

Emerging Problems in Infectious Diseases

Antimicrobial resistance surveillance of *Salmonella* isolates from the First People's Hospital of Yunnan Province, China

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Abstract

Introduction: Typhoid fever is a common disease in Yunnan province; however, the resistant phenotype and epidemic characteristics of *Salmonella* in this area are still unclear. In this study, a 15-year surveillance of antimicrobial susceptibility of *Salmonella* is reported.

Methodology: From January 1999 to December 2013, *Salmonella* isolates were recovered from patients in the First People's Hospital of Yunnan Province. Antimicrobial susceptibility was detected and data were analyzed using WHONET5.6.

Results: A total of 845 *Salmonella* isolates were recovered between 1999 and 2013. The most frequently isolated *Salmonella* serovar was *S. Paratyphi A* (93%), and 75.1% (635/845) of the isolates were from the young and middle-aged population. The resistance rates of *Salmonella* spp. to ciprofloxacin, ampicillin, and ceftriaxone increased dramatically during the 15 years. Carbapenems retained the highest and most stable activity against isolates. The resistance rates of all *Salmonella* isolates to chloramphenicol and sulfamethoxazole were 0.4% (3/845) and 1.8% (15/845), respectively.

Conclusions: As *Salmonella* isolates have been observed to be resistant to first-line antibiotics, antimicrobial agents should be used rationally and prescriptions should be based on case-by-case susceptibility testing.

Key words: *Salmonella*; antimicrobial drug resistance; China.

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Introduction

The genus *Salmonella* was identified for the first time by Eberth in the middle of the 18th century. Members of this genus are motile, Gram-negative, facultative anaerobic bacteria in the family *Enterobacteriaceae*. Despite the progress in medicine, enteric fever is still a public health threat throughout the world, particularly in developing countries such as China. It is estimated that more than 90% of typhoid fever cases were reported in South and Southeast Asian countries [1]. Typhoid fever is a common disease in Yunnan province, but little is known about the epidemic characteristics and antimicrobial resistance pattern in this area. The objective of this study was to investigate the antimicrobial resistance profile of 845 *Salmonella* isolates recovered during the period of 1999–2013 from patients at the First People's Hospital of Yunnan Province, China.

Methodology

Clinical isolates

In total, 845 *Salmonella* strains isolated from January 1999 to December 2013 were included in this retrospective study.

Antimicrobial susceptibility

During the period 1999–2010, the disk diffusion method was used to detect the susceptibility of *Salmonella* isolates against antibiotics, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI), and from 2011 to 2013, minimum inhibitory concentrations (MICs) for each antibiotic was determined by VITEK 2 compact (bioMérieux, Marcy l'Etoile, France). Susceptibility interpretations were based on CLSI M100-S23 clinical breakpoints [2]. The antibiotics used included carbapenems (imipenem, meropenem, and ertapenem), penicillins (ampicillin), cephalosporins (ceftriaxone and cephazidime), fluoroquinolones (ciprofloxacin and

levofloxacin), sulphonamides (sulphamethoxazole), and chloramphenicol. However, some of these antibiotics (meropenem and ertapenem) were not tested over the whole study period due to a change in the hospital’s treatment plan. *Escherichia coli* ATCC 25922 was used as a reference strain for quality control (QC). Results were included in the analysis only when the corresponding QC isolates tested were within the acceptable range according to CLSI guidelines.

Statistical analysis

The data were statistically analyzed using WHONET 5.6.

Results

Distribution of isolated strains

From 1999 to 2013, a total of 845 *Salmonellae* were detected at the First People’s Hospital of Yunnan Province, including 784 *S. Paratyphi A*, 37 *S. Typhi*, 5 *S. Enteritidis*, 2 *S. Typhimurium*, 2 *S. Choleraesuis*, 1 *S. Falkensee*, and another 14 unknown *Salmonella* serovars. The patients’ ages ranged from 6 months to 80 years. The majority of the isolates, 75.1% (635/845), were from the young and middle-aged population, and 6.0% (51/845) were from children (< 10 years of age). The average age of presentation was 29.5 years, of which 423 (50.1%) and 422 (49.9%) were males and females, respectively.

Table 1. Resistance rates of *Salmonella* isolates in 1999–2013 in this teaching hospital

Drug	Number of tested strains	Total resistance rates %	Resistance rates in every single year														
			1999 n=85	2000 n=39	2001 n=55	2002 n=42	2003 n=63	2004 n=212	2005 n=87	2006 n=69	2007 n=42	2008 n=27	2009 n=33	2010 n=17	2011 n=14	2012 n=20	2013 n=40
Ciprofloxacin	845	6.0	0	0	0	0	0	0	0	1.4 (1)	0	3.7 (1)	9.0 (3)	11.8 (2)	14.2 (2)	55 (11)	77.5 (31)
Levofloxacin	845	1.1	0	0	0	0	0	0	0	1.4 (1)	0	3.7 (1)	0	0	7.1 (1)	20 (4)	5 (2)
Sulfamethoxazole	845	1.8	0	0	0	0	0	0.9 (2)	0	2.9 (2)	7.1 (3)	3.7 (1)	0	0	0	25 (5)	5 (2)
Chloramphenicol	845	0.4	0	5.1 (2)	0	0	0	0	0	1.4 (1)	0	0	0	0	0	0	0
Ampicillin	845	4.3	0	0	5.5 (3)	0	1.6 (1)	0.9 (2)	1 (1)	5.8 (4)	7.1 (3)	7.4 (2)	6.0 (2)	11.8 (2)	14.3 (2)	25 (5)	22.5 (9)
Ceftriaxone	845	1.3	0	0	0	0	0	0.5 (1)	0	1.4 (1)	0	0	0	0	7.1 (1)	20 (4)	10 (4)
Ceftazidime	845	0.1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.5 (1)
Ertapenem	74	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0	0	0
Imipenem	845	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Meropenem	74	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0	0	0

The numbers in parentheses represent the numbers of resistant strains; ND: not detected.

Table 2. Resistance rates of *Salmonella Typhi* and *Salmonella Paratyphi A*

Drug	Tested <i>Salmonella Typhi</i> isolates	<i>Salmonella Typhi</i> resistance rates (%)	Tested <i>Salmonella Paratyphi A</i> isolates	<i>Salmonella Paratyphi A</i> resistance rates (%)
Ciprofloxacin	37	13.5 (5)	784	5.9 (46)
Levofloxacin	37	5.4 (2)	784	0.8 (6)
Sulfamethoxazole	37	5.4 (2)	784	1.4 (11)
Chloramphenicol	37	5.4 (2)	784	0.1 (1)
Ampicillin	37	10.8 (4)	784	2.0 (16)
Ceftriaxone	37	5.4 (2)	784	1.0 (8)
Ceftazidime	37	0.0	784	0.0
Ertapenem	37	0.0	784	0.0
Imipenem	37	0.0	784	0.0
Meropenem	37	0.0	784	0.0

The numbers in parentheses represent the numbers of resistant strains.

Antimicrobial susceptibility

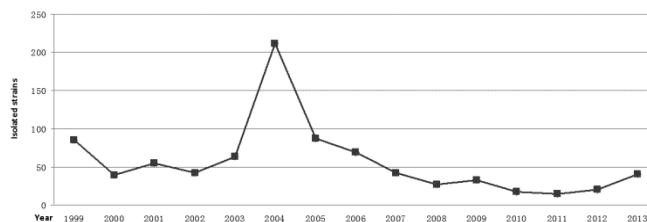
Imipenem, meropenem, and ertapenem retained the highest and most stable activity against all *Salmonella* isolates tested. Throughout the study period, none of the isolates was found to be resistant against imipenem. Similarly, resistance was not detected against the other carbapenems (meropenem and ertapenem), which were only tested during 2011, 2012, and 2013. The highest resistance rate was recorded for ampicillin compared to other tested β -lactams. Dramatic increases were found in the resistance rate to ceftriaxone after 2011. An increase in resistance rates to ceftriaxone, from 0% (0/85 isolates) in 1999 to 10% (4/40 isolates) in 2013, was observed. However, ceftazidime maintained a strong activity against 99.9% (844/845) of the studied isolates. As for fluoroquinolones, a significant increase in resistance rates to ciprofloxacin, from 0% (0/85) in 1999 to 77.5% (31/40) in 2013, was observed. Comparatively, levofloxacin showed stable activity with slightly increased resistance rates. Low resistance rates of 1.8% and 0.4% were recorded for sulfamethoxazole and chloramphenicol, respectively (Table 1). The resistance rates of *S. Typhi* and *S. Paratyphi A* are shown separately in Table 2.

Discussion

Our data show that salmonellosis is a common infectious disease in Yunnan province, affecting all age groups. A total of 75.1% of the isolates were recovered from the young and middle-aged population. This is different from what had been reported previously for Typhimurium infection in Spain and enteric fever in India, where most of the isolates (76% and 90.2%, respectively) were recovered from children [3,4]. Humans may be exposed to *Salmonella* through consumption of contaminated water and food [5]. With the rising public standard of living, a gradual reduction in the frequency of *Salmonella* isolation was observed during the study period (Figure 1), which may be due to the reduced likelihood of exposure to contaminated water and food.

Due to its changing modes of presentation, as well as the development of multidrug resistance (MDR), typhoid fever is becoming increasingly difficult to diagnose and treat. Quinolone antibiotics had been used widely in the treatment of enteric fever [6]. The resistance to quinolones is caused by amino acid substitutions in the quinolone resistance determining region of the DNA gyrase, subunit *gyrA*, *gyrB*, or DNA topoisomerase IV. Single mutations in *gyrA* are

Figure 1. Isolated *Salmonella* strains during 1999-2013



responsible for a decreased susceptibility of *Salmonella* to ciprofloxacin, whereas a combination of two or more mutations in *gyrA*, *gyrB*, *parC*, and *parE* makes them resistant [7]. For this reason, CLSI, in 2012, modified the breakpoints of ciprofloxacin from $\leq 1 \mu\text{g/mL}$ to $\leq 0.06 \mu\text{g/mL}$, which limited the clinical application of ciprofloxacin as an empirical therapy against typhoid fever. Previous reports on the antibiotic resistance of *Salmonella* in China described *S. Typhi* and *S. Paratyphi A* strains isolated in Jiangsu Province and showed the rate of resistance to nalidixic acid as 66.91% and the MDR rate as 30% [8]. In Guangxi Province, the results of a surveillance showed that almost all *S. Paratyphi A* isolates were resistant to nalidixic acid [9]. The resistance rates of nalidixic acid to *S. Typhi* and *S. Paratyphi A* isolated from a hospital in Shenzhen City were 52.0% and 95.3%, respectively [10]. It is evident that the resistance of *Salmonella* in China is serious. In 2013, the resistance rate of *Salmonella* to ciprofloxacin was 77.5% in the First People's Hospital of Yunnan Province compared to 0.0% in 1999; this observation may be explained by the use of ciprofloxacin as the first-line antibiotic in this teaching hospital. Comparatively, the low resistance rate of levofloxacin (1.1%) makes it an alternative option for treatment of adult patients.

Besides fluoroquinolones, β -lactams could be a choice for the treatment of enteric fever. Ampicillin is usually used as an empirical treatment in this teaching hospital, and the resistance rates and the degree of resistance are increasing yearly. Therefore, choosing ampicillin to treat enteric fever should be based on the results of a drug susceptibility test for each case. A total of eleven *Salmonella* isolates were resistant to ceftriaxone, including eight *S. Paratyphi A* strains, two *S. Typhi* strains, and one unknown *Salmonella* serovar. Nine of the eleven strains' MIC values of ceftriaxone were estimated, and all were above $64 \mu\text{g/mL}$. Relatively, ceftazidime had a more effective activity against *Salmonella* than did ceftriaxone. Only one unknown *Salmonella* serovar was resistant to it; this strain was also resistant to ceftriaxone. This may be due to the fact that *Salmonella* strains harbor the CTX-

M type extended spectrum beta-lactamase (ESBL) [11], which has a stronger hydrolyzing activity against ceftriaxone. Although a small number of *Salmonella* isolates had developed resistance to ceftriaxone, our data showed that third-generation cephalosporins maintained a good activity against *Salmonella*. The resistance rates of ceftazidime and ceftriaxone were 0.1% and 1.3%, respectively, and could be used as first-line antibiotics to treat enteric fever. Carbapenems have always been considered an ideal treatment option for infections caused by Gram-negative bacilli. In this study, carbapenems, including imipenem, ertapenem, and meropenem, maintained the highest and the most stable activity against *Salmonella* isolates. But the presence of one NDM-1-bearing *Salmonella* strain isolated from Zhejiang province should trigger an alert [12].

Enteric fever tends to be treated in the same way regardless of the causal agent (*S. Paratyphi A* or *S. Typhi*), but in some regions, there are differences in the susceptibility of *S. Paratyphi A* or *S. Typhi*; typhoid fever and paratyphoid fever, therefore, need to be treated as different diseases [13]. Our data indicated that *S. Typhi* showed a higher resistance rate than did *S. Paratyphi A* to most of the tested antibiotics (Table 2). The resistance rates to first-line antibiotics, such as levofloxacin, chloramphenicol, ampicillin, and ceftriaxone, were fivefold higher than the resistance rates of *S. Paratyphi A*. Therefore, *S. Typhi* should be treated with caution, and ceftazidime and carbapenems could be reliable option as empiric treatment in this region.

Chloramphenicol has its defects that cannot be overcome; its problematic side effects dictate that it should not be used extensively, even if it maintains good *in vitro* activity. Widespread use of fluoroquinolones and β -lactam antibacterial drugs as first-line treatment will likely result in the rapid re-emergence of MDR strains. Fortunately, no MDR profile of fluoroquinolones, β -lactams, and sulfamethoxazole was detected in this study.

Conclusions

The current survey indicated that resistance of *Salmonella* to first-line antibiotics was displayed by one or more of the studied isolates. To avoid the occurrence of *Salmonella* resistance, it is critically important to monitor the drug resistance of *Salmonella* and to follow a rational prescription of antibacterial agents. Governmental and non-governmental agencies should take measures to limit the overuse of antibacterial drugs.

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