

Characterization of *Salmonella Enterica* Serotype Typhimurium from Outpatients of 28 Hospitals in Henan Province in 2006¹

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Objective To characterize the diarrheal patients with *Salmonella* typhimurium (*S. typhimurium*) infections and to set up the first baseline for *S. typhimurium* pulsed-field gel electrophoresis (PFGE) patterns in Henan province, thus laying a foundation for comprehensive surveillance of *Salmonella* in human as well as foods. **Methods** *S. typhimurium* isolates recovered from outpatients with diarrhea in Henan province from May to October of 2006 were characterized. Antimicrobial susceptibility tests of 8 antimicrobial agents and PFGE were carried out to analyze the *S. typhimurium* isolates. **Results** Twenty-four (0.9%) *S. typhimurium* isolates were identified from 2661 stool specimens of diarrheal cases. Eighty-eight percent of isolates showed resistance to at least one antimicrobial agent. The resistance to chloramphenicol (79%) was most common. Fifty-eight percent of isolates were resistant to ciprofloxacin. All the 14 ciprofloxacin-resistant isolates were resistant to more than five antimicrobial agents. Thirty-three percent of *S. typhimurium* isolates were resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (R-type ACSSuT). Eight antimicrobial-resistant phenotypes were found among the 24 isolates in 16 PFGE patterns. **Conclusion** The rate of multidrug-resistant *S. typhimurium* is relatively high in *S. typhimurium* PFGE patterns of Henan province. Multidrug-resistant *S. typhimurium* should be considered a public health threat.

Key words: *Salmonella* typhimurium; Outpatients; Ciprofloxacin; Multidrug-Resistant; Pulsed-field gel electrophoresis

INTRODUCTION

Salmonellosis is a common cause of food-borne diseases worldwide. Each year in the United States, approximately 1.4 million persons are infected with *Salmonella*^[1]. More than 2500 *Salmonella* serotypes have been identified^[2]. *Salmonella* typhimurium is one of the leading serotypes causing salmonellosis worldwide^[3]. *Salmonella* infections are particularly severe in vulnerable persons such as young children, elderly people and immuno-suppressed patients. Fluoroquinolones such as ciprofloxacin are strongly recommended for the treatment of severe infections^[4]. However, there are rising concerns with the increased occurrence of multidrug-resistant (MDR) *Salmonella* in China and other countries^[5].

The World Health Organization Global Salm-Surv (WHO GSS) Program launched in January 2000 is a

global network of laboratories and individuals involved in surveillance, isolation, identification of *Salmonella* and detection of antimicrobial susceptibility to *Salmonella* (<http://www.who.int/salmsurv/en/>). Worldwide, there are over 850 WHO GSS members in 142 countries^[6]. In 2005, WHO GSS launched an Enhanced *Salmonella* Surveillance Project in Henan province and six other provinces of China. This project is a cooperative program between the Chinese Center for Disease Control and Prevention (China CDC), the United States CDC, and the WHO GSS. Henan province is located in the mid-east of China with an area of over 160 000 square kilometers, and a population of around 93.8 million, accounting for 7% of the total Chinese population.

In this study, we characterized the *S. typhimurium* isolates recovered from Henan province in 2006 using antimicrobial susceptibility tests and pulsed-field gel electrophoresis (PFGE).

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MATERIALS AND METHODS

Sample Collection and Salmonella Isolation

From May to October 2006, 28 sentinel hospitals in Henan province participated in WHO GSS for the Enhanced *Salmonella* Surveillance Project. The participants included 22 hospitals in urban areas accounting for 1.6% of the total population in Henan province and 6 hospitals in rural areas accounting for 0.4% of the total population in Henan province. Diarrheal cases recruited into the study had three or more episodes of loose stools in a 24-h period. Stool specimen was transferred to 10 mL selenite brilliant-green (SBG) enrichment broth (Becton Dickinson and Comp., Sparks, MD) and incubated at 37 °C for 16 h. After incubation, the culture was struck onto CHROMagar *Salmonella* agar (CHROMagar Co., Paris, France) and incubated at 37 °C for 18-24 h. *S. typhimurium* was identified using *Appareils et Procédés d'Identification 20 Entrobacteriaceae* (API 20E) strips (BioMérieux Sa, Inc., Marcy l'Etoile, France) and serotyped according to the Kauffmann-White serotyping scheme (2001).

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility was determined with the Kirby-Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI)^[7]. The antimicrobial susceptibility to the eight antimicrobial agents was measured, including ampicillin (AMP), cefoxitin (FOX), ciprofloxacin (CIP), nalidixic acid (NAL), tetracycline (TCY), chloramphenicol (CHL), streptomycin (STR), and sulfamethoxazole (SXT). Extended spectrum beta-lactamases (ESBL) producing isolates were screened using the double-disk test as recommended by CLSI^[7]. All the isolates with intermediate susceptibility were categorized as susceptible to our analysis. Multi-drug resistance was defined as resistance to three or more antimicrobial agents. *Escherichia coli* ATCC 25922 was used as the quality control organism.

Pulsed-field Gel Electrophoresis

PFGE was performed to determine the genomic DNA fingerprinting patterns of *S. typhimurium* isolates resulting from digestion by restriction enzymes *Xba*I (Roche, Indianapolis, IN) according to the procedures developed from the CDC PulseNet program. The PFGE patterns were interpreted using the BioNumerics 4.6 software (Applied Maths, BVBA, Belgium). A dendrogram of PFGE patterns was generated from dice coefficients with the

un-weighted pair group method using arithmetic averages (UPGMA). *Salmonella* Braenderup H9812 was used as the control strain.

RESULTS

Salmonella Isolation and Serotyping

During the study, 2661 stool specimens were collected from diarrheal cases (1449 from urban hospitals and 1212 from rural hospitals). *S. typhimurium* was isolated from 24 (0.9%) of the stool specimens [0.8% from urban hospitals and 1% from rural hospitals (χ^2 , $P>0.05$)]. The isolation frequency was 2.4% in June, 0.8% in July, and 1.1% in August and September, respectively.

Antimicrobial Susceptibility Testing

The resistance of the 24 *S. typhimurium* isolates to chloramphenicol, nalidixic acid, tetracycline, sulfamethoxazole, ampicillin, ciprofloxacin, and streptomycin was 79%, 75%, 75%, 75%, 75%, 58%, and 46%, respectively. All isolates were susceptible to cefoxitin. Of the 24 *S. typhimurium* isolates, 21 (88%) showed resistance to at least one antimicrobial agent [10 of 12 (83%) from urban hospitals, 11 of 12 (92%) from rural hospitals (χ^2 , $P>0.05$)], and 19 (79%) isolates showed resistance to multi-drugs, including 6 isolates that were resistant to seven antimicrobial agents. All the 14 ciprofloxacin-resistant isolates were resistant to more than 5 antimicrobial agents. Eight antimicrobial resistant phenotypes were observed. Resistant phenotype AMP-CHL-SXT-TCY-CIP-NAL was the most often observed resistant phenotype (7 isolates)

TABLE 1

Antimicrobial Resistance Profiles among the 21 Resistant *Salmonella typhimurium* Isolates from Outpatients in Henan province, China

The Number of Resistant Antimicrobial Agents	Antimicrobial Resistant Phenotypes	Antimicrobial Resistant Isolates No
1	STR	2
4	CHL-STR-SXT-TCY	1
	AMP-CHL-SXT-NAL	1
5	AMP-CHL-SXT-TCY-NAL	1
	AMP-CHL-TCY-CIP-NAL	1
6	AMP-CHL-SXT-TCY-CIP-NAL	7
	AMP-CHL-STR-SXT-TCY-NAL	2
7	AMP-CHL-STR-SXT-TCY-CIP-NAL	6
	Total	21

Note. AMP, Ampicillin; FOX, Cefoxitin; CIP, Ciprofloxacin; NAL, Nalidixic Acid; TCY, Tetracycline; CHL, Chloramphenicol; STR, Streptomycin; SXT, Sulfamethoxazole.

followed by resistant phenotype AMP-CHL-STR-SXT-TCY-CIP-NAL (6 isolates). Phenotype resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole, and tetracycline (R-type ACSSuT) was found in eight (33%) *S. typhimurium* isolates. No ESBL-producing isolate was identified (Table 1).

Pulsed-field Gel Electrophoresis

Sixteen unique PFGE patterns were identified

with 13-18 resolvable bands at approximately 25 kb to 1200 kb in 24 *S. typhimurium* isolates, 13 PFGE patterns were identified in 19 chloramphenicol-resistant *S. typhimurium* isolates, and 11 PFGE patterns were identified in 14 ciprofloxacin-resistant *S. typhimurium* isolates, respectively. Among the eight *S. typhimurium* isolates with ACSSuT resistance, seven PFGE patterns were identified. For the same PFGE pattern, the resistance was similar to antimicrobial agents except for streptomycin (Fig. 1).

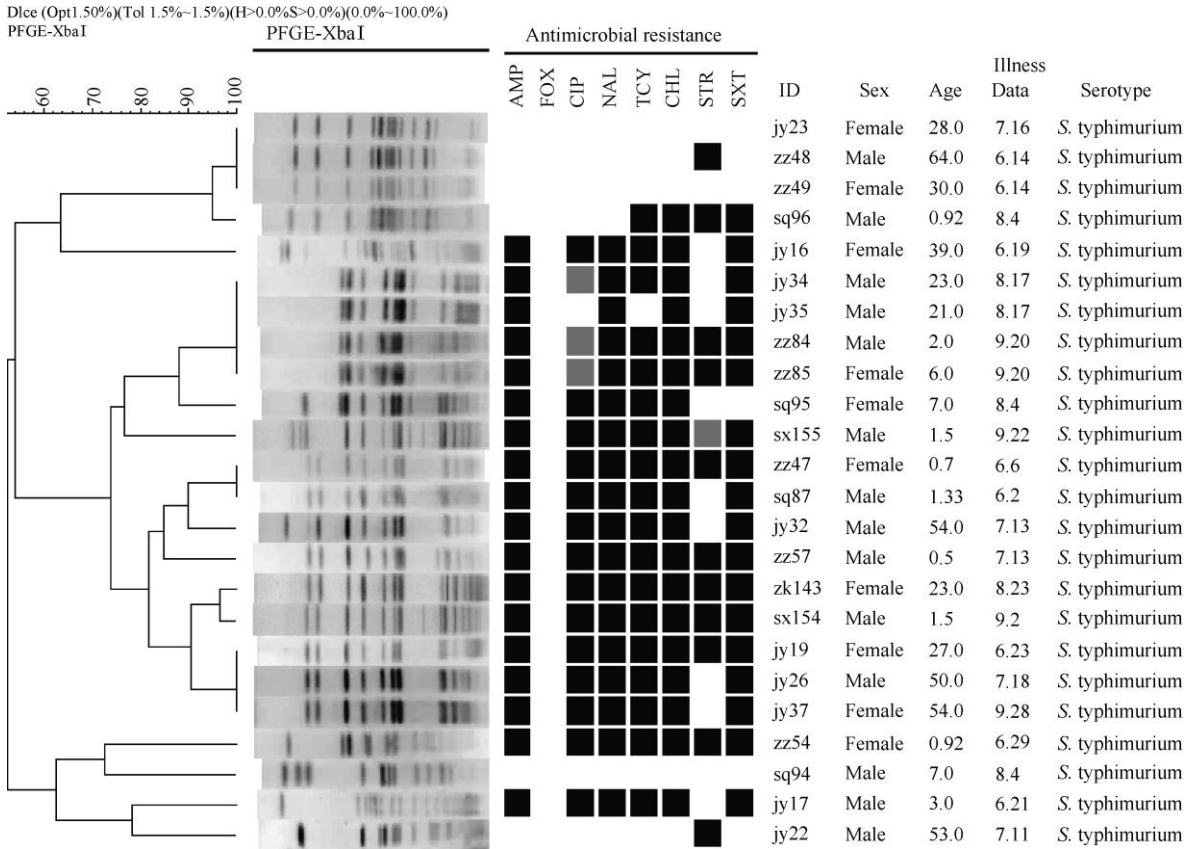


FIG. 1. Dendrogram of pulsed-field gel electrophoresis (PFGE) types for *Salmonella typhimurium* isolates in Henan province (2006). The corresponding antimicrobial resistant phenotypes (a black box indicates resistance to the particular antimicrobial and a gray box indicates intermediate susceptibility), ID, sex, age, illness data, and the serotypes are listed.

DISCUSSION

The predominant *Salmonella* serotype identified from outpatients in Henan province from May to October 2006 was *typhimurium*, which is consistent with the reported data^[3, 6, 8-9]. A food survey in Henan province conducted in 2006 also showed that this serotype is common in foods such as raw uncooked meats, aquatic products, etc. The high isolation rate of *S. typhimurium* indicates that it is a major public

health problem. In accordance with the illness pyramid, Foodborne Diseases Active Surveillance Network (FoodNet) used symptom-specific multipliers (care seeking, stool submission, laboratory testing, and culture-method sensitivity) to estimate the *Salmonella* infections in the United States in 1996-1999. It was extrapolated that for each laboratory-confirmed case of *Salmonella* there would be 68 cases in the community^[1]. Up to now, no study on *Salmonella* infections is available in China, but

such problems should exist in China. It is, therefore, worthwhile to enhance surveillance for *S. typhimurium*.

The resistance of *S. typhimurium* isolates from outpatients in Henan province to microbial agents was higher than that of enteric bacteria to microbial agents detected by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS)^[10]. In particular, we found that 58% of human *S. typhimurium* isolates were resistant to ciprofloxacin, suggesting that ciprofloxacin is a drug of choice for the treatment of serious *Salmonella* infections^[11]. If patients are infected with *Salmonella* isolates with decreased susceptibility to fluoroquinolones, treatment with fluoroquinolone may fail^[12]. It was reported that ciprofloxacin-resistant *S. typhimurium* isolates are usually resistant to multiple drugs^[13]. Resistance to ciprofloxacin and nalidixic acid is a serious problem across the world, especially in Asia^[14-16]. In this study, all ciprofloxacin-resistant *S. typhimurium* isolates were resistant to 4-6 additional antimicrobial agents, indicating that fluoroquinolone resistant *S. typhimurium* isolates are widely disseminated in local communities.

The high incidence of fluoroquinolone-resistant *S. typhimurium* isolates in this study might be affected by the following factors, including application of other antimicrobial agents that may contribute to the spreading of fluoroquinolone-resistant *S. typhimurium* isolates^[17], dissemination of ciprofloxacin-resistant *S. typhimurium* isolates which might be facilitated by the use of fluoroquinolones in livestock product products, a common cause of salmonellosis^[13].

In the present study, eight MDR *S. typhimurium* isolates characterized by ACSSuT resistance, had additional resistances to ciprofloxacin and nalidixic acid with similar resistant phenotypes to MDR DT 104 strains which are internationally distributed. MDR DT 104 has caused outbreaks of infection in Ireland, Denmark, Germany, Austria, France, Czech, Italy, Sweden, Trinidad, South Africa, Netherlands, Northern Ireland, United Arab Emirates, Philippines, and Israel^[18]. In the United States, the proportion of *S. typhimurium* R-type ACSSuT isolates increased from 0.6% in 1979-1980 to 34% in 1996 and 30% in 2001^[19]. Illnesses caused by MDR *S. typhimurium* R-type ACSSuT isolates was more severe, resulting in longer hospitalization and more deaths^[20]. Therefore, spreading of MDR *S. typhimurium* R-type ACSSuT infection in Henan province is of great concern and requires further investigation.

In this study, more heterogeneities were found in PFGE types within *S. typhimurium* isolates. *S. typhimurium* isolates were grouped into 5 ciprofloxacin susceptible patterns and 11

ciprofloxacin resistant patterns. Similar distribution patterns of the high fluoroquinolone-resistant *S. typhimurium* isolates have also been observed in isolates from Japan^[21]. The wide diversity of PFGE types suggests that the isolates are from sporadic *Salmonella* infections. In this study, the resistance of *S. typhimurium* isolates to antimicrobial agents was due to the dissemination of many clones rather than a few clones. *S. typhimurium* zz84 and zz85 isolated on the same day from the same location were identical in antimicrobial resistance and PFGE pattern, suggesting that an outbreak of *Salmonella* infections may occur in Henan province.

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