Prevalence, serotypes, and antimicrobial resistance of *Salmonella* isolates from patients with diarrhea in Shenzhen, China

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Abstract

Background : *Salmonella* is one of the main causative agents of diarrhea which results in substantial disease burden. To determine the prevalence, serotype distribution, and antimicrobial resistance profiles of clinical *Salmonella* isolates in Shenzhen, a 6-year surveillance study was conducted. **Methods:** Three sentinel hospitals were chosen and patients who visited the gastroenteritis clinics with acute diarrhea were enrolled. *Salmonella* isolates from the stool samples were subjected to serotyping, antimicrobial susceptibility testing to 24 antibiotics, and detection of resistance genes. **Results:** A total of 297 (5.7%) *Salmonella* strains were isolated from stool samples from 5,239 patients. Among the 42 serovars identified, serovar Typhimurium was the most common serotype which represented 39.7% of the isolates (118), followed by serovar Enteritidis (71, 23.9%), London (12, 4.0%), 4, 5, 12: i - (11, 3.7%), and Senftenberg (8, 2.7%). A high frequency of resistance was found in ampicillin (70.6%), piperacillin (64.5%), tetracycline (63.5%), and streptomycin (54.3%). Resistance to ampicillin and tetracycline was observed in 95.3% of *S. Typhimurium* isolates; and nalidixic acid in 93.1% of *S. Enteritidis* isolates. Resistance to 5 or more antimicrobial agents was found in 78.8% of *S. Typhimurium* and 69.0% of *S. Enteritidis* isolates. **Conclusions:** *Salmonella* is an important cause of acute gastroenteritis and a broad range of serotypes are prevalent in Shenzhen, with Enteritidis and Typhimurium being the most common serovars. The high level of antibiotic resistance is of public health significance and ongoing monitoring combined with rational use of antibiotics are recommended.

Background

Salmonella is a main foodborne pathogen worldwide which causes an annual death of 230,000 due to foodborne disease [1]. *Salmonella* is also a common cause of foodborne outbreaks and transmitted by contaminated food such as beef, pork, tomato, and cucumbers [2-5]. *Salmonella* is prevalent throughout the year, but it was reported the most commonly detected between April and October in China [6]. Children less than 5 years old accounted for the largest proportion of infections [7, 8].

Over 2500 serovars have been reported [9], with serovar Enteritidis and Typhimurium being the most common serotypes causing gastroenteritis [10]. *Salmonella* isolates with different serovars vary in the pathogenicity, prevalence, and sensitivity to antibiotics. *Salmonella* Typhi and *Salmonella* Paratyphi are usually associated with higher mortality [11]. Some serovars were reported in only one single region of the world, such as *Salmonella* Rissen and *Salmonella* Weltevreden were only identified in Asia [12, 13]. Multi-drug resistance was the most common in *S. Typhimurium* [14].

Salmonellosis is not a severe infection, but the emergence of third-generation cephalosporins and multi-drug resistant isolates has raised concerns [15, 16]. The wide use of antibiotics in poultry and for empirical treatment of salmonellosis has led to the rising of drug resistance rate of *Salmonella*. A high resistance rate to at least one clinically important antimicrobial was found in the clinical and animal-derived isolates [16, 17]. A wide range of mechanisms were associated with cephalosporins and

quinolone resistance, including mutation in the quinolone-resistance determining regions (QRDRs), overexpression of an efflux pump and acquisition of drug resistance plasmids [18].

A one-year surveillance was conducted in our previous research and *Salmonella* was found one of the main causes (12.1%) of acute infectious diarrhea in Shenzhen [19]. Nevertheless, the long-term trend of serotype distribution and antimicrobial resistance pattern of the *Salmonella* isolates were not defined. To improve our understanding of the prevalence of *Salmonella* in this region and thus provide basis for designing prevention and control strategies, we investigated the serotypes and antibiotic resistance of the isolates obtained from the surveillance network during 2013 and 2018.

Materials And Methods

Stool samples collection. Peking University Shenzhen Hospital (PKUSZH), Shenzhen Children's Hospital (SCH), and The Eighth Affiliated Hospital, Sun Yat-Sen University (SYSU8H) were selected as sentinel hospitals. Stool samples were collected from outpatients who visited gastroenteritis clinic due to acute infectious diarrhea and agreed to take part in this study. Acute diarrhea was defined as over 3 passages of loose, mucus-, watery, or bloody-stools during 24-hour period. The stool samples were examined for *Salmonella* sp. using CHROM agars. The clinical signs and demographic information were collected from electronic medical records.

A total of 5,239 cases (2,749 of whom were male) were included during study period. Among these patients, 3,870, 425, and 944 cases were enrolled from PKUSZH, SCH, and SYSU8H, respectively. Patients ranged in age from 0 to 96 years (median 30 years). Local population accounted for 60.4% of the patients. Of all the patients, 310 (5.9%) had fever, 635 (12.1%) had vomiting, and 50 (1.0%) had blood in stools, respectively. Of the patients over 5 years old, 2,098 (54.1%) had abdominal pain (Table 1). Overall, *Salmonella* was isolated from 297 (5.7%) of all cases.

Serotyping and antimicrobial susceptibility testing. The *Salmonella* sp. isolates were identified using Vitek-2 compact system (bioMérieux, France) and serotyped with a commercial serotyping kit (S&A company, S&A Reagent Lab, Bangkok, Thailand).

A total of 197 *Salmonella* isolates representing 26 serotypes were selected for antimicrobial susceptibility testing. Twenty-four antimicrobials (Oxoid, UK) including amikacin (30 micrograms (mcg), AK), ampicillin/sulbactam (20 mcg, SAM), ampicillin (10 mcg, AMP), aztreonam (30 mcg, ATM), cefepime (30 mcg, FEP), cefotaxime (30 mcg, CTX), ceftazidime (30 mcg, CAZ), ceftriaxone (30 mcg, CRO), cephazolin (30 mcg, KZ), chloramphenicol (30 mcg, C), ciprofloxacin (5 mcg, CIP), gentamicin (10 mcg, CN), imipenem (10 mcg, IPM), levofloxacin (5 mcg, LEV), meropenem (10 mcg, MEM), nalidixic acid (30 mcg, NA), nitrofurantoin (300 mcg, F), norfloxacin (10 mcg, NOR), piperacillin (100 mcg, PRL), streptomycin (10 mcg, S), sulphamethoxazole/trimethoprim (25 mcg, SXT), tetracycline (30 mcg, TE), and trimethoprim (5 mcg, W) were tested using disk diffusion method.

The MacFarland 0.5 inoculums were prepared and swabbed on the entire surface of Mueller-Hinton agar (Huankai, China) and left to dry for 3-5 min. Antimicrobial susceptibility test discs (Oxoid, UK) were placed on the inoculated agar plate with a disc dispenser (Oxoid, UK) and incubated at 37°C for 24h. After incubation, the diameter of inhibition zone was measured and the results were interpreted as susceptible, intermediate, and resistant according to the Clinical and Laboratory Standards Institute guideline (CLSI, 2019)[20]. *Escherichia coli* strain ATCC 25922 was used as quality control.

The multiple antibiotic resistance (MAR) index was the ratio between the number of antibiotics to which the organism was resistant and the number of antibiotics tested. Multi-drug resistant (MDR) was defined as resistant to three or more different classes of antimicrobial agents.

Detection of target gene mutations. A total of 21 fluoroquinolone resistant isolates were chosen to screen for the mutations of the *gyrA*, *gyrB*, *parC*, and *parE* genes in the QRDRs. The bacterial nucleic acid was extracted using QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany) and subjected to PCR amplification using *Taq* PCR Master Mix Kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer's recommended protocols. The PCR products were sent to Sangon (Shanghai, China) for sequencing and the results were analyzed using BLAST (PubMed). The primers used for PCR amplification and sequencing were listed in Table 2.

Results

Serotyping results. A total of 42 serovars were identified in the 285 *Salmonella* isolates and additional 12 strains were un-typable. Serovar Typhimurium (118, 39.7%) was the most common serotype, followed by serovar Enteritidis (71, 23.9%), London (12, 4.0%), 4, 5, 12: i - (11, 3.7%), and Senftenberg (8, 2.7%). A total of 46 isolates with uncommon serotypes were found, including serovar Virchow, Corvallis, Vilvoorde, and Sarajane (Table 3).

Of the 118 *S. Typhimurium* isolates, 87 (73.7%) were recovered from children aged below 5 years old. Eight of 11 serovar 4, 5, 12: i - strains were isolated from this age group, while similar detection rate of *S. Enteritidis* strains was observed in each age group. Of the 72 isolates with other serotypes, 51 (70.8%) were isolated from adults aged between 20 ~ 59 years old (Table 4).

Age and monthly distribution. The highest detection rate was observed in the age group of 5 ~ 9 years (15.1%), followed by <5 years (10.1%) and 30 ~ 39 years (4.9%). The age group of over 60 years showed the lowest prevalence (2.8%). A high prevalence of *S. Typhimurium* infection was seen in the young children aged less than 5 years (6.4%) and 5~9 years old (5.5%). In the age group of 5 ~ 9 years, serovar Enteritidis and 4, 5, 12: i - were the other two common serotypes (Table 4).

The highest detection rate was seen in August (11.01%), followed by September (8.56%), June (8.49%), and October (7.88%). The detection rate between May and November (8.19%) was significantly higher than that between December and April (1.89%). Similar seasonal distribution was also observed in the serovar Typhimurium isolates (Figure 1).

Antimicrobial resistance profile. The highest rate of resistance was found in ampicillin (139, 70.6%), followed by piperacillin (127, 64.5%), tetracycline (125, 63.5%), streptomycin (107, 54.3%), cefazolin (87, 44.2%), and sulphamethoxazole/trimethoprim (75, 38.1%). The resistance to ampicillin and tetracycline was observed in 81 (95.3%) of *S. Typhimurium* isolates. Twenty-seven (93.1%) of *S. Enteritidis* isolates were resistant to nalidixic acid (Table 5). Five out of 8 *S. Senftenberg* isolates were susceptible to all the tested antibiotics. Among the 163 isolates resistant to three or more antimicrobial agents, 82 and 23 were found in *S. Typhimurium* and *S. Enteritidis* isolates, respectively. The MAR index of 123 isolates was over 0.21. The highest MAR index (0.71) was found in a serogroup B un-typable strain.

Mutations with QRDRs. The mutation of amino acids 87 in *gyrA* was seen in 14 isolates. Ten strains were identified with mutation in *gyrB*. A single base change in amino acids 67, 75, 77, 117, and 123 in *parC* were found in 5 of the tested strains. The mutation of codons 500 and 509 in *parE* was found in 3 and 7 isolates, respectively (Table 6).

Discussion

Salmonella is an important causative microorganism of acute gastroenteritis, which was shown in the detection rate during 2013 and 2018 (4.2%~6.8%). The detection rate was lower compared with our previous study (12.1%) which incorporated PCR method [19], but it was comparable to another study (4.8%) conducted in Guangzhou using conventional assay [23]. A rate of 4.5% was observed in a large laboratory-based surveillance study in Guangdong province during 2009 and 2012, suggesting the continued prevalence in this region [17].

A broad distribution of serotypes was also reported in another study conducted in Shenzhen [24]. Apart from the detection rate, this study provided the trend of prevalence over a long period and antimicrobial resistance data, and identified some rare serotypes.

The detection rate between May and November was significantly higher compared with that in other months, which might be associated with high temperature. Hot weather contributed to bacterial growth and the chance of consumption of insufficiently heat-treated food or salad was higher. A linear association between temperature and the number of reported cases of salmonellosis was found and it was proven that higher temperature was associated with *Salmonella* infections [25]. As a result, the sporadic cases and salmonellosis outbreak were commonly found during this period [17].

The high incidence of *Salmonella* infection in children was consistent with another study where a recovery rate of 17.2% in children was reported [15]. The high frequency of infections in children could be attributed to the behavior such as frequent contact with contaminated limbs, consumption of contaminated food or water, or close contacts with an asymptomatic caretaker [23]. *S. Typhimurium* and *S. Enteritidis* were the most common serovars in children aged below 5 years old, which represented 75.4% (104/138) of the isolates, in accordance with other studies [15, 17]. The high frequency of multi-drug resistance in *S. Typhimurium* posed the difficulties in treating pediatric patients. Some measures

such as good hygiene, proper hand washing, and education of their guardians were recommended to reduce the disease burden of salmonellosis in children.

Wide distribution of serotypes might be another reason of the continued prevalence of *Salmonella* infections. Apart from *S. Typhimurium* and *S. Enteritidis*, a broad range of uncommon serovars including Vilvoorde and Sarajane were identified, indicating the wide sources of *Salmonella*. As one of the major pathogenic bacteria in Chinese food commodities [26], *Salmonella* was commonly isolated from beef, pork, and poultry meat [27, 28]. Agona, Corvallis, and Kentucky were reported the dominant serotypes in chicken samples, while Typhimurium, Rissen, and Derby were the most common in pork samples [16]. Serovar Gallinarum-pullorum was isolated from a 24-year-old male patient with mild diarrhea. *S. Gallinarum-pullorum* was generally regarded as chicken-derived which causing little public health concern. However, occasional infections in human were reported following consumption of heavily contaminated food and low detection rate was found in humans between 1982 and 1992 [29]. Transient illness caused by large number of *S. Gallinarum-pullorum* was observed in both volunteers [30] and our case. Although the contaminated raw meat was not considered as a food safety risk due to the thorough cooking tradition, it was often associated with direct exposure to enteric pathogens and cross-contamination of ready-to-eat foods [31]. As a result, contamination from food handlers, or the consumption of contaminated or cross contaminated food may lead to the *Salmonella* infections.

Salmonella infections are normally associated with self-limiting diarrhea and antimicrobial therapy was not indicated, but appropriate antimicrobial treatment could be life-saving in severe cases. In addition, antibiotic treatment with ciprofloxacin or fluoroquinolones was recommended for *Salmonella* infections in infants less than 3 months of age due to the high risk of bacteremia and extraintestinal complications [32, 33]. The occurrence of multiple drug resistant strains raised the importance of antibiotics resistance surveillance. The high level of resistance to the first-line agents: ampicillin, sulphamethoxazole/trimethoprim, and chloramphenicol was also observed in other studies [23]. A similar profile was also found in the isolates from food-producing animals [34], suggesting the careful use of antibiotics in breeding industry was necessary.

The frequency of multiple drug resistance was common. Resistance to 5 or more antibiotics was commonly observed in *S. Enteritidis* (69.0%) and *S. Typhimurium* (78.8%). The occurrence of multidrug resistant (MDR) strains was reported to be associated with coexistence of resistance-related genes [14] and their transmission led to great difficulties in treatments. The resistance to first-line drugs of treating severe infections, such as third-generation cephalosporins and fluoroquinolones were of clinical concerns. The emergence of MDR isolates and increasing resistance to important antibiotics suggested the prevention measures and ongoing surveillance of antibiotic resistance were needed to control the infections.

The resistance to ciprofloxacin was mediated by multiple mechanisms [35], and the main mechanism of mutations in QRDRs were investigated in this study. A high frequency of mutations in the *gyrA*, *gyrB*, *parC*, and *parE* gene were found, indicating the need of long-term surveillance on QRDRs. In the isolates

absence of chromosomal mutations, other mechanisms such as overexpression of efflux pump and mutations of other elements might contribute to the resistance [36, 37]. Due to the small sample size and complexity of resistance mechanisms, the association between the detected mutations and resistant phenotypes was difficult to identify. But it was investigated in other studies, such as isolates with a mutation in both *gyrA* and *parC* were more susceptible to ciprofloxacin than the isolates with a mutation in *gyrA* alone [38].

Conclusion

Salmonella was an important causative microorganism of acute diarrhea in this region. A total of 42 serovars and 12 un-typable strains were identified from the 297 isolates during 2013 and 2018. *S. Typhimurium* and *S. Enteritidis* were the most common serotypes. The highest detection rate was found in the age group of less than 9 years old and during June and October. The highest rate of resistance was found in ampicillin (70.6%), followed by piperacillin (64.5%), tetracycline (63.5%), streptomycin (54.3%), cefazolin (44.2%), and sulphamethoxazole/trimethoprim (38.1%). Resistant to 5 or more antibiotics was most common in serotype *Typhimurium* (78.8%) and *Enteritidis* (69.0%). An increasing trend of resistant rate to third-generation cephalosporins and multi-drug resistance, and a high frequency of mutations in QRDRs were observed, which raised the awareness of rational use of antibiotics and ongoing surveillance.

Declarations

Ethical approval.

All aspects of the study were performed in accordance with national ethics regulations and approved by the Ethics Committees of the participated organizations, including Shenzhen Center for Disease Control and Prevention, Futian District Center for Disease Control and Prevention, and the sentinel hospitals. Participants received information regarding the purpose of this study and of their right to confidentiality. Written consent was obtained from the participants or the parents or guardians of the pediatric patients.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding authors on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

HS, JZ, and YO were involved in the collection of samples and detection of *Salmonella* isolates. TH detected the antibiotic resistant genes. HC collected the clinical data. WM, SC, and LZ helped analyzed data. QH and YZ designed this study. HS and YZ drafted and revised this manuscript.

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Figures

Figure 1

The highest detection rate was seen in August (11.01%), followed by September (8.56%), June (8.49%), and October (7.88%). The detection rate between May and November (8.19%) was significantly higher than that between December and April (1.89%). Similar seasonal distribution was also observed in the serovar Typhimurium isolates