Infection and Drug Resistance
Dovepress
open access to scientific and medical research

Open Access Full Text Article
ORIGINAL RESEARCH

# A Food Poisoning Caused by *Salmonella Enterica* (*S. Enteritidis*) ST11 Carrying Multi-Antimicrobial Resistance Genes in 2019, China

Zhiyi Zhang[1,2,*], Baisheng Li[3,4,*], Huitao Huang[5], Yanmei Fang[5], Wenqiang Yang[5]

[1]School of Public Health, Guangdong Pharmaceutical University, Guangzhou, People's Republic of China; [2]Faculty of Health Sciences, University of Macau, Macao, People's Republic of China; [3]Institute of Microbiology, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, People's Republic of China; [4]Guangdong Provincial Key Laboratory of Pathogen Detection for Emerging Infectious Disease Response, Guangdong Provincial Center for Disease Control and Prevention, Guangzhou, People's Republic of China; [5]Institute of Microbiology, Zhuhai Center for Disease Control and Prevention, Zhuhai, People's Republic of China

*These authors contributed equally to this work

Correspondence: Wenqiang Yang, Zhuhai Center for Disease Control and Prevention, Zhuhai, 519000, People's Republic of China, Email 917461182@qq.com

**Purpose:** This study was to identify and analyze the pathogen responsible for food poisoning in a tourist group traveling from Macao to Zhuhai.

**Patients and Methods:** Samples were obtained from 27 patients of 96 cases, as well as samples of contaminated food in Macau. The collected samples were subjected to serological identification, drug sensitivity analysis, drug resistance gene identification, virulence factor analysis, and tracing.

**Results:** Twenty-six isolates and the salad isolate were *S. enteritidis* ST11. Isolates from patients were exhibited significant resistance to Penicillin AMP (Ampicillin) and quinolones NAL (Nalidixic acid). Among these isolates, 21 strains were resistant to two or more antibiotics, indicating the multi-drug resistance (MDR). Genomic characteristics and phylogenetic analysis were performed on 9 of the isolates using whole genome sequencing (WGS). The analysis revealed that the resistance to AMP and NAL was primarily caused by a gryA mutation D87Y (9/9, 100%), and the presence of beta-lactam resistance genes blaOXA-1 (1/9, 11.11%), blaTEM-141 (1/9, 11.11%), and blaTEM-1B (8/9, 88.89%). It was also found a strains isolated from patients had two resistance genes to quinolones or beta-lactam drugs (1/8, 12.5%), respectively. The strains were found to possess 165 virulence genes, one adherence class virulence factor, one invasion class virulence factor and various pathogenicity islands, including SPI-1, SPI-2, SPI-3, SPI-4, SPI-5, SPI-9, SPI-10, SPI-13, SPI-14, SPI-15, SGI 1, CS54_island, and C63PI-1. Additionally, the virulence plasmids were detected, including IncFIB(s)-IncFII(s)-IncX1 (55.56%), IncFIB(s)-IncFII(s) (33.33%), and IncFIB(s)-IncFII(s)-IncHI2-IncHI2A (11.11%). PFGE (Pulsed Field Gel Electrophoresis) and phylogenetic tree analysis revealed a high degree of similarity between Salmonella isolates from patients and food samples from Macao.

**Conclusion:** This study identified Salmonella *enterica* ST11 as the cause of the food poisoning outbreak. The findings highlight the importance of phenotypic characterization and next-generation sequencing (NGS) tools in epidemiological studies and emphasize the potential risk of a new emerging multi-antibiotic ST11 clone for *S. enteritidis*.

**Keywords:** salmonella food poisoning, sequence type 11, whole-genome sequencing, antimicrobial resistance, multi-locus sequence typing

## Introduction

Foodborne diseases are a significant global public health issue, contributing to both mortality and morbidity rates.[1] Salmonellosis is a commonly reported foodborne disease worldwide.[2] In China, Salmonella is responsible for 70% of diarrheal cases caused by foodborne diseases.[3] Additionally, Salmonella is one of the four main global causes of diarrheal diseases.[4] Salmonella, known for causing foodborne diseases, has been responsible for numerous outbreaks of food poisoning.[5] For instance, there have been reported incidents of Salmonella infection in various countries and regions



Received: 13 December 2023
Accepted: 27 March 2024
Published: 6 May 2024

© 2024 Zhang et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v3.0) License (http://creativecommons.org/licenses/by-nc/3.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

worldwide, including the outbreak of Salmonella infection in canned sardines in Colombia,[6] and the contamination of water leading to Salmonella infection in Greece.[7] Previous research had shown that America is frequently affected by Salmonella outbreaks since there have been incidents related to Salmonella contamination in onions, sweetened puffed wheat, and packaged leafy greens.[8–10] In China, retail meat in supermarkets and open-air markets in Guangxi, and two universities in Xiamen were also reported Salmonella food contamination and poisoning.[11,12]

Salmonella is a gram-negative bacterium that primarily affects children, pregnant women, and the elderly.[13] According to the White-Kaufman Scheme, Salmonella is classified into two types: Salmonella *bongori* and Salmonella *enterica* (*S. enteritidis*).[14] There are more than 2500 known serotypes of Salmonella.[15] The global impact of non-typhoid Salmonella on human health is significant, with an estimated 93.8 million patients, out of which approximately 80.3 million are foodborne cases, leading to 155,000 deaths annually.[16] Furthermore, antimicrobial resistance is a major global public health concern, and Salmonella is one of the microorganisms where resistant serotypes have emerged, affecting the food chain.[4]

Salmonella strains with resistance to antimicrobial drugs are now widespread worldwide.[17] Previous research had shown that nalidixic acid, sulfamethoxazole, and ciprofloxacin were the top three antibiotics to which resistance genes to quinolone resistance were mostly mediated by plasmids quinolone resistance genes [aac(6 ')-Ib-cr, qnrA, qnrB, oqxAB, qepA, qnrS, and qnrD, showed mutations in the determination region of quinolone resistance [gyrA, parC, and parE]. The most common β-lactamase gene was blaTEM-1, followed by blaCTX-M-55, blaCMY-2, and blaxa-1 in China.[18] Others have indicated that almost all types of Salmonella are resistant to AMP, CHL, and SXT. The drug resistance rate of typhimurium and enteritis serotype isolates to AMP were significantly higher compared to other serotypes, and the drug resistance rate of enteritis serotype isolates to SXT was also significantly higher than that of other serotypes.[19] The prevalence of these resistant genes demonstrates the tendency of Salmonella bacteria to respond to nalidixic acid, sulfamethoxazole, and ciprofloxacin, providing evidences for treatment of Salmonella bacteria and warning governments to reduce the use of these antibiotics.

On July 24th, 2019, a food poisoning caused by Salmonella *enterica* (*S. enteritidis*) ST11 occurred in five tour groups traveling from Zhuhai to Macao, involving 96 persons, and the main clinical manifestations of the 96 clinical cases were abdominal pain (94%), diarrhea (95%), nausea (80%), fever (75%) and so on. However, there are few reports of associated cases in mainland China and Macau. In this study, we aimed to investigate the genomic characteristics of these ST11 strains by using whole-genome sequencing (WGS). In particular, we analyzed the isolates for virulence, drug resistance, phenotype, and plasmids. Moreover, we conducted a phylogenetic analysis of these *S. enteritidis* strains along with the Singapore ST11 strains from food reported by other studies,[20] aiming to gain a better understanding of the characteristics and potential risks associated with the Salmonella strain responsible for the outbreak.

# Methods and Materials

## Epidemiological Investigation

The Zhuhai People's Hospital notified the center that they had received and treated a group of tourists, and the total number of people was 848, and 96 of them were experiencing vomiting and diarrhea. In response, an epidemiological investigation was launched. These cases consisted of both suspected and confirmed cases. Suspected cases were defined as individuals who exhibited symptoms such as fever, abdominal pain, diarrhea, and nausea. These symptoms were observed among mainland tourists who had traveled to Hong Kong and Macao between July 23 and 26, 2019. The confirmed cases were identified as *S. enteritidis* through laboratory culture of clinical cases. Demographic data of the patients were collected through face-to-face interviews with tour guides, tourists, and receiving doctors.

## Isolation and Identification of Salmonella

Samples were collected from willing patients, as well as from salad collected by the Centers for Disease Control in Macao for testing. In total, there were 30 samples including 27 Anal swabs, two vomit, and 1 salad. Samples from patients were obtained through anal swabs, which were then placed in sampling tubes and transported to the laboratory within 2 hours of collection using an ice box. The salad sample was from Macau which was marked and placed in a sterile plastic bag, and also transported to the laboratory within 2 hours of collection using an ice box. The isolation and identification of Salmonella followed the technical specifications of the National Pathogen Identification Network.[21] To experiment, a sterilized swab was used to insert

the sample into a 7mL SBG broth tube,put into a 36±1°C incubator (not CO2) incubated for 18 to 24 hours. On the second day, the sample was inoculated with a 10ul inoculated ring bacteria solution and transferred to the Salmonella Comaca chromogenic medium. The medium was then incubated at a temperature of 36 ± 1°C for 18–24 hours. Subsequently, the plate was cultured overnight to observe the presence of any suspected Salmonella colonies. At least 3–5 suspicious colonies were selected from each specimen and transferred to KIA and MIU. These colonies were then incubated at a temperature of 36 ± 1°C for 18 to 24 hours. The selected colonies were identified based on their K/A, gas production, H2S+ / -, motility, indigo matrix, and urea characteristics for ONPG (O-Nitrophenyl-β-D-Galactopyranoside) identification test.

## Salmonella Serotyping

The specific O and H antisera of bacteria and Salmonella were tested using the slide agglutination test to determine the serotypes. The agglutination test was first performed with polyvalent serum and then with monovalent serum factors. It is important to note that H-phase antigens are divided into H1 phase and H2 phase antigens, and these two phase antigens appear in no particular order. If only one phase of the antigen shows agglutination, the other phase of the antigen needs to be induced in reverse (ie, the induced serum of the known phase of the antigen is used to block the emergence of the H antigen, allowing the expression of the other phase of the antigen). The final serotype is reported for those that match the characteristics of Salmonella through biochemistry and serology according to Kauffman-White Salmonella Antigen List, 9th edition, 2008.

## Antibiotic Susceptibility Testing and Investigation of the Mechanisms Mediating Resistance

Antimicrobial resistance test: In this study, several colonies were selected and placed in 2~3mL of sterile normal saline. The turbidity of the colonies was compared with a 0.5 McGillister turbidimetric tube, and the concentration of the bacterial solution prepared was approximately $1.5\times10^8$cfu/mL. Nutrient broth medium and drug-sensitive medium were then added as negative controls. Next, 60μL of the bacterial solution was taken and mixed into the drug-sensitive culture medium for dilution. Subsequently, an automatic sampling device was used to add the diluted solution to a micro-drug-sensitive slats with 96 holes. The slats were placed in a constant temperature incubator at 35°C for 18 to 20 hours. After incubation, the strips were removed from the incubator and the bottom was gently wiped with a dry towel. The results and analysis report were then read by the microbial identification drug sensitivity analysis system. Alternatively, a visual interpretation of each hole's negative or positive results was conducted, where turbidity indicated a positive result and clarity indicated a negative result. The obtained results were inputted into the software for further analysis.

To investigate drug resistance genes (AMR genes), eight Salmonella strains selected randomly from patients in different travel groups and one from salad sample were subjected for whole genome sequencing. Nextera XT DNA library preparation kit was used to establish the DNA library for sequencing. gDNA was labeled with Nextera transposons and segmented, and then DNA was added with splitter sequences line tag. The labeled DNA was then amplified using a limited cycle PCR procedure, followed by the use of AMPure XP microbeads to purify the library DNA and remove shorter library fragments. Run 1 microliter undiluted library on Agilent Technology 2100 Bioanalyzer using a highly sensitive DNA chip. The number of each library is calibrated, and finally, an equal number of standardized libraries are combined in a test tube. After mixing, the hybrid library is diluted and terminatively denatured before being loaded into the library for sequencing runs. The obtained sequences were then analyzed using the Resfinder website.

## Investigation of the Mechanisms Mediating Resistance and Virulence Gene

The virulence genes of the sequences were investigated by using abricate software and VFDB (Virulence Factor Database) website.

## Multilocus Sequence Typing (MLST) and Pulsed-Field Gel Electrophoresis (PFGE)

The genetic correlation of 27 isolates was studied using pulsed-field gel electrophoresis (PFGE). Additionally, 9 strains with the complete genetic sequence were tested using multi-locus, sequence typing (MLST), and phylogenetic tree analysis. For the multi-locus sequence typing (MLST) of the completely sequenced bacterial genomes, short sequence reads were firstly assembled to draft genomes.[22] For the Whole Genome MLST scheme, the MLST allele of each locus was aligned to the genome using BLAST. After that, the ST was determined by a combination of the MLST alleles after close-matching of the

selected alleles. The MLST typing of all the 27 isolates was based on the comparison of internal sequences of the Salmonella seven housekeeping gene fragments (aroC, dnaN, hemD, hisD, purE, sucA, and thrA). For the PFGE analysis, patterns were generated for the Salmonella isolates following the technical specifications of the National Pathogen Identification Network in China 2017.[21] The procedure involved resuscitating and transferring colonies, preparing a bacterial suspension, and creating a gel with a standard strain. The gel block of cells were then lysed. The gel block was cleaned, and subjected to pre-digestion. Subsequently, the gel was cut, enzyme cutting buffer was added, the electrophoretic glue was made, put electrophoretic solution and electrophoresis was performed. The resulting PFGE data were analyzed using BioNumerics software version 5.0. Finally, BioNumerics 7.0 was used to build the phylotree, and the original data of 9 strains (numbering, ST information, 7 alleles information, etc.) are then constructed according to the software instructions.

# Result

## Descriptive Epidemiology

The prevalence of the disease in the tour group was 11.32% (96/848). Sixty-four of the clinical cases were investigated and found that the main clinical manifestations were abdominal pain (94%), diarrhea (95%), nausea (80%), fever (75%) and so on. Abdominal pain was mainly periumbilical pain (52%). 69% had diarrhea 3–9 times; The main fever was moderate (43%). The male-to-female ratio of the 64 cases was 16:48, and the age range was 16–66. All of them were not severe cases. Thirty samples were collected including 27 anal swabs, 2 vomitus, and a salad. And 27 anal swabs were Salmonella positive (27/29, 93.10%). Another food sample (salad) was also positive, so we selected 27 positive anal swab samples and one salad sample, a total of 28 samples for subsequent analysis.

## Serotyping of Salmonella and Antibiotic Resistance

Twenty-eight isolates were analyzed by the classical serotyping slide agglutination test, following the White–Kauffmann–Le Minor scheme,[23] and all the isolates tested were *S. enteritidis* (1,9,12:g,m:-). Based on the phenotypic antimicrobial resistance pattern of the isolates against the 27 different antimicrobial drugs, only 26 strains did the antimicrobial resistance test since one of the samples' data from the patient was missing and the food sample from Macau only provided serotype and sequence. All the tested strains were found to show resistance to 2 or more antibiotics tested and 21 of them showed multiple drug resistance (21/26, 80.76%) (Table 1). All tested strains were completely resistant to nalidixic acid and Ampicillin, with varying degrees of resistance to other antibiotics. (Figure 1). Moreover, the antimicrobial agent with the most prevalent multidrug resistance spectrum among these 26 strains of *S. enteritidis* was AMP-AMS-TET-CFZ-NAL-Sul-DOX-STR (6/26,23.08%) (Table 2).

**Table 1** The Characterization of Salmonella

| Isolate ID | Source | Serotype | Predicted Antibiotic Phenotype Based on AMR Genes | Sequence Typing (ST) |
|---|---|---|---|---|
| ZH19_SAL_124 | Anal swab | *S. enteritidis* | AMP——————NAL——————————— | ST11 |
| ZH19_SAL_125 | Anal swab | *S. enteritidis* | AMP——————NAL——————————— | ST11 |
| ZH19_SAL_126 | Anal swab | *S. enteritidis* | AMP–TET—————NAL–Sul———————DOX–STR– | ST11 |
| ZH19_SAL_127 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ———NAL–Sul–AMC——————DOX–STR– | ST11 |
| ZH19_SAL_128 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ———NAL–Sul———————DOX–STR– | ST11 |
| ZH19_SAL_129 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ———NAL–Sul———————DOX–STR– | ST11 |

(Continued)

**Table 1** (Continued).

| Isolate ID | Source | Serotype | Predicted Antibiotic Phenotype Based on AMR Genes | Sequence Typing (ST) |
|---|---|---|---|---|
| ZH19_SAL_130 | Anal swab | *S. enteritidis* | AMP-AMS——CFZ————NAL——————————————— | ST11 |
| ZH19_SAL_131 | Anal swab | *S. enteritidis* | AMP———CFZ————NAL——————————————— | ST11 |
| ZH19_SAL_132 | Anal swab | *S. enteritidis* | AMP———CFZ————NAL——————————————— | ST11 |
| ZH19_SAL_133 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ————NAL–Sul——————————DOX–STR– | ST11 |
| ZH19_SAL_134 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ————NAL————————————DOX–STR– | ST11 |
| ZH19_SAL_135 | Anal swab | *S. enteritidis* | AMP-AMS-TET——————NAL————————————DOX–STR– | ST11 |
| ZH19_SAL_136 | Anal swab | *S. enteritidis* | AMP-AMS-TET——————NAL————————————DOX–STR– | ST11 |
| ZH19_SAL_137 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ————NAL–Sul——————————DOX–STR– | ST11 |
| ZH19_SAL_138 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ————NAL–Sul——————————DOX–STR– | ST11 |
| ZH19_SAL_139 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ————NAL–Sul——————————DOX–STR– | ST11 |
| ZH19_SAL_140 | Anal swab | *S. enteritidis* | AMP-AMS———————NAL————————————STR– | ST11 |
| ZH19_SAL_141 | Anal swab | *S. enteritidis* | AMP–TET——————NAL——————————————— | ST11 |
| ZH19_SAL_142 | Anal swab | *S. enteritidis* | AMP–TET—CFZ————NAL——————————————— | ST11 |
| ZH19_SAL_143 | Anal swab | *S. enteritidis* | AMP-AMS-TET—CFZ————NAL————————————DOX–STR– | ST11 |
| ZH19_SAL_144 | Anal swab | *S. enteritidis* | AMP–TET——————NAL–Sul——————————DOX–STR– | ST11 |
| ZH19_SAL_145 | Anal swab | *S. enteritidis* | AMP————————NAL——————————————— | ST11 |
| ZH19_SAL_146 | Anal swab | *S. enteritidis* | AMP–TET——————NAL————————————DOX–STR– | ST11 |
| ZH19_SAL_147 | Anal swab | *S. enteritidis* | AMP–TET——————NAL——————MIN————DOX–STR– | ST11 |
| ZH19_SAL_148 | Anal swab | *S. enteritidis* | AMP————————NAL——————————————— | ST11 |

*(Continued)*

**Table 1** (Continued).

| Isolate ID | Source | Serotype | Predicted Antibiotic Phenotype Based on AMR Genes | Sequence Typing (ST) |
|---|---|---|---|---|
| ZH19_SAL_149 | Anal swab | *S. enteritidis* | —————————————————————————— | ST11 |
| ZH19_SAL_150 | Anal swab | *S. enteritidis* | AMP————————NAL———————————————— | ST11 |
| 19salad_2019_Sal | Food | *S. enteritidis* | —————————————————————————— | ST11 |

**Abbreviations**: AMP, Ampicillin; NAL, Nalidixic; CHL, Chloramphenicol; AMS, Ampicillin – Sulbactame; CTX, Cefotaxime; CFX, Ceftazidime; CTX, Cefoxitin; GEN, Gentamicin; IMI, Imipenem; AZI, Azithromycin; CT, Colistin; PB, Polymyxin B; AMI, Amikacin; AZM, Aztreonam; FEP, Cefepime; MEM, Meropenem; LEV, Levofloxacin; KAN, Kanamycin; TET, Tetracycline; SXT, Cotrimoxazole; CFZ, Cefazolin; CAZ, Ceftazidime; AMC, Amoxicillin-clavulanic acid; DOX, Doxycycline, STR, Streptomycin; Sul, Sulfamisoxazole; CIP, Ciprofloxacin.

## Detection of AMR Genes

To investigate the mechanism of drug resistance to AMP and NAL (class quinolone), we analyzed the resistance gene of 9 Salmonella strains using Resfinder 4.1. Our findings revealed that all 9 strains exhibited a D87Y mutation in the gene

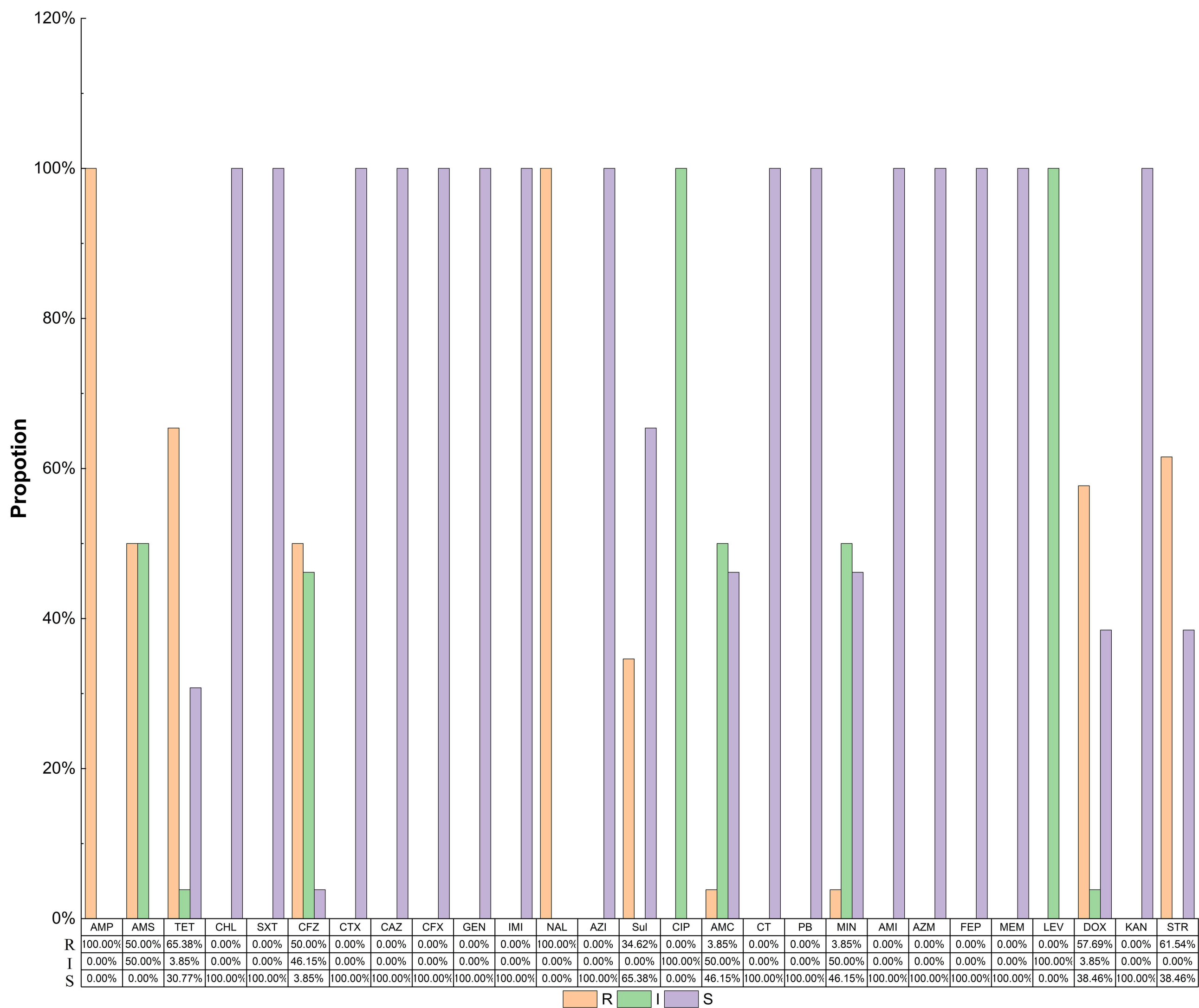


**Figure 1** Distribution of drug resistance in Salmonella *enterica* (*S. enteritidis*).

**Table 2** Drug Resistance Gene Spectrum of Salmonella *Enterica* (*S. Enteritidis*)

| Number of Isolate (%) | Predicted Antibiotic Phenotype Based on AMR Genes |
|---|---|
| 5 (19.23) | AMP——NAL—— |
| 2 (7.69) | AMP–TET——NAL–Sul——DOX–STR– |
| 1 (3.85) | AMP-AMS-TET—CFZ——NAL–Sul–AMC——DOX–STR– |
| 2 (7.69) | AMP-AMS-TET—CFZ——NAL——DOX–STR– |
| 1 (3.85) | AMP-AMS——CFZ——NAL—— |
| 2 (7.69) | AMP——CFZ——NAL—— |
| 2 (7.69) | AMP-AMS-TET——NAL——DOX–STR– |
| 6 (23.08) | AMP-AMS-TET—CFZ——NAL–Sul——DOX–STR– |
| 1 (3.85) | AMP-AMS——NAL——STR– |
| 1 (3.85) | AMP–TET——NAL—— |
| 1 (3.85) | AMP–TET—CFZ——NAL—— |
| 1 (3.85) | AMP–TET——NAL——DOX–STR– |
| 1 (3.85) | AMP–TET——NAL——MIN——DOX–STR– |

**Abbreviations**: AMP, Ampicillin; NAL, Nalidixic; CHL, Chloramphenicol; AMS, Ampicillin – Sulbactame; CTX, Cefotaxime; CFX, Ceftazidime; CTX, Cefoxitin; GEN, Gentamicin; IMI, Imipenem; AZI, Azithromycin; CT, Colistin; PB, Polymyxin B; AMI, Amikacin; AZM, Aztreonam; FEP, Cefepime; MEM, Meropenem; LEV, Levofloxacin; KAN, Kanamycin; TET, Tetracycline; SXT, Cotrimoxazole; CFZ, Cefazolin; CAZ, Ceftazidime; AMC, Amoxicillin-clavulanic acid; DOX, Doxycycline, STR, Streptomycin; Sul, Sulfamisoxazole; CIP, Ciprofloxacin.

gyrA. Additionally, plasmid-mediated Quinolone resistance (PMQR) determinants were detected, specifically qnrA1 + aac(6')-Ib-cr (1/9, 11.11%) (Table 3). However, no mutations were found in gyrB and parC across all strains. According to the 9 strains result, only one strain displayed both gryA mutations and plasmid-mediated quinolone resistance to qnrA1 and aac(6')-Ib-cr, while the others exhibited quinolone resistance solely due to gryA mutations. At the same time, all 9 strains were found to carry the β-lactam AMP resistance gene, and 8 (88.89%) strains were found to contain blaTEM-1B, and 1 (11.11%) contained blaTEM-141 enzyme, respectively. It was also found that 1 (11.11%) strains contained both blaOXA-1 and blaTEM-1B enzymes (Table 3).

## Identification of Virulence Genes and Salmonella Pathogenicity Islands

To investigate and predict the virulence genes of 9 strains, we utilized the VFDB database for comparison. Our analysis revealed a total of 165 virulence genes and one Adherence class virulence factor (see Supplementary Table 1). All 9 *S. enteritidis* isolates contained Salmonella pathogenicity island, SPI-1, SPI-2, SPI-3 to SPI-5, SPI-9, SPI-10, SPI-13, SPI-14, SGI 1, CS54_island and C63PI-1 (see Supplementary Table 2). Specifically, we identified 1 Adherence class virulence factor, one invasion class virulence factor, 15 Autotransporter gene, 54 Fimbrial adherence determinants genes, 2 Macrophage

**Table 3** The Number of Strains with Multiple AMR Genes

| Source | No.of Source | Multiple PMQR Genes in the Strains (%) | Multiple Beta-lactam Genes in the Strains (%) | | | |
|---|---|---|---|---|---|---|
| | | qnrA1+ aac(6')-Ib-cr | blaTEM-1B | blaTEM-141 | blaOXA-1 | blaOXA-1+blaTEM-1B |
| People | 8 | 1 (12.5%) | 7(87.5%) | 1(12.5%) | 1 (12.5%) | 1(12.5%) |
| Food | 1 | 0 (0%) | 1(100%) | 0 (0%) | 0 (0%) | 0 (0%) |

inducible genes, 2 Magnesium uptake genes, 4 Nonfimbrial adherence determinants genes, 2 Regulation genes, 83 genes in virulence island, 1 Serum resistance gene, 1 Stress adaptation gene, and 1 Toxin gene. All 9 strains were found to contain plasmids. Theobserved plasmid profiles were IncFIB(s)-IncFII(s)-IncX1, which accounted for 55.56% of the total 9 strains. Three strains possessed IncFIB(s)-IncFII(s) plasmids, representing 33.33% of the total. Additionally, one strain harbored IncFIB(s)-IncFII(s)-IncHI2-IncHI2A plasmids, making up 11.11% of the strains (see Supplementary Table 3).

## Multi-Locus Sequence Typing Analysis, Pulse-Field Gel Electrophoresis, and Phylogenetic Tree

The 27 S. enteritidis were all assigned to ST11. The XbaI and BIn I digestion was successfully performed on all the isolates according to the previous study.[24] All 27 isolates revealing 12 and 14 DNA bands. With the conditions of 2.2-63.8s and 18-19h, the PFGE analysis produced two pulsotypes, all from the patients, and they shared a similarity score in the region of 96% and 100%, respectively. (Figure 2). Further analysis of isolated *S. enteritidis* strains in the phylogenetic tree and showed that isolates from anal swabs were closely clustered with strains isolated in Salad collected from Macau. (Figures 2 and 3).

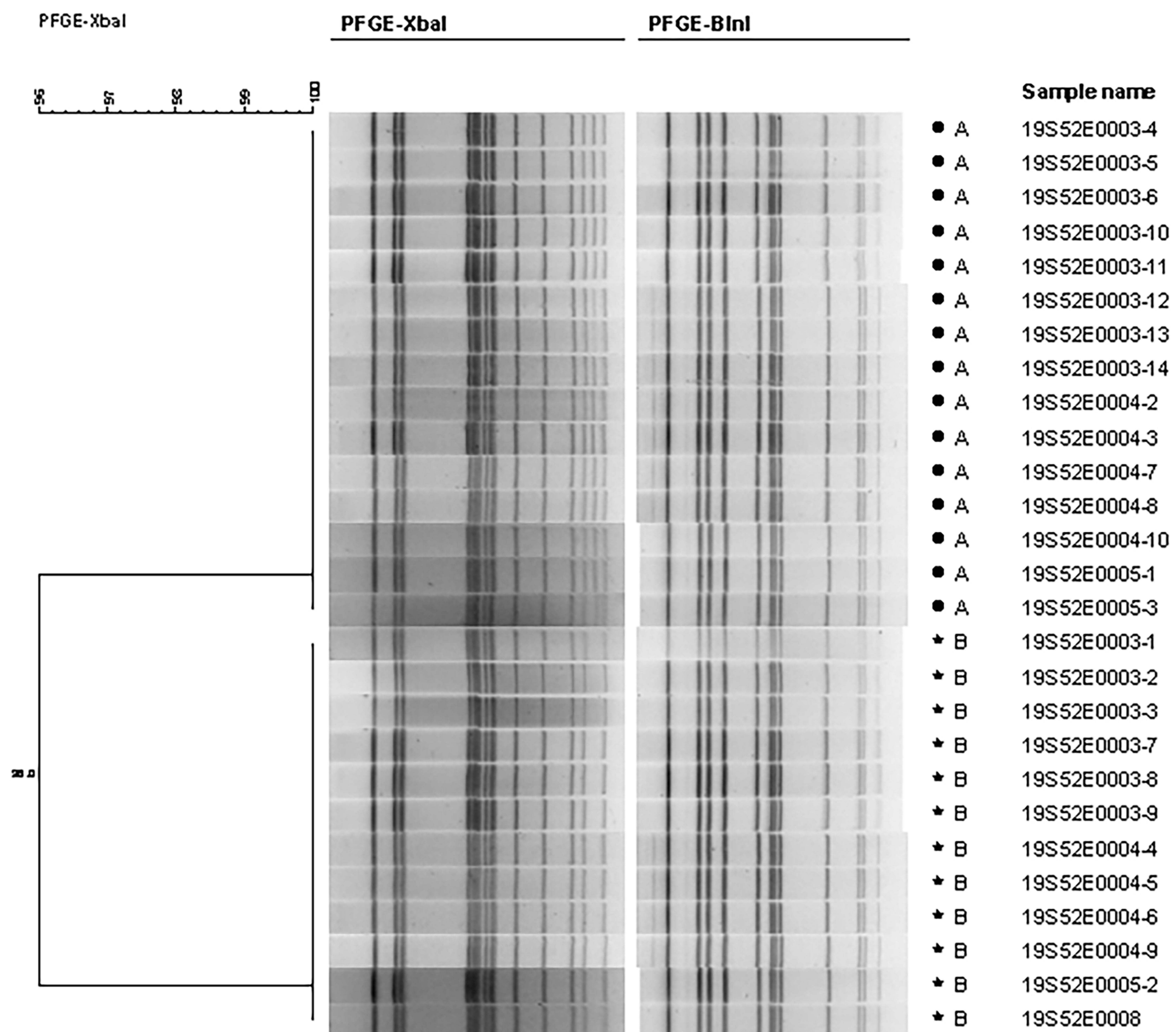


**Figure 2** The PFGE of Salmonella *enterica* (*S. enteritidis*) (**A** and **B** represent different types).

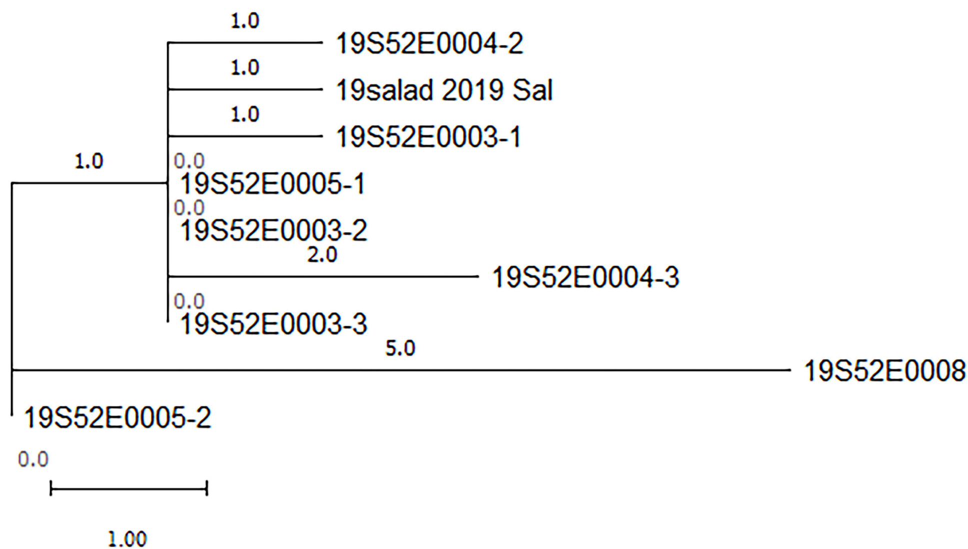


**Figure 3** Phylotree of Salmonella *enterica* (*S. enteritidis*).

## Discussion

Food safety is of utmost importance. According to the World Health Organization, an estimated 1.6 million people worldwide fall ill every day due to consuming unsafe food. Therefore, ensuring the safety of food is crucial for maintaining human health.[25] In this study, the rate of Salmonella cases among the tourists in mainland tour groups to Hong Kong and Macao was found to be 11.31%. This rate is similar to the rate reported by *S. enteritidis* in the UK (14.41%), but significantly lower than the rate reported in Beijing (69.4%).[26,27] Due to the high infection rate of Salmonella and its strong pathogenicity, it still needs to be taken seriously. The joint prevention and control of food supervision in mainland China, Hong Kong, and Macao is of great importance. All regions should intensify their efforts in food supervision. The Salmonella serotype identified examined in this study was *S. enteritidis*. Similarly, two other reports also identified *S. enteritidis* as the causative agent of food poisoning in tourist groups, which the average prevalence rate is 46.67% and 69.29%,[28,29] it can be seen that Salmonella infection is relatively common, and the impact cannot be ignored. This should attract the attention of the regulatory authorities, regulatory authorities are supposed to prioritize the detection of food disease caused by Salmonella bacteria to implement more focused strategies while ensuring food safety.

In this article, the cause of food poisoning was salad. Additionally, another case of food poisoning caused by beef contaminated with Salmonella Hvittingfoss was reported in a Hong Kong-Macau tour group in Zhuhai in 2019.[30] The occurrence of these incidents highlights the need for increased attention and strict supervision by the governments of Guangdong, Hong Kong, and Macao in terms of food safety and the monitoring of raw materials such as salad, and beef that may be potential sources of Salmonella. Given the transboundary nature of these incidents, collaboration between health authorities, food safety agencies, and tourism boards across Hong Kong, Macau, and mainland China is crucial. Sharing information, best practices, and implementing consistent food safety regulations can help mitigate the risk of future outbreaks. Tourists and locals alike should be educated about the risks associated with consuming contaminated food and empowered to make informed choices.

In this study, it was observed that 26 isolates of *S. enteritidis* exhibited the highest resistance to AMP and NAL, which aligns with the findings of two previous studies[31,32] In addition, AMP-AMS-TET-CFZ-NAL-Sul-DOX-STR resistance spectrum had the highest frequency, which was consistent with the existing ST11 Salmonella studies that found a higher proportion of isolates resistant to benzacillin, norfloxacin, piperacillin, and ampicillin/sulbactam, and the studies show higher resistance to ampicillin, piperacillin, and the ampicillin/sulbactam combination.[20] These results serve as a reminder to authorities about the importance of reducing the misuse of NAL and AMP. Furthermore, in the region responsible for determining quinolone resistance in Salmonella enteritis, only the D87Y mutation was identified in gryA, while no mutations were detected in gryB, ParB, and ParC. This finding deviates slightly from previous studies.[20,33] It is

possible that this discrepancy is due to less frequent mutations in gyrB, parC, and parE, or it could be that the gyrA mutations in these nine strains did not result in mutations in the other three quinolone resistance determinants. Only one strain possessed both QRDR and PMQR (qnrA1 and aac(6')-Ib-cr), and the rest were quinolone resistance caused by gryA's D87Y mutation in QRDR, indicating that the D87Y mutation in gryA was the main cause of quinolone resistance in this batch of strains. Similar to the literature, blaTEM and blaOXA enzymes have also been identified in *S. enteritidis*, which are responsible for resistance to Ampicillin.[31]

Salmonella possesses various virulence factors that are primarily responsible for causing illness in individuals. According to the result shows by VFDB website, the prevalence of the SpvC (0%) is lower compared to the previous study.[34] In line with prior research, all nine Salmonella enteritidis isolates included Salmonella pathogenic islands, namely SPI-1, SPI-3-SPI-5, SPI-13, SPI-14, and centisome 63 pathogenic islands (C63PI-1).[20] None of the nine Salmonella strains tested positive for spvC, but a higher prevalence of spvB (66.67%) was observed, which contradicts existing literature.[35] At the same time, we also found that the prevalence of invA in 9 strains reached 100%, which was the same as the results of the previous study, while the prevalence of msgA was 0%, which was contradictory to the existing results.[36,37] These discrepancies may be attributed to variations in genetic diversity and pathogenicity of Salmonella across different regions.

MLST analysis identified *S. enteritidis* as the cause of the food poisoning (ST11), which is consistent with the MLST type of the major non-typhoid Salmonella affecting sub-Saharan African countries reported in one literature.[38] The results of PFGE and phylotree analysis showed a high degree of similarity among the samples, particularly between the Salmonella extracted from the salad provided by Macau and the Salmonella obtained from the patient. This indicates a possible link between the patient and the contaminated food in this food poisoning incident. These findings are also significant for the joint efforts of Zhuhai and Macau in preventing Salmonella outbreaks. The clustering of isolates from patients and those from food in Macau indicated a close phylogenetic relationship between these two isolates, suggesting a possible common chain of transmission. This finding, along with previous evidence, suggests that Macanese food may play an important role in the epidemiology of *S. enteritidis* in tour groups. The high homology between Salmonella strains isolated from the food poisoning incident and the food sample from Macau suggests a direct link between the contaminated food source and the illness. This finding emphasizes the importance of thorough food testing and surveillance to identify and mitigate potential risks to consumer health. More specific epidemiological information and more isolates are needed to better understand disease transmission routes. Whole-genome sequencing of large numbers of Salmonella isolates can be further complemented by the application of mathematical models, combined with machine-learning algorithms that can identify patterns in complex datasets, for better estimates.

This study investigates a case of food poisoning in a tourist group visiting from Macao to Zhuhai, and first reports the ross-border public health emergency stemming from food poisoning in Zhuhai and Macao. The research revealed that the strains exhibited multi-drug resistance, with the rate of multi-drug resistance reaching as high as 80.76% in the samples. What's more, the study identified multi-drug resistance genes, with two strains having two resistance genes to quinolones and beta-lactam drugs, respectively. Additionally, the study finds a high homology between the Salmonella found in the poisoning incident and the food samples provided by Macao. Although the number of sequencing samples used in this study are relatively small, they are still representative to a certain extent. At the same time, this study also provides strong support for strengthening the cooperation between laboratories in Zhuhai and Macao, conducting thematic research on food-borne disease outbreaks in the two districts, understanding the transmission characteristics and trends of the epidemic, and providing relevant departments with effective epidemic prevention and control strategies.

This study is the first to report a cross-border food poisoning emergency in Zhuhai and Macao, emphasizing the potential public health risks that require high attention from both governments.

## Conclusion

The enteritis serotype that causes food poisoning in tourist groups is mainly resistant to AMP and NAL. The virulence genes are mostly located on the virulence island of Salmonella. The Salmonella bacteria extracted from the patient and the food salad sampled in Macau show high homology, providing evidence that the Salmonella food poisoning is a result of food hygiene issues in Macau.

## Ethical Statement and Informed Consent

Our study was reviewed and approved by the ethical committee of the Zhuhai Center for Disease Control and Prevention. All sample collecting was conducted with the informed consent of the participants. The guidelines outlined in the Declaration of Helsinki were followed when we designed the research.

## Acknowledgments

The study was supported by the Zhuhai Center for Disease Control and Prevention, Project number: 2320004000164, and anal swabs and salad samples were collected with the consent of patients and restaurants. Open access funding enabled and organized by Project Countermeasures of biosafety risk assessment in Zhuhai High Level Biosafety Laboratory. Also thanks to the VFDB database and Resfinder website.

## Disclosure

The authors report no conflicts of interest in this work.

## References

1. Chen L, Sun L, Zhang R, Liao N, Qi X, Chen J. Surveillance for foodborne disease outbreaks in Zhejiang Province, China, 2015–2020. *BMC Public Health*. 2022;22(1):135. doi:10.1186/s12889-022-12568-4
2. World Health Organization. Call for experts on the microbiological risk assessment of non-typhoidal Salmonella spp. and Campylobacter spp. in poultry meat. Available from: https://www.who.int/news-room/articles-detail/call-for-experts-on-The-microbiological-risk-assessment-of-non-typhoidal-salmonella-spp.-and-campylobacter-spp.-in-poultry-meat. Accessed April 3, 2024.
3. Cai Y, Tao J, Jiao Y, et al. Phenotypic characteristics and genotypic correlation between Salmonella isolates from a slaughterhouse and retail markets in Yangzhou, China. *Int J Food Microbiol*. 2016;222:56–64. doi:10.1016/j.ijfoodmicro.2016.01.020
4. World Health Organization. Salmonella (non-typhoidal). Available from: https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal). Accessed April 3, 2024.
5. Shen Y, Xu L, Li Y. Biosensors for rapid detection of Salmonella in food: a review. *Compr Rev Food Sci Food Saf*. 2021;20(1):149–197. doi:10.1111/1541-4337.12662
6. Flórez NY, Arévalo SA, Rodríguez EC, et al. Brote de Salmonella *enterica* subsp. *enterica* serovar Give asociado con enfermedad transmitida por alimentos en Vichada, Colombia, 2015 [An outbreak of Salmonella *enterica* subsp. *enterica* serovar Give associated with foodborne illness in the department of Vichada, Colombia, 2015]. *Biomedica*. 2021;41(1):41–51. doi:10.7705/biomedica.5206
7. Mellou K, Sideroglou T, Kefaloudi C, et al. Waterborne outbreak in a rural area in Greece during the COVID-19 pandemic: contribution of community pharmacies. *Rural Remote Health*. 2021;21(3):6630. doi:10.22605/rrh6630
8. McCormic ZD, Patel K, Higa J, et al. Bi-national outbreak of Salmonella Newport infections linked to onions: the United States experience. *Epidemiol Infect*. 2022;150:e199. doi:10.1017/s0950268822001571
9. Keaton AA, Schwensohn CA, Brandenburg JM, et al. Multistate outbreak of Salmonella Mbandaka infections linked to sweetened puffed wheat cereal - United States, 2018. *Epidemiol Infect*. 2022;150:e135. doi:10.1017/s095026882200108x
10. McClure M, Whitney B, Gardenhire I, et al. An outbreak investigation of salmonella typhimurium illnesses in the United States linked to packaged leafy greens produced at a controlled environment agriculture indoor hydroponic operation – 2021. *J Food Prot*. 2023;86(5):100079. doi:10.1016/j.jfp.2023.100079
11. Xu Z, Wang M, Zhou C, et al. Prevalence and antimicrobial resistance of retail-meat-borne Salmonella in southern China during the years 2009–2016: the diversity of contamination and the resistance evolution of multidrug-resistant isolates. *Int J Food Microbiol*. 2020;333:108790. doi:10.1016/j.ijfoodmicro.2020.108790
12. Guo Z, Su C, Huang J, Niu J. A food-borne outbreak of gastroenteritis caused by different Salmonella serotypes in 2 universities in Xiamen, Fujian, China, in 2012. *Jpn J Infect Dis*. 2015;68(3):187–191. doi:10.7883/yoken.JJID.2014.235
13. Pradhan D, Devi Negi V. Stress-induced adaptations in Salmonella: a ground for shaping its pathogenesis. *Microbiol Res*. 2019;229:126311. doi:10.1016/j.micres.2019.126311
14. Ferrari RG, Rosario DKA, Cunha-Neto A, Mano SB, Figueiredo EES, Conte-Junior CA. Worldwide epidemiology of salmonella serovars in animal-based foods: a meta-analysis. *Appl Environ Microbiol*. 2019;85:14.
15. Hayford AE, Brown EW, Zhao S, et al. Genetic and resistance phenotypic subtyping of Salmonella Saintpaul isolates from various food sources and humans: phylogenetic concordance in combinatory analyses. *Infect Genet Evol*. 2015;36:92–107. doi:10.1016/j.meegid.2015.08.022
16. Majowicz SE, Musto J, Scallan E, et al. The global burden of nontyphoidal Salmonella gastroenteritis. *Clin Infect Dis*. 2010;50(6):882–889. doi:10.1086/650733
17. Threlfall EJ. Antimicrobial drug resistance in Salmonella: problems and perspectives in food- and water-borne infections. *FEMS Microbiol Rev*. 2002;26(2):141–148. doi:10.1111/j.1574-6976.2002.tb00606.x
18. Zhan Z, Xu X, Gu Z, et al. Molecular epidemiology and antimicrobial resistance of invasive non-typhoidal Salmonella in China, 2007–2016. *Infect Drug Resist*. 2019;12:2885–2897. doi:10.2147/idr.S210961
19. 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. doi:10.1016/j.jiph.2018.10.012
20. 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.
21. Prevention CCfDCa. Technical specification of National Pathogen identification network; 2017.

22. Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total-genome-sequenced bacteria. *J Clin Microbiol*. 2012;50 (4):1355–1361. doi:10.1128/jcm.06094-11
23. Grimont PADaW, F.X. Antigenic formulae of the Salmonella serovars 9th ed; 2007.
24. Bahmani N, Mirnejad R, Arabestani MR, et al. Comparison of PCR-RFLP and PFGE for determining the clonality of Brucella isolates from human and livestock specimens. *Saudi J Biol Sci*. 2019;26(2):256–262. doi:10.1016/j.sjbs.2017.08.017
25. World Health Organization. Opening statement by the WHO Director-General at the media briefing on 8; 2023. Available from: https://www.who.int/zh/director-general/speeches/detail/who-director-general-s-opening-remarks-at-The-media-briefing—8-june-2023. Accessed April 3, 2024.
26. Benson HE, Reeve L, Findlater L, et al. Local Salmonella Enteritidis restaurant outbreak investigation in England provides further evidence for eggs as source in widespread international cluster, March to April 2023. *Euro Surveill*. 2023;28:2.
27. Zhang Y, Liu K, Zhang Z, et al. A severe gastroenteritis outbreak of Salmonella *enterica* serovar enteritidis linked to contaminated egg fried rice, China, 2021. *Front Microbiol*. 2021;12:779749. doi:10.3389/fmicb.2021.779749
28. Gao Z, Mahe M, Tuohetamu S, et al. Survey of traveler's diarrhea: epidemiology and testing reveal the source. *Can J Infect Dis Med Microbiol*. 2019;2019:3569840. doi:10.1155/2019/3569840
29. Guérin PJ, Nygård K, Siitonen A, et al. Emerging salmonella enteritidis anaerogenic phage type 14b: outbreak in Norwegian, Swedish and Finnish travellers returning from Greece. *Euro Surveill*. 2006;11(2):5–6. doi:10.2807/esm.11.02.00599-en
30. Fang Y, Huang H, Li B, et al. An outbreak of Salmonella Hvittingfoss infection in a tourist group back from Hong Kong, Southeast China. *J Infect Apr*. 2022;84(4):e28–e30. doi:10.1016/j.jinf.2022.02.007
31. Dai W, Zhang Y, Zhang J, et al. Analysis of antibiotic-induced drug resistance of Salmonella enteritidis and its biofilm formation mechanism. *Bioengineered*. 2021;12(2):10254–10263. doi:10.1080/21655979.2021.1988251
32. Ohmani F, Khedid K, Britel S, et al. Antimicrobial resistance in Salmonella *enterica* serovar Enteritidis in Morocco. *J Infect Dev Ctries*. 2010;4 (12):804–809. doi:10.3855/jidc.806
33. Ngoi ST, Thong KL. High resolution melting analysis for rapid mutation screening in gyrase and Topoisomerase IV genes in quinolone-resistant Salmonella *enterica*. *Biomed Res Int*. 2014;2014:718084. doi:10.1155/2014/718084
34. Bahramianfard H, Derakhshandeh A, Naziri Z, Khaltabadi Farahani R. Prevalence, virulence factor and antimicrobial resistance analysis of Salmonella Enteritidis from poultry and egg samples in Iran. *BMC Vet Res*. 2021;17(1):196. doi:10.1186/s12917-021-02900-2
35. Lu Y, Sun P, Shao W, et al. Detection and molecular identification of salmonella pathogenic islands and virulence plasmid genes of salmonella in xuzhou raw meat products. *J Food Prot*. 2022;85(12):1790–1796. doi:10.4315/jfp-22-169
36. Farahani RK, Ehsani P, Ebrahimi-Rad M, Khaledi A. Molecular detection, virulence genes, biofilm formation, and antibiotic resistance of salmonella *enterica* serotype enteritidis isolated from poultry and clinical samples. *Jundishapur J Microbiol*. 2018;11(10):e69504. doi:10.5812/jjm.69504
37. Skyberg JA, Logue CM, Nolan LK. Virulence genotyping of Salmonella spp. with multiplex PCR. *Avian Dis*. 2006;50(1):77–81. doi:10.1637/7417.1
38. Crump JA, Nyirenda TS, Kalonji LM, et al. Nontyphoidal salmonella invasive disease: challenges and solutions. *Open Forum Infect Dis*. 2023;10 (Suppl 1):S32–S37. doi:10.1093/ofid/ofad020



**Infection and Drug Resistance** Dovepress

## 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