Characterization of Clinical *Salmonella enterica* Strains in Huzhou, China

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**Abstract**

*Salmonella enterica* subspecies *enterica* causes salmonellosis in humans and animals and is an important antecedent of food infections worldwide. This study collected 105 clinical *S*. *enterica* isolates from diarrhoea samples from six sentinel hospitals for active surveillance of foodborne diseases in Huzhou, China, between 2018 and 2020. These represented all the *Salmonella* isolates collected in Huzhou during that period. Methods. The isolates were characterized by serovar determination, antimicrobial susceptibility tests, and pulse-field gel electrophoresis (PFGE) typing. Results. The 105 *Salmonella* strains were mainly *S*. *typhimurium* (35.24%, 95% CI from 25.95 to 44.53%) and *S*. *enteritidis* (18.10%, 95% CI from 10.61 to 25.58%). Testing indicated that the resistance rate of the *Salmonella* strains ranged from 0.00% to 70.48%, and the highest resistance rate was for ampicillin (70.48%; 74/105), followed by tetracycline (67.62%; 71/105) and doxycycline (65.71%; 69/105). Following XbaI digestion, the 105 strains yielded 93 PFGE patterns, and 15 clones had similarity values >85.00%. Conclusions. Our analyses revealed the serovar distribution of isolates recovered from diarrhoea patients and the characteristics of resistant strains in Huzhou from 2018 to 2020. Our results highlight a serovar shift and a concerning number of multidrug-resistant (MDR) strains. Continued surveillance of *Salmonella* and their MDR profiles and efforts to control the rapid increase in antimicrobial resistance among *Salmonella* in Huzhou are needed.

**1. Introduction**

*Salmonella* is an important zoonotic pathogen in Enterobacteriaceae. It can survive for long periods in meat, eggs, and related products, and frequently causes human gastroenteritis and other types of food poisoning, especially in developing countries [1]. *Salmonella* can contaminate the entire food chain and eventually infect people during *Salmonella* outbreaks [2]. *Salmonella* is the major pathogen causing foodborne diseases [3, 4]. Salmonellosis causes approximately 93.8 million cases of gastroenteritis and 155,000 deaths per year worldwide [5] and often acts in coinfection with other enteric pathogens [6]. *Salmonella* infection-related hospitalizations and deaths dominated foodborne disease outbreaks in the United States in 2011, in both active and passive surveillance systems [3]. In China, 9.035 million cases of foodborne nontyphoid salmonellosis were reported every year, with 792 deaths every year [7]. Salmonellosis affects both human health and the economy.

Serotyping is the traditional method for subtyping and differentiating *Salmonella* isolates based on the Kauffmann–White (KW) scheme. Over 2,700 *Salmonella* serotypes are known [8]. However, only 40–50 serotypes have been isolated from humans, animals, and food [9]. The main serotypes of gastroenteritis cases are *Salmonella enterica serotype enteritidis* and *typhimurium*, while the main serotype in animals/animal products is Indiana [10, 11]. While the traditional serotyping method is mainly used to identify *Salmonella* serotypes, it cannot identify different strains of the same serotype [12]. Pulsed-field gel electrophoresis (PFGE) determines the kinship of strains isolated by other means, based on the principle that individuals from the same parent have common genetic material and the same PFGE fingerprints. Determining the etiological relationships
among cases can compensate for serotyping deficiencies [12, 13].

Antibiotics are commonly used to treat Salmonella infection, but extensive use of antibiotics has increased the number of Salmonella serotypes resistant to various antibiotics [2, 14]. Outbreaks of drug-resistant Salmonella (and changes in the drug resistance spectrum) are difficult to treat and threaten public health. To understand Salmonella’s serotypes, drug resistance, and molecular typing characteristics in Huzhou, 105 strains of Salmonella isolated from diarrhoea cases in Huzhou from 2018 to 2020 were typed serologically, and drug resistance analysis and PFGE typing of the strains were performed.

2. Materials and Methods

2.1. Bacterial Isolates. The study examined 105 Salmonella strains isolated from six active foodborne surveillance sentinel hospitals in Huzhou, Zhejiang from 2018 to 2020 (31, 49, and 25 strains, respectively). The standard Salmonella enterica strain for PFGE is serotype Braenderup (H9812), from the Zhejiang Center for Disease Control and Prevention.

2.2. Isolation and Identification of Bacteria. Diarrhoea (anus swab) specimens were grown in selenite brilliant green sulphate enrichment broth and then inoculated in Salmonella chromogenic medium for separation. Suspicious colonies were identified after the pure culture. Finally, a Vitek automatic bacterial identification instrument (bio Merieux, Inc., Marcy-l’Étoile, France) was used for the biochemical identification of the Salmonella strains.

2.3. Serotyping. The isolated, purified positive strains were inoculated on blood plates and cultured at 37 for 18h. A single colony was selected for O antigen slide agglutination. Then, Salmonella H phase induced agar was used for H1 and H2 phase flagellar induction and serum agglutination. The K-W serotyping table was searched for the obtained antigen formula to determine the serotype. Normal saline was used as a self-coagulation control.

2.4. Antimicrobial Susceptibility Testing. The antimicrobial susceptibilities of the 105 clinical Salmonella strains were tested using the broth microdilution method and classified as sensitive, intermediate, or resistant according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints for Salmonella strains. Escherichia coli ATCC 25922 was used as a control. The results were analysed according to the CLSI breakpoints.

The identification board contains the following 30 antibiotics: ampicillin (AMP), AMP/sulbactam (AMS), tetracycline (TET), chloramphenicol (CHL), cotrimoxazole (SXT), cefazolin (CFZ), cefotaxime (CTX), cefazidime (CAZ), cefoxitin (CFX), gentamicin (GEN), imipenem (IMI), naphthalic acid (NAL), azithromycin (AZI), sulfisoxazole (Sul), ciprofloxacin (CIP), amoxicillin-clavulanic acid (AMC), cefotaxime-clavulanic acid (CTX/C), ceftazidime-clavulanic acid (CAZ/C), polymyxin E (CT), polymyxin B (PB), minocycline (MIN), amikacin (AN), aztreonam (ATM), cefepime (FEP), meropenem (MEM), levofloxacin (LEV), doxycycline (DOX), kanamycin (KAN), streptomycin (STR), and gemifloxacin (GMI).

2.5. Pulsed-Field Gel Electrophoresis. The Salmonella isolates were subjected to PFGE analysis according to the standard non-typhoid Salmonella PFGE method of the National Pathogen Identification Network. The Salmonella standard strain H9812 was used as the standard. Briefly, the chromosomal DNA was digested with XbaI. The restriction fragments were resolved with 1% SeaKem gold agarose gels in 0.5% Tris-boric acid-EDTA buffer using the CHEF Mapper XA system (Bio-Rad Laboratories, Richmond, CA, USA). The PFGE patterns were analyzed using BIONUMERICS 7.1. Clustering was performed using the unweighted pair group method and the Dice correlation coefficient with a position tolerance of 1.5%. Clusters were defined using a 90% similarity cutoff [15].

3. Results

3.1. Serotyping. From 2018 to 2020, the Huzhou Foodborne Disease Surveillance System isolated 105 Salmonella strains. These were divided into 26 serotypes belonging to six groups: groups B (6 serotypes), C1 (7 serotypes), C2 (1 serotype), C3 (4 serotypes), D (4 serotypes), and E1 (4 serotypes). There were 46, 26, and 15 strains from groups B, D, and E1, respectively. S. enterica serovar typhimurium was the most common in group B. The prevalence was 35.24% (95% CI, from 25.95 to 44.53%). The prevalence of S. enterica serovar enteritidis, the most common in group D, was 18.10% (95% CI, 10.61–25.58). S. enterica serovar London, however, was the most common in group E1 with a prevalence of 8.57% (95% CI, 3.13–14.01). Salmonella Thompson and Salmonella Tennessee, however, were the most common in group C with a prevalence of 2.86% (95% CI, 0–6.1). The other Salmonella strains accounted for low proportions (Table 1).

3.2. Antimicrobial Resistance Profile. Table 2 shows the results of drug sensitivity testing of the 105 Salmonella strains to 30 antibiotics. The sensitivity ranged from 19.05% to 100%. The greatest sensitivity was to imipenem (100%), followed by azithromycin and amikacin (both 98.10%). The sensitivities to cefoxitin, cefotaxime-clavulanic acid, ceftazidime-clavulanic acid, aztreonam, cefepime, meropenem, and kanamycin all exceeded 90.00%. The rates for levofloxacin and AMP/sulbactam were 66.67% and 60.95%, respectively. The drug resistance rates for the 30 antibiotics ranged from 0.00% to 70.48%. AMP (70.48%) had the highest drug resistance rate, followed by TET (67.62%) and DOX (65.71%).

Of the 105 Salmonella strains, 80 were resistant to three or more antibiotics, and the total multiple drug resistance (MDR) rate was 76.19% (80/105). Drug resistance profiles
were identified for 44 of the 105 Salmonella strains; the dominant drug resistance profile was AMP-TET-NAL-DOX-STR (8 strains). The Salmonella resistant to five antibiotics accounted for 65.91% (29/44) of the MDR strains, and the most drug-resistant strains were two Salmonella strains detected in 2019; both were resistant to 12 antibiotics (Table 3).

### 3.3. PFGE and Cluster Analysis

The 105 Salmonella strains were digested with the restriction endonuclease XbaI, and PFGE and cluster analysis were performed for all 105 strains (Figure 1). The band pattern similarity was 28.5% to 100.0%. Based on the number and location of bands, there were 93 different PFGE types, with one type containing up to five strains (Salmonella enteritidis, isolated in 2020). Fifteen clones had similarities exceeding 85.00%. Different PFGE bands may occur within the same serotype. Each serovar corresponded to a single clade, while a few isolates clustered in other serovar clades. Salmonella enteritis and Salmonella typhimurium showed clusters were observed.

### 4. Discussion

Salmonella is an important and widespread zoonotic pathogen that causes food poisoning and infectious diarrhoea [16]. About 70–80% of patients with foodborne diseases in China have Salmonella infection, mainly nontyphoid Salmonella [17, 18]. All Salmonella serotypes can cause potentially life-threatening diseases. Therefore, knowledge of the distribution of Salmonella serotypes in a given area can help prevent Salmonella epidemics. The 105 Salmonella strains isolated by the Huzhou Food-borne Disease Surveillance System from 2018 to 2020 were all nontyphoid Salmonella. Twenty-six serotypes were isolated, among which Salmonella typhimurium was the most...
common, followed by *Salmonella enteritidis*; these are the dominant food-borne *Salmonella* serotypes in many parts of China [19, 20]. With the improvement in living standards, food consumption is becoming increasingly diversified. Therefore, continuous *Salmonella* monitoring is necessary.

Bacterial drug resistance has become an important problem. The widespread use of antibiotics in agriculture and the irrational use of antibiotics in clinical practice lead to drug resistance in bacteria, including *Salmonella*. We showed that the drug resistance of *Salmonella* in the Huzhou area was serious; only 7 of the 105 *Salmonella* strains were sensitive to all 30 antibiotics and the remaining 98 strains were resistant to at least 1 antibiotic. Four antibiotics had drug resistance rates of over 50%: AMP (70.48%; 74/105), TET (67.62%; 71/105), DOX (65.71; 69/105), and STR (62.86; 66/105). These findings were similar to the drug resistance rates of *Salmonella* seen in other cities [12, 21, 22]. The drug resistance rate to NAL in this study (36.19%) was different from that of Zhang et al. [22] (66.67%), which may be related to the difference in clinical medication in

| Antibiotic resistant species (species) | Drug-resistant spectrum | Number of isolates (n) | Proportion (%) |
|---------------------------------------|-------------------------|------------------------|---------------|
| 3                                     | TET-DOX-STR             | 5                      | 4.76          |
|                                       | TET-CHL-DOX             | 1                      | 0.95          |
|                                       | AMP-TET-DOX             | 1                      | 0.95          |
| 4                                     | TET-Sul-DOX-STR         | 1                      | 0.95          |
|                                       | TET-CHL-Sul-STR         | 1                      | 0.95          |
|                                       | NAL-CT-PB-STR           | 1                      | 0.95          |
|                                       | AMP-NAL-CT-STR          | 2                      | 1.90          |
| 5                                     | AMP-TET-DOX-STR         | 1                      | 0.95          |
|                                       | AMP-TET-CHL-DOX-STR     | 4                      | 3.81          |
|                                       | AMP-TET-DOX-STR         | 3                      | 2.86          |
|                                       | TET-Sul-DOX-STR         | 1                      | 0.95          |
| 6                                     | AMP-TET-CHL-Sul-DOX-STR | 8                      | 7.62          |
|                                       | AMP-NAL-AZM-FEP-KAN     | 1                      | 0.95          |
|                                       | AMP-NAL-Sul-CT-STR      | 2                      | 1.90          |
|                                       | TET-CHL-Sul-CIP-DOX     | 1                      | 0.95          |
|                                       | AMP-TET-NAL-DOX-STR     | 5                      | 4.76          |
| 7                                     | AMP-TET-CHL-GEN-DOX-STR | 1                      | 0.95          |
|                                       | AMP-TET-CHL-GEN-CIP-DOX | 1                      | 0.95          |
|                                       | AMP-TET-CTX-AZM-DOX-STR | 1                      | 0.95          |
|                                       | AMP-TET-NAL-CT-DOX-STR  | 1                      | 0.95          |
| 8                                     | AMP-TET-CHL-Sul-DOX-KAN | 1                      | 0.95          |
|                                       | AMP-TET-CHL-GEN-Sul-STR | 1                      | 0.95          |
|                                       | AMP-TET-CTX-AZM-FEP-DOX | 1                      | 0.95          |
|                                       | AMP-TET-NAL-CT-DOX-STR  | 1                      | 0.95          |
| 9                                     | AMP-TET-CHL-GEN-DOX-KAN | 2                      | 1.90          |
| 10                                    | AMP-TET-CHL-GEN-DOX-KAN | 1                      | 0.95          |
| 12                                    | AMP-TET-CHL-CTX-GEN-DOX-KAN | 2                  | 1.90          |
Figure 1: PFGE cluster analysis of 105 Salmonella strains collected from 2018 to 2020 in six sentinel hospitals from Huzhou, Zhejiang, China.
different regions. The drug resistance rate to cephalosporins was low, consistent with other reports [23].

The multidrug resistance of Salmonella is becoming increasingly serious. Of the 105 Salmonella strains, 80 were MDR strains. They were highly resistant to AMP and TET; 29 were resistant to six or more antibiotics, and one was resistant to 12 antibiotics. These MDR data are consistent with domestic reports [19]. Drug resistance monitoring of Salmonella helps elucidate temporal changes in drug resistance and can guide clinical use. Supervision of food production and processing should also be enhanced to prevent the spread of MDR strains.

PFGE analyzes the relationships among strains at the molecular level and can monitor, trace, and identify strains [24]. It is considered the "gold standard" for bacterial molecular typing because of its high repeatability and reliability. Using PFGE, a Salmonella database can be established to trace the source of foodborne disease outbreaks quickly, prevent the spread of disease and clarify the genetic relationships among Salmonella from different regions and years, and assess the epidemiological characteristics of Salmonella. This study shows that the patterns of S. enteritidis and S. enterica typhimurium showed two clusters. The molecular types of Salmonella typhimurium were mainly clustered in the upper half of Figure 1, while Salmonella enteritidis was mainly clustered in the lower half, consistent with Zhang et al. [22].

Other Salmonella types, such as Salmonella Zvenigorod, were also clustered, although not in large numbers. These clusters exist across regions and years, posing challenges to the prevention of foodborne outbreaks.

While the molecular types of different strains of the same serotype are similar, they are not completely consistent, which may be due to the horizontal transfer of antigen-determining genes between strains with distant genetic relationships; although the serotype is the same, there are obvious genetic differences [25]. We also found that the antibiotic resistance of strains with similar molecular types was highly comparable, such as HUZ20-56-60 and other strains. In some cases, the drug resistance spectrum can be determined by the molecular type.

5. Conclusions

This study analyzed the characteristics of Salmonella enteritidis strains in diarrhoea samples from patients in Huzhou, Zhejiang. Different serotypes were detected in the clinical isolates. Drug resistance in Salmonella typhimurium was serious in Huzhou and multidrug-resistant strains were common. It is necessary to pay close attention to the emergence of antimicrobial-resistant strains and enhance antimicrobial management. The data in this study will be useful for controlling and treating food-borne illnesses caused by Salmonella enterica in Huzhou, Zhejiang.

Data Availability

Data supporting the results of our study can be found in our manuscript.

Ethical Approval

Institutional review board approval was not required; the only human materials used were stool samples collected from patients.

Consent

Patient consent was not required as samples were tested using routine laboratory protocols and patient demographic information was not included in the analysis.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Deshun Xu and Lei Ji contributed equally to this work.

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References

[1] L. Z. Liu, S. Lu, S. L. Zhao et al., “Drug resistance of major Salmonella of different serotypes other than Salmonella typhoid in China,” Disease Surveillance, vol. 28, no. 6, pp. 459–463, 2013.
[2] H. Xu, W. Zhang, C. Guo et al., “Prevalence, serotypes, and antimicrobial resistance profiles among Salmonella isolated from food catering workers in Nantong, China,” Foodborne Pathogens and Disease, vol. 16, no. 5, pp. 346–351, 2019.
[3] E. Scallan, R. M. Hoekstra, F. J. Angulo et al., “Foodborne illness acquired in the United States-major pathogens,” Emerging Infectious Diseases, vol. 17, no. 1, pp. 7–15, 2011.
[4] L. Wang, X. Huo, W. Qi, Z. Xia, Y. Li, and J. Lin, “Rapid and sensitive detection of Salmonella Typhimurium using nickel nanowire bridge for electrochemical impedance amplification,” Talanta, vol. 211, Article ID 120715, 2020.
[5] S. E. Majowicz, J. Musto, E. Scallan et al., “The global burden of Nontyphoidal salmonella gastroenteritis,” Clinical Infectious Diseases, vol. 50, no. 6, pp. 882–889, 2010.
[6] S.-X. Zhang, Y.-M. Zhou, W. Xu et al., “Impact of co-infections with enteric pathogens on children suffering from acute diarrhea in southwest China,” Infectious Diseases of Poverty, vol. 5, no. 1, p. 64, 2016.
[7] S. X. Zhang, C. L. Yang, W. P. Gu et al., “Case–control study of diarrheal disease etiology in individuals over 5 years in southwest China,” Gut Pathogens, vol. 58, 2016.
[8] J. Zhao, Y. Zhang, Z. Xie et al., “Characteristics of drug resistance and molecular typing research for Salmonella derby and Salmonella agony isolated in Henan Province,” Journal of Pathogen Biology, vol. 11, no. 6, pp. 517–521, 2016.
[9] K. Shao, “Study on types and actives surveillance of foodborne Salmonellaspp,” in Shandong ProvinceShandong University, Jinan, China, 2011.
[10] European Food Safety Authority(EFDA) and European Centre for Disease Prevention and Control (ECDC), “The European Union Summary Report on trends and sources of zoonoses,
zoonotic agents, and food-borne outbreaks in 2012,” EFSA Journal, vol. 12, pp. 1–312, 2014.

[11] L. Zheng, L. W. Zhu, X. J. Guo, and P. Chen, “Research progress of antimicrobial resistance of major epidemic serotypes of Salmonella,” Jiangsu Agricultural Sciences, vol. 48, no. 6, pp. 8–12, 2020.

[12] Y. Q. Hu, L. Y. Zhang, and Y. Li, “Serotypes, drug resistance and PFGE fingerprinting of Salmonella in Wenzhou,” Preventive Medicine, vol. 31, no. 6, pp. 640–642, 2019.

[13] J. Bai, K. Yin, and W. Liu, “Serotype distribution, molecular type, and antimicrobial resistance of Salmonella isolated in Haidian district of Beijing, 2016–2019,” DisSurveil, vol. 36, no. 5, pp. 1–7, 2021.

[14] Y. J. Hu, C. Liu, and M. M. Wang, “Resistance characteristic analysis for foodborne Salmonella isolates from China, 2016,” Chinese Journal of Food Hygiene, vol. 30, no. 5, pp. 456–461, 2018.

[15] S.-e. Tsai, K.-J. Jong, Y. H. Tey et al., “Molecular characterization of clinical and environmental Vibrio para-haemolyticus isolates in Taiwan,” International Journal of Food Microbiology, vol. 165, no. 1, pp. 18–26, 2013.

[16] A. F. Silva, A. R. dos Santos, D. A. Coelho Trevisan et al., “Cinnamaldehyde induces changes in the protein profile of Salmonella typhimurium biofilm,” Research in Microbiology, vol. 169, no. 1, pp. 33–43, 2018.

[17] X. D. Mao and L. X. M. Hu, “Epidemiological characteristics of bacterial foodborne disease during the years 2003–2007 in China,” Chinese Journal of Food Hygiene, vol. 22, no. 3, pp. 224–228, 2010.

[18] R. Elgroud, S. A. Granier, M. Marault et al., “Contribution of avian Salmonella enterica isolates to human salmonellosis cases in Constantine (Algeria),” BioMed Research International, Article ID 352029, 8 pages, 2015.

[19] Y. Zhou, W. Zheng, and J. C. Chen, “Epidemiological characteristics and molecular typing of Salmonella in Hangzhou in 2013,” Chinese Journal of Epidemiology, vol. 36, no. 8, pp. 907–909, 2015.

[20] D. Z. Zhang, J. Y. Huang, Q. Zhou et al., “Analysis on serotypes and antibiotic resistance characteristics of food-borne Salmonella strains in Guizhou Province from 2016 to 2018,” Chinese Journal of Microbiology and Immunology, vol. 39, no. 10, pp. 737–742, 2019.

[21] K. Guo, X. L. Liu, W. D. Wang, and J. Qu, “Molecular typing and drug resistance of Salmonella in diarrhea cases in Qingdao, Shandong, 2014–2018,” DisSurveil, vol. 35, no. 4, pp. 345–349, 2020.

[22] S.-X. Zhang, Y.-M. Zhou, L.-G. Tian et al., “Antibiotic resistance and molecular characterization of diarrheogenic Escherichia coli and non-typhoidal Salmonella strains isolated from infections in Southwest China,” Infectious Diseases of Poverty, vol. 7, no. 1, p. 53, 2018.

[23] B. Chen, X. B. Ma, and G. L. Hong, “Analysis of serotype distribution and drug resistance of Salmonella in Xiamen area,” Chinese Journal of ClinicalRational Drug Use, vol. 12, pp. 38–39, 2019.

[24] S. M. Fadlallah, M. Shehab, K. Cheaito et al., “PulseNet Lebanon: an overview of its activities, outbreak investigations, and challenges,” Foodborne Pathogens and Disease, vol. 16, no. 7, pp. 498–503, 2019.

[25] J. Lou, B. W. Diao, J. Li, and M. Y. Yan, “Correlation between pulsed-field gel electrophoresis profiles and Salmonella serotypes,” Chinese Journal of Epidemiology, vol. 34, no. 6, pp. 618–621, 2013.