patients with multiple underlying diseases, likely related to impaired immunity. Host factors that increase susceptibility to invasive non-typhoidal *Salmonella* infections include immunosuppression, corticosteroids, hematological malignancies, bone marrow and solid organ transplantation.^{22,23}

Further, the high frequency of resistance to ampicillin and ampicillin/sulbactam is similar to previous studies.²⁴ Thus, these antibiotics should be cautiously prescribed for the clinical treatment of infections caused by these strains. Fortunately, the observed frequencies of resistance towards aztreonam, third- and fourth-generation cephalosporins were lower than those previously reported.^{20,25} Additionally, the distribution of MDR strains observed in this study was lower than that reported in Argentina,²⁴ the USA,²⁶ and China.²⁰ This suggests that the third- and fourth-generation cephalosporins may still be effective for the clinical treatment of *Salmonella* infections in our hospital. The high prevalence of *bla*TEM-1B in our study is in line with previous studies.²⁷ Such widespread distribution of *bla*TEM-1B should be monitored, as the extensive IS26-associated gene amplification of *bla*TEM-1 mediates resistance to piper-acillin/tazobactam, cefoperazone/sulbactam, and ampicillin/sulbactam.²⁸ Furthermore, only three *qnrS1* genes were identified in this study, confirming that mutations in the quinolone resistance-determining regions of *gyrA* and *parC* were the primary mechanisms responsible for fluoroquinolone resistance. It is worth noting that the identification of multiple ARGs alerts us the potential public health issue posed by MDR *Salmonella* spp. due to their rapid spread among humans and within animal production chains.¹¹ Therefore, the urgent need to enhance surveillance of such strain in both of human medicine and veterinary practice is highlighted.

Moreover, multiple plasmid replicons identified in our study may be associated with the spread of VFs and/or ARGs. It is important to note that IncFIB(S) and IncFII(S) replicons were exclusively detected in *S*. Enteritidis and *S*. Typhimurium, which is in accordance with previous research.^{27,29} These plasmid replicons are associated with ARGs, cytotoxins, and adhesion factors, which confer hypervirulent bacterial fitness.³⁰ The plasmid profile IncFIB(S)-IncFII(S)-IncX1, which was present in most of the 19*S*. Enteritidis isolates, was similar to the profile found in human and chicken isolates in Singapore.³¹ Given that all IncFIB(S)-IncFII(S)-IncX1 sequences were located in a single contig, it is possible that this is a compatible plasmid that contributes to bacterial diversification and adaptation through horizontal gene transfer.³²

More importantly, 16 out of the 22 BSIs cases in our study were caused by *S*. Enteritidis ST11, which exhibited clonal dissemination. As we know, *S*. Enteritidis ST11 is the most common clone^{33,34} and the primary cause of bacteremia in children and adults in Africa.^{35,36} However, *Salmonella*-related BSIs are rare in China. To the best of our knowledge, this is the first report of clonally disseminated *S*. Enteritidis ST11 causing BSIs. Unfortunately, epidemiological contact surveys were not implemented in a timely manner when patient clinical information was available. Therefore, it is unclear whether these patients were infected by the consumption of contaminated food or by another route. Furthermore, we found that the same STs exhibited significant genetic similarity, highlighting the need to strengthen infection control measures to prevent outbreaks. In addition, we report for the first time that *S*. Diguel and *S*. Muenster cause human BSIs. Moreover, in contrast to a previous study that demonstrated a direct correlation between serovars and STs,³⁷ the Typhimurium serovar in this study had various STs, suggesting the rapid evolution of Typhimurium. To the best of our knowledge, this is the first time that *S*. Senftenberg ST2390 and *S*. Diguel ST5494 were identified.

The pathogenicity of Salmonella spp, closely related to the expression of VFs, significantly affects the severity of human infections caused by this pathogen.³⁸ Our study detected a large number of VFs in all Salmonella strains, indicating the potential pathogenicity of these strains in our hospital. Additionally, a considerable portion of adhesionrelated genes contributed to biofilm formation, enhanced bacterial adhesion and colonization abilities, which are crucial for bacterial pathogenicity. However, strong biofilm formation ability was not observed in these BSI-causing isolates. Although all isolates harbored typical VFs from SPI-1 and SPI-2, which are involved in host cell invasion, host cell apoptosis inhibition, intracellular survival, and replication promotion,³⁹ the growth and anti-serum killing ability evaluated in our study did not appear to be strong regarding the VFs present in the strains. These differences may be due to variations in the expression of VFs. Moreover, all S. Enteritidis isolates carried the invA, hilA, sipA, sipC, sefA, sopB, ssrA, sopE, and sopE2 genes, which is consistent with previous research,^{6,40} indicating that these VFs may form the basis for the basic pathogenic ability in S. Enteritidis. It is important to note that strains causing BSIs have a higher prevalence of seven VFs. Among them, *ssel* may attenuate the host's ability to clear bacteria by obstructing the migration of host immune cells;⁴¹ Rck, as an outer membrane protein, mimics natural host cell ligands and triggers engulfment of the bacterium by interacting with the epidermal growth factor receptor;⁴² SpvB is a pLST-encoded cytotoxic protein associated with enhanced Salmonella survival and intracellular replication.⁴³ Whereas the functions of the remaining 4 VFs remain unknown. Taking all factors into account, as most of the patients with BSIs in our study were of advanced age and had multiple underlying diseases, combined with the pathogenicity of the bacteria, we speculate that the occurrence of BSIs is mainly due to the weakened immune systems of the patients.

Our study has limitations. Firstly, the sample size was small, with less than 40 cases, making it difficult to draw accurate and reliable statistical Conclusions. Secondly, it was a single-centre study, which limits the generalisability of our Results.

Conclusions

The BSIs were mainly caused by *S*. Enteritidis ST11, which not only carried multiple ARGs, VFs and plasmid replicons, but also showed clonal spread. However, these isolates did not exhibit strong abilities in growth, biofilm formation or anti-serum killing. This is the first time we have provided data on clonally disseminated *S*. Enteritidis ST11 causing BSIs, highlighting the urgency of implementing infection control measures.

Nucleotide Sequence GenBank BioProject Numbers

All the sequences were deposited into the NCBI Sequence Read Archive (SRA). The Whole Genome Shotgun BioProject for these isolates has been deposited at GenBank, the BioProject number was PRJNA882512.

Abbreviations

AMP, Ampicillin; ANI, Average Nucleotide Identity; ARGs, Antibiotic-Resistance Genes; AST, Antimicrobial Susceptibility Testing; ATM, Aztreonam; BSIs, Bloodstream Infections; CAZ, Ceftazidime; CIP, Ciprofloxacin; CLSI, Clinical Laboratory Standard Institute; CRO, Ceftriaxone; DALYs, Disability-adjusted life years; ETP, Ertapenem; FEP, Cefepime; IPM, Imipenem; LEV, Levofloxacin; LPS, Lipopolysaccharide; MIC, Minimum Inhibitory Concentration; MDR, Multi-drug resistant; MLST, Multi-locus sequence typing; VFDB, Virulence factor database; PMQRs, Plasmid mediated quinolone resistance genes; pSTV, Virulence-associated plasmid; SAM, Ampicillin/sulbactam; SCF, Cefoperazone/sulbactam; SNP, Single nucleotide polymorphism; SPIs, *Salmonella* pathogenicity islands; ST, Sequence typing; STs, Sequence types; SXT, Sulfamethoxazole/trimethoprim; T3SS, Type III secretion systems; TZP, Piperacillin/ tazobactam; VFs, Virulence factors; WGS, Whole Genome Sequencing; WHO, World Health Organization.

Data Sharing Statement

All data generated or analysed during this study are included in this published article.

Ethics Approval

This program has been approved by the Ethics Committee of the Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University (The approval number is 2023-390). The need of informed consent was waived by the Ethics Committee of the Nanjing Drum Tower Hospital due to anonymous and retrospective study design. All methods were performed in accordance with the relevant guidelines and regulations.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was funded by the National Natural Science Foundation of China (Grant numbers 81902124) and National Key research & development Plan Project (SQ2018YFGH000345).

Disclosure

The authors declare no competing interests in this work.

References

1. Jajere SM. A review of Salmonella enterica with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. *Vet World*. 2019;12(4):504–521. published Online First: 20190406. doi:10.14202/vetworld.2019.504-521

Andino A, Hanning I. Salmonella enterica: survival, colonization, and virulence differences among serovars. Sci World J. 2015;2015;520179. published Online First: 20150113. doi:10.1155/2015/520179

Graham SM, Molyneux EM, Walsh AL, Cheesbrough JS, Molyneux ME, Hart CA. Nontyphoidal Salmonella infections of children in tropical Africa. *Pediatr Infect Dis J.* 2000;19(12):1189–1196. doi:10.1097/00006454-200012000-00016

^{4.} Parisi A, Crump JA, Glass K, et al. Health outcomes from multidrug-resistant salmonella infections in high-income countries: a systematic review and meta-analysis. *Foodborne Pathog Dis.* 2018;15(7):428–436.published Online First: 20180406. doi:10.1089/fpd.2017.2403

Ma Y, Li M, Xu X, et al. High-levels of resistance to quinolone and cephalosporin antibiotics in MDR-ACSSuT Salmonella enterica serovar Enteritidis mainly isolated from patients and foods in Shanghai, China. Int J Food Microbiol. 2018;286:190–196. doi:10.1016/j.ijfoodmicro.2018.09.022

- Zou M, Keelara S, Thakur S. Molecular characterization of Salmonella enterica serotype Enteritidis isolates from humans by antimicrobial resistance, virulence genes, and pulsed-field gel electrophoresis. *Foodborne Pathog Dis.* 2012;9(3):232–238. published Online First: 20120127. doi:10.1089/fpd.2011.1012
- Fardsanei F, Soltan Dallal MM, Douraghi M, et al. Genetic diversity and virulence genes of Salmonella enterica subspecies enterica serotype Enteritidis isolated from meats and eggs. *Microb Pathog*. 2017;107:451–456. published Online First: 20170420. doi:10.1016/j.micpath.2017.04.026
- 8. Coburn B, Grassl GA, Finlay BB. Salmonella, the host and disease: a brief review. *Immunol Cell Biol*. 2007;85(2):112–118. published Online First: 20061205. doi:10.1038/sj.icb.7100007
- 9. Perez JC, Groisman EA. Transcription factor function and promoter architecture govern the evolution of bacterial regulons. *Proc Natl Acad Sci* U S A. 2009;106(11):4319–4324. published Online First: 2009/03/03. doi:10.1073/pnas.0810343106
- 10. Paudyal N, Pan H, Elbediwi M, et al. Characterization of Salmonella Dublin isolated from bovine and human hosts. *BMC Microbiol.* 2019;19 (1):226.published Online First: 20191016. doi:10.1186/s12866-019-1598-0
- D VTN, Venkitanarayanan K, Kollanoor Johny A. Antibiotic-resistant salmonella in the food supply and the potential role of antibiotic alternatives for control. Foods. 2018;7(10). published Online First: 20181011. doi:10.3390/foods7100167
- 12. Timsit JF, Ruppe E, Barbier F, Tabah A, Bassetti M. Bloodstream infections in critically ill patients: an expert statement. *Intensive Care Med.* 2020;46(2):266–284. published Online First: 20200211. doi:10.1007/s00134-020-05950-6
- 13. Performance standards for antimicrobial susceptibility testing, M100, 33rd ed. Wayne, PA: Clinical and Laboratory Standards Institute ; 2023. www.clsi.org.
- Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics*. 2014;30(14):2068–2069. published Online First: 20140318. doi:10.1093/ bioinformatics/btu153
- Chen L, Yang J, Yu J, et al. VFDB: a reference database for bacterial virulence factors. Nucleic Acids Res. 2005;33:D325–8. doi:10.1093/nar/ gki008
- Brynildsrud O, Bohlin J, Scheffer L, Eldholm V. Erratum to: rapid scoring of genes in microbial pan-genome-wide association studies with Scoary. Genome Biol. 2016;17(1):262. published Online First: 20161219. doi:10.1186/s13059-016-1132-8
- 17. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*. 2014;30(9):1312–1313. published Online First: 20140121. doi:10.1093/bioinformatics/btu033
- Mughini-Gras L, Pijnacker R, Duijster J, et al. Changing epidemiology of invasive non-typhoid Salmonella infection: a nationwide population-based registry study. *Clin Microbiol Infect*. 2020;26(7):941 e9–41 e14.published Online First: 20191121. doi:10.1016/j.cmi.2019.11.015
- Liang B, Xie Y, He S, et al. Prevalence, serotypes, and drug resistance of nontyphoidal Salmonella among paediatric patients in a tertiary hospital in Guangzhou, China, 2014–2016. J Infect Public Health. 2019;12(2):252–257.published Online First: 20181119. doi:10.1016/j.jiph.2018.10.012
- Yue M, Liu D, Li X, et al. Epidemiology, serotype and resistance of salmonella isolates from a children's hospital in Hangzhou, Zhejiang, China, 2006–2021. Infect Drug Resist. 2022;15:4735–4748. published Online First: 20220822. doi:10.2147/IDR.S374658
- 21. Medalla F, Gu W, Friedman CR, et al. increased incidence of antimicrobial-resistant nontyphoidal salmonella infections, United States, 2004–2016. Emerg Infect Dis. 2021;27(6):1662–1672. doi:10.3201/eid2706.204486
- 22. Fierer J. Invasive Non-typhoidal Salmonella (iNTS) Infections. Clin Infect Dis. 2022;75(4):732-738. doi:10.1093/cid/ciac035
- Gordon MA. Salmonella infections in immunocompromised adults. J Infect. 2008;56(6):413–422. published Online First: 20080512. doi:10.1016/j. jinf.2008.03.012
- 24. Parada J, Galas M, Faccone D, Tamiozzo P, Carranza A. Antibiotic resistance and associated resistance determinants in different Salmonella enterica serovars isolated from pigs in Argentina. Vet World. 2022;15(5):1215–1220. published Online First: 20220520. doi:10.14202/vetworld.2022.1215-1220
- 25. Zeng S, Zhuo Z, Huang Y, et al. Prevalence of Chromosomally Located bla(CTX-M-55) in Salmonella Typhimurium ST34 Isolates Recovered from a Tertiary Hospital in Guangzhou, China. *Microbiol Spectr.* 2022;10(3):e0277121.published Online First: 20220526. doi:10.1128/spectrum.02771-21
- 26. Xu Y, Tao S, Hinkle N, Harrison M, Chen J. Salmonella, including antibiotic-resistant Salmonella, from flies captured from cattle farms in Georgia, U.S.A. Sci Total Environ. 2018;616–617:90–96. published Online First: 20171116. doi:10.1016/j.scitotenv.2017.10.324
- Chen J, Ed-Dra A, Zhou H, Wu B, Zhang Y, Yue M. Antimicrobial resistance and genomic investigation of non-typhoidal Salmonella isolated from outpatients in Shaoxing city, China. Front Public Health. 2022;10:988317. published Online First: 20220913. doi:10.3389/fpubh.2022.988317
- Hubbard ATM, Mason J, Roberts P, et al. Piperacillin/tazobactam resistance in a clinical isolate of Escherichia coli due to IS26-mediated amplification of bla(TEM-1B). Nat Commun. 2020;11(1):4915.published Online First: 20201001. doi:10.1038/s41467-020-18668-2
- 29. Li Y, Kang X, Ed-Dra A, et al. Genome-Based Assessment of Antimicrobial Resistance and Virulence Potential of Isolates of Non-Pullorum/ Gallinarum Salmonella Serovars Recovered from Dead Poultry in China. *Microbiol Spectr.* 2022;10(4):e0096522.published Online First: 20220621. doi:10.1128/spectrum.00965-22
- 30. Zhao Q, Feng Y, Zong Z. An integrated IncFIB/IncFII plasmid confers hypervirulence and its fitness cost and stability. Eur J Clin Microbiol Infect Dis. 2022;41(4):681–684. published Online First: 20220119. doi:10.1007/s10096-022-04407-6
- 31. Aung KT, Khor WC, Ong KH, et al. Characterisation of Salmonella Enteritidis ST11 and ST1925 Associated with Human Intestinal and Extra-Intestinal Infections in Singapore. Int J Environ Res Public Health. 2022;19(9). published Online First: 20220506. doi:10.3390/ ijerph19095671
- 32. Muthuirulandi Sethuvel DP, Anandan S, Devanga Ragupathi NK, et al. IncFII plasmid carrying antimicrobial resistance genes in Shigella flexneri: vehicle for dissemination. J Glob Antimicrob Resist. 2019;16:215–219. published Online First: 20181018. doi:10.1016/j.jgar.2018.10.014
- Yan S, Zhang W, Li C, et al. Serotyping, MLST, and Core Genome MLST Analysis of Salmonella enterica From Different Sources in China During 2004–2019. Front Microbiol. 2021;12:688614. published Online First: 20210916. doi:10.3389/fmicb.2021.688614
- 34. Park CJ, Li J, Zhang X, Gao F, Benton CS, Andam CP. Diverse lineages of multidrug resistant clinical Salmonella enterica and a cryptic outbreak in New Hampshire, USA revealed from a year-long genomic surveillance. *Infect Genet Evol.* 2021;87:104645. published Online First: 20201125. doi:10.1016/j.meegid.2020.104645
- 35. Feasey NA, Dougan G, Kingsley RA, Heyderman RS, Gordon MA. Invasive non-typhoidal salmonella disease: an emerging and neglected tropical disease in Africa. *Lancet*. 2012;379(9835):2489–2499. published Online First: 20120514. doi:10.1016/S0140-6736(11)61752-2
- 36. Park SE, Pham DT, Pak GD, et al. The genomic epidemiology of multi-drug resistant invasive non-typhoidal Salmonella in selected sub-Saharan African countries. *BMJ Glob Health*. 2021;6(8). doi:10.1136/bmjgh-2021-005659

- 37. Ben Hassena A, Haendiges J, Zormati S, et al. Virulence and resistance genes profiles and clonal relationships of non-typhoidal food-borne Salmonella strains isolated in Tunisia by whole genome sequencing. Int J Food Microbiol. 2021;337:108941. published Online First: 20201028. doi:10.1016/j.ijfoodmicro.2020.108941
- Dos Santos AMP, Ferrari RG, Conte-Junior CA. Virulence factors in salmonella typhimurium: the sagacity of a bacterium. *Curr Microbiol*. 2019;76 (6):762–773. published Online First: 20180521. doi:10.1007/s00284-018-1510-4
- Deng W, Marshall NC, Rowland JL, et al. Assembly, structure, function and regulation of type III secretion systems. *Nat Rev Microbiol*. 2017;15 (6):323–337.published Online First: 20170410. doi:10.1038/nrmicro.2017.20
- 40. Campioni F, Moratto Bergamini AM, Falcao JP. Genetic diversity, virulence genes and antimicrobial resistance of Salmonella Entertitidis isolated from food and humans over a 24-year period in Brazil. *Food Microbiol.* 2012;32(2):254–264. published Online First: 20120713. doi:10.1016/j. fm.2012.06.008
- McLaughlin LM, Govoni GR, Gerke C, et al. The Salmonella SPI2 effector Ssel mediates long-term systemic infection by modulating host cell migration. *PLoS Pathog.* 2009;5(11):e1000671.published Online First: 20091126. doi:10.1371/journal.ppat.1000671
- 42. Mambu J, Virlogeux-Payant I, Holbert S, Grepinet O, Velge P, Wiedemann A. An updated view on the rck invasin of salmonella: still much to discover. *Front Cell Infect Microbiol.* 2017;7:500. published Online First: 20171208. doi:10.3389/fcimb.2017.00500
- 43. Yang S, Deng Q, Sun L, et al. Salmonella Effector SpvB Inhibits NF-kappaB Activity via KEAP1-Mediated Downregulation of IKKbeta. *Front Cell Infect Microbiol*. 2021;11:641412. published Online First: 20210318. doi:10.3389/fcimb.2021.641412

Infection and Drug Resistance

Dovepress

DovePress

2377

F 🔰

in 🗖

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/infection-and-drug-resistance-journal