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Original Article

Characteristic diversity and antimicrobial resistance of *Salmonella* from gastroenteritis

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ABSTRACT

Salmonella is a leading cause of foodborne disease worldwide and may cause to gastroenteritis. The aim of this study was to determine the prevalence, serotypes, virulence genes, molecular subtyping, and antibiotic resistance phenotype of *Salmonella* from gastroenteritis in Hubei, China. Of 500 patients stools samples collected from January 2015 to January 2016, 52 (10.40%) samples were contaminated by *Salmonella*. The results showed that most of the isolates were positive for eight virulence genes that appear on pathogenicity islands, prophages, plasmid, and fimbrial. A total of twelve serotypes were found. Antimicrobial susceptibility results indicated that most strains were resistant to ampicillin (57.69%), kanamycin (53.85%), and tetracycline (40.38%). There were 33 STs on MLST types, and were grouped into two clusters. Thus, our findings provided insights into the dissemination of antibiotic resistant strains, genetic diversity, and improved our knowledge of microbiological risk assessment in *Salmonella* from gastroenteritis.

1. Introduction

Gastroenteritis caused by *Salmonella* is a global public health concern. As the report, this organism accounting for a large number of patients [1,2]. The primary sources of salmonellosis are food-producing animals including poultry, pig, and cattle. This pathogen is also mainly disseminated by trade in animals and uncooked animal food products [3]. The report showed that about 80 million laboratory-confirmed cases of infection of *Salmonella* are annually recorded, globally [4]. In China, it also an important foodborne pathogen and contributed to approximately 70–80% of foodborne pathogenic outbreaks [5]. *Salmonella* mostly causes self-limiting gastroenteritis, although severe systemic infections do occur in infants, the elderly and immune compromised individuals [6].

The correct typing of *Salmonella* is essential for epidemiological investigation in infections and outbreaks. As a helpful method for characterizing *Salmonella*, virulotyping is beginning to inform our understanding of how various virulence gene repertoires reflect bacterial properties and cause specific disease syndromes. The pathogenic ability of this organism can be distinguished by virulence genes they carry [7,8]. To date, more than 2600 serotypes have been reported [9]. What is more, the prevalence of *Salmonella* serovars in different countries and also over time. Some serovars also be found that can emerge within a country or region for a period and then disappear with no obvious cause or intervention measure [10,11]. Multilocus sequence typing (MLST), which is based on sequence analysis of selected housekeeping genes, is becoming an important method for investigation of the evolution and epidemiology of various *Salmonella* owing to its high repeatability [12,13].

Traditionally, *Salmonella* is considered susceptible to antimicrobials. However, during the past few decades, antimicrobial resistance has emerged and evolved in many bacterial as the excessive use of antimicrobials in human and aquaculture systems [14,15]. It was reported that *Salmonella* strains with resistance to clinically important antimicrobial agents have been frequently isolated [16,17]. For example, chloramphenicol and trimethoprim-sulfamethoxazole are recommended as antibiotics in the treatment of severe *Salmonella* infections, but the resistance strains also been found [18]. Therefore, the potential presence of antibiotic-resistant *Salmonella* isolates may be an important public health

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problem related to disease management and control on gastroenteritis.

Although some *Salmonella* serovar, virulotyping studies and antimicrobial resistance have been undertaken, little is known of the distribution of this strains from gastroenteritis in China. In summary, our study is the first comprehensive study that described characteristic in these strains. The serovar and virulotyping was related to the pathogenicity; MLST typing showed genetic diversity, and he antimicrobial resistance patterns revealed the resistance strains. The information generated in this study will provide insights into the distribution and population of *Salmonella* from clinical.

2. Materials and methods

2.1. Sample collection, detection and isolation of Salmonella

From January 2015 to January 2016, a total of 500 patients stools samples were collected from hospital in Hubei, China. All the patients had been diagnosed with gastroenteritis.

Briefly, a 25 g sample was collected from each stools samples and was pre-enriched in 225 ml of buffered peptone broth, One millilitre cultures were incubated in 10 ml of selenite cystine broth (SC) at 37 °C and 10 ml of tetrathionate brilliant green broth (TTB) at 42 °C for 24 h, respectively. Loopfuls of SC and TTB cultures were streaked onto xyloselysine-tergitol 4 (XLT4) selective agar plates and chromogenic Salmonella agar plates, then incubated at 37 °C for 24 h. Presumptive colonies were picked from each plate, stabbed into a triple sugar iron slant, and incubated at 37 °C for 24 h. Isolates with typical Salmonella phenotypes were further confirmed using API 20E test strips (BioMerieux Co., French) [13].

2.2. Detection of virulence genes

Genomic DNA was extracted from collected *Salmonella* isolates by using a Bacterial Genomic DNA Purification kit (Dongsheng Biotech, Guangzhou, China) according to the manufacturer's instruction.

Eight virulence genes were individually detected in collected isolates with the PCR technique. Three (*avrA*, *ssaQ* and *sopB*) were found on the *Salmonella* pathogenicity islands (SPIs); *gipA* was on prophages, Gene *spvC* and *pefA* were located on a virulence plasmid, and two (*bcfC*, *steB*) were appear on the fimbrial cluster. The primer concentrations and amplification conditions used were as described previously [8,11,19].

2.3. Serotyping of Salmonella isolates

All the *Salmonella* isolates were serotyped by slide agglutination using commercial O and H antisera in accordance with the Kauffmanne-White scheme [18].

2.4. MLST

MLST analysis was conducted via the *Salmonella* MLST website and database (http://mlst.warwick.ac.uk/mlst) [20]. PCR conditions were denaturation at 96 °C for 5 min; annealing at 55 °C for 1 min; and extension at 72 °C for 1 min, for 35 cycles; with a final extension step at 72 °C for 10 min. PCR was performed with a Bio-Rad PTC-200 Thermal Cycler (Bio-Rad, California, USA) according to the manufacturer's directions. PCR products were sequenced on BGI instrument (Shenzhen, China). The alignments of these sequences were determined using BioEdit. The sequences were analyzed online (http://mlst.warwick.ac.uk/mlst/dbs/Senterica/) to assign allele numbers and define STs. The evolution tree of the concatenated sequences of the seven loci was built based on the method of the Kimura-2-parameter in Mega 6.0 [21].

2.5. Antimicrobial susceptibility testing

The susceptibility of the *Salmonella* isolates to antibiotics was examined by the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2014). Briefly, Muller-Hinton agar and a panel of 12 antibiotics disks were selected for resistance tests. These 12 antibiotic disks (Oxoid, Hampshire, UK) were ampicillin (10 μg), azithromycin (15 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), norfloxacin (10 μg), streptomycin (10 μg), trimethoprim-sulfamethoxazole (25 μg), and tetracycline (30 μg). The results were expressed as sensitive (S), intermediate (I), or resistant (R) following the methods of the CLSI.

3. Results

3.1. Salmonella in gastroenteritis

Of the 500 samples tested, 52 (10.40%) were positive for *Salmonella*. We also got 52 *Salmonella* isolates. All strains were stored at −80 °C in trypticase soy broth with 20% glycerol and all positive samples information was shown in Table S1.

3.2. Results of virulence genes

All of the isolates were further characterized by examination of eight virulence genes with the PCR technique. As shown in Table 1, most of the *Salmonella* isolates harboured *avrA*, *ssaQ, sopB* and *bcfC* genes, the ratio reach to 94.23%, 96.15%, 98.08% and 94.23%. These were 42 (80.77%) *Salmonella* strains detected *spvC* gene. The *steB*, *pefA* and *gipA* positive isolate were lower, but it still reached 61.54%, 32.69%, and 25.00%, respectively.

3.3. Serotype distribution

Fifty-two *Salmonella* isolates were serotyped into 12 distinct serovars, including *S. Pllorum* (n = 12), *S. Enteritidis* (n = 8), *S. Derby* (n = 7), *S. Meleagridis* (n = 5), *S. Rissen* (n = 4), *S. Give* (n = 3), *S. London* (n = 3), *S. Senftenberg* (n = 3), *S. Agona* (n = 2), *S. Heidelberg* (n = 2), *S. Weltevreden* (n = 2), and *S. Stanley* (n = 1).

3.4. Antibiotic susceptibility

According the diameter of zone of inhibition, all 52 *Salmonella* isolates were classified as sensitive, intermediate, and resistant using the breakpoints specified by the CLSI. The result shown in Table 2. The highest proportion isolates were resistant to ampicillin, with resistance was 57.69%. The resistance to kanamycin, tetracycline, streptomycin, chloramphenicol, nalidixic acid, trimethoprim-sulfamethoxazole, gentamicin, azithromycin, and ciprofloxacin reached 53.85%, 40.38%, 34.62%, 19.23%, 19.23%, 15.38%, 13.46%, 11.54% and 11.54%, respectively. None of the strains showed

Table 1
Detection results of virulence genes.

No.	Name	No. of isolates	No.	Name	No. of isolates
1	*avrA*	49 (94.23%)	5	*spvC*	42 (80.77%)
2	*ssaQ*	50 (96.15%)	6	*pefA*	17 (32.69%)
3	*sopB*	51 (98.08%)	7	*bcfC*	49 (94.23%)
4	*gipA*	13 (25.00%)	8	*steB*	32 (61.54%)

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