Original Article

Epidemiological Characteristics and Clinical Treatment Outcome of Typhoid Fever in Ningbo, China, 2005–2014: Pulsed-Field Gel Electrophoresis Results Revealing Great Proportion of Common Transmission Sources

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SUMMARY: We aimed to describe the molecular epidemiological characteristics and clinical treatment outcome of typhoid fever in Ningbo, China during 2005–2014. Eighty-eight Salmonella Typhi isolates were obtained from 307 hospitalized patients. Three prevalent pulsed-field gel electrophoresis (PFGE) patterns of 54 isolates from 3 outbreaks were identified. Overall, there were 64 (72.7%) isolates from clustered cases and 24 (27.3%) isolates from sporadic cases. Resistance to nalidixic acid (NAL) (n = 47; 53.4%) and ampicillin (AMP) (n = 40; 45.4%) and rare resistance to tetracycline (TET) (n = 2; 2.3%) and gentamicin (GEN) (n = 2; 2.3%) were observed. No isolates resistant to cefotaxime (CTX), chloramphenicol (CL), ciprofloxacin (CIP), and trimethoprim-sulfamethoxazole (SXT) were found. The occurrence of reduced sensitivity to CIP was 52.3% (n = 46). The medians of fever clearance time in cases with and without complications were 7 (interquartile range (IQR): 4–10) and 5 (IQR: 3–7) days (P = 0.001), respectively, when patients were treated with CIP or levofloxacin (LEV) and/or third-generation cephalosporins (CEP). Rates of serious complications were at low levels: peritonitis (2.3%), intestinal hemorrhage (6.8%), and intestinal perforation (1.1%). The present study revealed a long-term clustering trend with respect to PFGE patterns, occasional outbreaks, and the rapid spread of AMP resistance and decreased CIP susceptibility among S. Typhi isolates in recent years.

INTRODUCTION

Typhoid fever, a potentially life-threatening disease, is transmitted mainly via the consumption of food or water that is fecally contaminated with the bacterium Salmonella enterica serotype Typhi. S. Typhi infections can cause bacteremia, sustained fever, malaise, and abdominal distress (1). Some patients may have serious complications, such as intestinal perforation and toxic myocarditis (2). Globally, this pathogen causes approximately 11.9–20.6 million cases and 223,000 annual deaths (3). In China, 11,998 cases of typhoid and paratyphoid fever were reported in 2012 and the incidence rate was 0.89 per 100,000 (4).

Typhoid fever is also accompanied by the threat of antimicrobial resistance. Particular concern for the development of resistance to first-line antimicrobials has arisen in recent decades (5). Decreased ciprofloxacin (CIP) susceptibility has been reported, which has a significant impact on clinical outcomes and future treatment options (6). Furthermore, international travel increases the spread of resistant S. Typhi isolates (6). In China, multidrug-resistant (MDR) S. Typhi isolates that are resistant to ampicillin (AMP), chloramphenicol (CL), and trimethoprim-sulfamethoxazole (SXT) have been found (7).

Information on the etiological characteristics of S. Typhi isolates and the relevant clinical treatment outcomes is important for the prevention and treatment of disease; however, there are few previous reports related to S. Typhi isolates and typhoid fever cases from China. The aim of our study was to investigate the etiological characteristics of S. Typhi isolates, including the genetic distribution by means of pulsed-field gel electrophoresis (PFGE) and resistance development, and to assess the clinical efficacy of antimicrobial treatment and complications in culture-confirmed patients.

MATERIALS AND METHODS

This study was conducted in Ningbo, which is located in mid-eastern China and is the largest bulk cargo port city in the world, with a population of about 10 million and land area of 9,780 square kilometers. We included hospitalized patients with a sustained fever along with characteristic features, such as malaise, lethargy, myalgia, and decreased white blood cells, and who were clinically diagnosed as having typhoid fever. Laboratory confirmation was conducted by means of a positive culture of S. Typhi or a fourfold increase in antibody titer by the Widal test. All procedures in the research involving human participants meet the 1964 Helsinki declaration and its later amendments.

The presumptive bacteria were identified using standard methods including the API20E biochemical identification system (bioMérieux, Etoile, France) and S. Typhi-specific serological agglutination test (polyvalent O, O9, Hd, and Vi antigen; Denka Seiken, Tokyo, Japan). Clinical information relevant to the cases was collect-
ed, including age, sex, clinical efficacy of antimicrobial treatment in terms of fever clearance time, complications (aminotransferase elevation, toxic myocarditis, typhoid encephalopathy, peritonitis, intestinal hemorrhage, or intestinal perforation), onset date, residence, and recent travel history. Patients with increased alanine aminotransferase and aspartate aminotransferase levels (both > 40 IU/L), negative serologic test results for viral hepatitis, and no other definite causes were considered to have elevated aminotransferase levels. Patients with electrocardiograms and/or elevation of myocardial enzymes that are characteristic of myocarditis and no other definite causes were considered to have toxic myocarditis.

PFGE was performed to investigate the genetic relatedness of isolates, following the PulseNet standardized protocol (8). Image data were imported into BioNumerics 6.6 software (Applied Maths, Kortrijk, Belgium) and analyzed to distinguish outbreaks and sporadic cases. A dendrogram of PFGE patterns was constructed using the unweighted pair-group method with arithmetic mean, with a position tolerance of 1.2%. PFGE patterns were defined by groups of bands formed at least 95% Dice similarity cutoffs on the dendrogram. In this study, an outbreak was defined as more than one case with identical PFGE patterns and epidemiological information on the distribution of disease that suggests a common transmission source. Clusters having only 2 cases with identical PFGE patterns and no such epidemiological information were considered minor clusters. Sporadic cases were defined as a unique PFGE pattern for one isolate.

Antimicrobial susceptibility testing (AST) was done using the Kirby-Bauer disc diffusion method specified in Clinical and Laboratory Standards Institute (CLSI) document M02-A12 (9). AST results were interpreted according to guidelines in CLSI document M100-S25 (10). The antibiotic discs (Oxoid, Basingstoke, England) included AMP (10 μg), CL (30 μg), tetracycline (TET) (30 μg), CIP (5 μg), nalidixic acid (NAL) (30 μg), gentamicin (GEN) (10 μg), cefotatime (CTX) (30 μg), and SXT (1.25/23.75 μg). *Escherichia coli* ATCC 25922 was used as a quality control isolate.

We used the Student t-test for age, Pearson’s chi-squared test for proportion of sex, and Mann-Whitney U test for fever clearance time, to compare these variables between cases with and without complications. Statistical significance was set to P-values < 0.05.

**RESULTS**

Overall, 707 typhoid cases were reported by the local infectious disease surveillance system in Ningbo during 2005–2014 (Fig. 1). A total of 88 isolates were randomly obtained from 307 of 707 patients admitted to hospitals; of these 307 cases, 180 were confirmed by Widal test and 127 by culture. Of the 88 isolates, 4 were isolated from stool and 84 from blood. Clinical information including age (mean: 34.3; range: 9–77 years old), sex (50 men and 38 women), and date of illness onset was obtained. No patients had traveled abroad within one month before onset date. Resistance to NAL (n = 27; 31.8%) and AMP (n = 40; 45.5%) was at high levels, followed by rare resistance to TET (n = 2; 2.3%) and CTX (n = 2; 2.3%). Forty-six isolates (52.3%) showed intermediate resistance to CIP. Isolates resistant to NAL and intermediate resistant to CIP were first found in 2006, the occurrence rate of which remained at low levels until 2011 (4/32; 12.5%) but changed to high levels beginning in 2012 (42/56; 75%). Isolates resistant to CTX, CL, CIP, and SXT were not found. Forty-three isolates (48.9%) were not resistant to any of the antimicrobial agents tested. The most prevalent complication was aminotransferase elevation (n = 28; 31.8%). The occurrence rates for toxic myocarditis, typhoid encephalopathy, peritonitis, hemorrhage, and perforation ranged from 1.1–6.8% (Table 1). Three types of antibiotic treatment regimens (CIP or levofloxacin [LEV]), third-generation CEP, and 2 concomitant drugs) were applied to treat the illness (Table 2). In total, the fever clearance time from treatment initiation ranged from 2 to 12 days, the shortest being 2 days and the longest 12 days.
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medians of which in cases with/without complications were 7 (interquartile range (IQR): 4–10) and 5 (IQR: 3–7) days ($P = 0.001$), respectively. All 88 patients were finally cured and none had relapsed within one month’s time.

Incidence rates ranged from 0.3 to 2.4 per 100,000 person-years, and a downward trend was observed over the study period (Fig. 1). The incidence rates peaked in 2005 (197 cases) and have generally declined since 2005; a minor increase was observed from 2013 to 2014 due to an outbreak.

Analysis of 88 isolates by PFGE resulted in 34 distinguishable patterns with Dice coefficient 95%. Three prevalent PFGE patterns of 54 isolates from 3 outbreaks, referred to as outbreaks A (5 cases in 2014), B (13 cases in 2005), and C (36 cases during 2013–2014), were

![Fig. 2. Dendrogram of PFGE patterns for 88 S. Typhi isolates detected in Ningbo city, China, 2005–2014.](image)

1. S, sporadic cases; C, clustering or outbreak cases at 95% similarity. 2. AMP, ampicillin; TET, tetracycline; NAL, nalidixic acid; GEN, gentamicin.
detected (Fig. 2, Table 3). The other clusters were 5 minor clusters of 2 cases each. Overall, there were 64 (72.7\%) isolates from clusters (marked “C” in Fig. 2) and 24 (27.3\%) isolates from sporadic cases (marked “S” in Fig. 2). Outbreaks A and B were not serious enough to raise concern at onset time; this situation led to no intervention measures being taken during the outbreak period. Outbreak C took place from June 2013 to May 2014 and was identified in March 2014 by the increasing incidence rate observed in the local infectious disease surveillance network, PFGE analysis of the isolates, and epidemiological investigation. Cases in Outbreak C occurred along a man-made river within a town with a population of 90,000 people. The incidence rate baseline rose from an average of 0.3 per 100,000 person-years (27 cases per year, 2009–2012) to 0.7 per 100,000 person-years (63 cases per year, 2013–2014).

Epidemiological and environmental investigation identified an incriminated river from which water was pumped to wash the cement platforms of 2 marketplaces where vegetables and seafood were sold. Bacterial surveillance of the river water showed a high carrier rate of *Salmonella* spp. In this way, pathogenic microbes in the river water entered the food chain, causing large numbers of people, who were otherwise not exposed individually, to be exposed to infection risk. This situation caused the outbreak to be persistent and widespread. After infectious disease control measures concerning the river were taken in April 2014, including stopping washing of platforms with river water, distribution of health information, and recommendations to avoid the consumption of raw seafood, the outbreak ended in June 2014. The incidence rate has since remained at its normal baseline level.

### DISCUSSION

Our study outlined the trend in typhoid fever incidence from 2005 to 2014, and further explored the rates of complication, clinical efficacy of antimicrobial treatment, antimicrobial resistance, and genetic distribution in 88 culture-confirmed cases.

Generally, typhoid incidence rates in the study city, Ningbo, have demonstrated a downward trend in recent years, to a baseline of about 0.3 per 100,000 person-years (Fig. 1). In 2012, of China’s 32 provinces, Yunnan had the highest incidence with 5.93/100,000; Zhejiang, with incidence 0.23/100,000, was among the 5 provinces with lowest incidence of the disease (4). Ningbo accounts for about one-fourth of Zhejiang Province in terms of population. The incidence in Ningbo was 0.25/100,000 in 2012, very close to the lowest national level. However, the reported annual typhoid fever incidence in China of 10–100 per 100,000 was based on data from only 2 of China’s more than 2,000 counties, located in the southwest of the country, which had high incidence during 1995–1996 and 2000–2001; these data are not representative of the country’s current situation (3). The significant decrease in disease incidence might be due to the increase in the supply of clean tap water in the study region, especially in rural areas, beginning in 2005. However, outbreaks still pose a threat to particular groups of people. There were 2 suspected outbreaks retrospectively identified by PFGE and at least one outbreak identified by both PFGE and epidemiological investigation, as well as some minor PFGE clusters, indicating that occasional outbreaks exist in specific subgroups of the population, e.g., in a particular area or among a population with a particular dietary habit. It is reasonable to speculate that outbreaks of this illness may be underestimated because some outbreaks are not serious enough to warrant epidemiological investigation. This situation results in some outbreaks going unnoticed, especially in low-incidence areas. In this study, 9 months passed before Outbreak C was identified and effective intervention measures were taken, owing to its scattered distribution and minor increase of incidence. By comparison, in high-incidence areas of sub-Saharan Africa and Southeast Asia, many typhoid fever cases occur within a short period and within narrow geographical boundaries, thereby making it easier to identify these outbreaks (11,12). For instance, an outbreak of 103 cases was identified within 4 months in one city in India (13).

In the present study, further etiological investigation using PFGE revealed a trend of high levels of clustering among the isolates, i.e., a large proportion of cases with identical PFGE patterns were observed. Overall, 72.7\% of 88 isolates had PFGE identical patterns over the entire study period (Fig. 2). This situation suggests a large proportion of cases might have a common pathogen transmission source, indicative of a risk of outbreaks existing geographically.

Rapid spread of β-lactam resistance and reduced fluoroquinolone susceptibility from 2012 onward was also observed. Overall, 45.4\% of isolates were resistant to AMP. Ampicillin resistance spread quickly in 2013 and persisted during 2013–2014, revealing the possible spread of resistant *S. Typhi* isolates in recent years. Reduced susceptibility to fluoroquinolones has become serious in recent years. A significant increasing trend in the occurrence of declining fluoroquinolone susceptibility was found. Although a number of previous studies have reported MDR *S. Typhi* isolates (14), our study found no MDR isolates; strains resistant to SXT and CL were not noted. Our findings also showed certain inconsistencies with *S. Typhi* resistance data in the National Antimicrobial Resistance Monitoring

### Table 3. Distribution of 88 *S. typhi* isolates from sporadic and clustering cases

| Distribution pattern | Frequency | No. of isolates | Onset year |
|----------------------|-----------|-----------------|------------|
| Isolates from sporadic cases | 24        | 24              | 2005, 2006, 2007, 2008, 2012, 2013, 2014 |
| Cluster of 2 identical PFGE isolates | 5         | 10              | 2006, 2012, 2013, 2014 |
| Cluster of 5 identical PFGE isolates | 1         | 5               | 2014 |
| Cluster of 14 identical PFGE isolates | 1         | 13              | 2005 |
| Cluster of 36 identical PFGE isolates | 1         | 36              | 2013, 2014 |
System for Enteric Bacteria (NARMS), released by the United States Centers for Disease Control and Prevention for the following drugs (15): CTX (0% in our study vs. 8.1% in NARMS); and an increase in AMP resistance since 2013 in our study vs. a downward trend in NARMS (from 26.5% in 2003 to 16.9% in 2012), which might result from AMP-resistant isolates in Outbreak C and 5 AMP-resistant isolates from endemic cases (Fig. 2). This inconsistency might be explained by considerable geographic variation in the resistance of S. Typhi isolates, with sites in India, Pakistan, and Vietnam having higher rates of resistance than sites in China and Indonesia (16).

Finally, owing to the timely application of effective drugs, treatment of the patients in our study was effective in terms of the high rate of cured cases and low rate of serious complications (Table 1 and Table 2). In some developing countries, the lack of local microbiological testing capabilities leads to difficulties in isolating S. Typhi from blood cultures and long delays in etiological confirmation. These conditions result in typhoid cases going undetected and causing high rates of serious complications. Up to 249 cases with intestinal perforation and 47 deaths occurred among 577 cases in a typhoid outbreak in Africa (12). In our study, fluoroquinolones and third-generation CEP were effective for the treatment of the illness, based on the AST results and all cases being cured. Accordingly, rates of complication were at low levels.

The main limitation of this study was that some isolates might appear to have identical PFGE patterns because they were indistinguishable by PFGE. Another limitation was that the study only included S. Typhi isolates from one region of China. Further studies should be performed to reach more general conclusions. However, the isolates studied here covered a long period and the typing results by PFGE can still provide valuable information, owing to the proven reliability and discriminatory capability of PFGE in subtyping of Salmonella species.

To conclude, this study showed a trend of geographic clustering in S. Typhi isolates over a long-term period, revealing many possible common transmission sources. In recent years, occasional outbreaks, rapidly spreading AMP resistance, and reduced susceptibility to fluoroquinolones appear to be a characteristic of Typhoid fever cases. We suggested strengthened surveillance be implemented to detect possible outbreaks and the development of antimicrobial resistance.

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Conflict of interest None to declare.

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