

Multilocus Sequence Typing of the Clinical Isolates of *Salmonella Enterica* Serovar Typhimurium in Tehran Hospitals

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What's Known

- Genetic linkage and sequence types of *Salmonella typhimurium* strains isolated from clinical samples were identified based on a typing method (MLST).

What's New

- We report the common sequence types of from Iran's hospitals for the first time. There is no previous study in this field in Iran.

Abstract

Background: *Salmonella enterica* serovar Typhimurium is one of the most important serovars of *Salmonella enterica* and is associated with human salmonellosis worldwide. Many epidemiological studies have focused on the characteristics of *Salmonella* Typhimurium in many countries as well as in Asia. This study was conducted to investigate the genetic characteristics of *Salmonella* Typhimurium using multilocus sequence typing (MLST).

Methods: Clinical samples (urine, blood, and stool) were collected from patients, who were admitted to 2 hospitals in Tehran between April and September, 2015. *Salmonella* Typhimurium strains were identified by conventional standard biochemical and serological testing. The antibiotic susceptibility patterns of the *Salmonella* Typhimurium isolates against 16 antibiotics was determined using the disk diffusion assay. The clonal relationship between the strains of *Salmonella* Typhimurium was analyzed using MLST.

Results: Among the 68 *Salmonella* isolates, 31% (n=21) were *Salmonella* Typhimurium. Of the total 21 *Salmonella* Typhimurium isolates, 76% (n=16) were multidrug-resistant and showed resistance to 3 or more antibiotic families. The *Salmonella* Typhimurium isolates were assigned to 2 sequence types: ST19 and ST328. ST19 was more common (86%). Both sequence types were further assigned to 1 eBURST group.

Conclusion: This is the first study of its kind in Iran to determine the sequence types of the clinical isolates of *Salmonella* Typhimurium in Tehran hospitals using MLST. ST19 was detected as the major sequence type of *Salmonella* Typhimurium.

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Keywords • *Salmonella* Typhimurium • Multilocus sequence typing • Iran

Introduction

Infectious diseases are considered the most common cause of morbidity and mortality in developing countries. Enteric infections constitute the second commonest medical problem after respiratory diseases.¹ *Salmonella enterica* (*S. enterica*) serovars are among the most frequent causes of enteric and gastrointestinal bacterial infections in animals and humans.² Food-borne diseases caused by non-typhoid *Salmonella* (NTS) can be a major public health problem.² Infections caused by *Salmonella*

species are increasing in Iran. Different serovars of *S. enterica* have been identified in Iran, and *S. enterica* serovar Typhimurium is one of the most prevalent serovars associated with human and animal diseases in this country.³

Although the infections caused by *Salmonella* Typhimurium are self-limiting in humans, a highly invasive form of NTS infections, associated with this organism, has been frequently reported in sub-Saharan Africa.^{2,4,5}

An important aspect in the characterization of bacteria is the molecular typing in determining the clonal and strain distributions among various environments. Traditional microbial typing methods, albeit generally considered to be variable, labor-intensive, and time-consuming, are of practical value in epidemiological investigations. Molecular detection and typing methods are mainly based on the analysis of the genetic material of microbial agents.⁶⁻⁸ The most commonly used methods include antimicrobial susceptibility phenotype analysis, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and virulence or resistance gene carriage.^{9,10} MLST has been known as a tool for epidemiological studies to investigate the evolutionary pathways and clonal lineages of bacteria. MLST differentiates strains into a sequence of 7 housekeeping genes with appropriate level of discrimination using allelic differences.⁹

Some studies have been previously conducted on the prevalence of *Salmonella* Typhimurium in clinical samples in Iran; however, most of the investigations in this country and other countries have focused on the prevalence of *Salmonella* Typhimurium in nonhuman hosts such as poultry, lobster, fish, and shrimp as sources of infection in the human host.¹¹⁻¹³

The present study, therefore, aimed at determining (a) the common clones of *Salmonella* Typhimurium in clinical isolates via MLST and (b) antibiotic resistance phenotypes using samples from a general hospital and a pediatric hospital in Tehran, Iran. In other words, the molecular epidemiology and genetic characteristics of this serovar in Iran were explored.

The main purpose of the current study was to investigate the genetic characteristics of the clinical strains of *Salmonella* Typhimurium using MLST in Tehran, Iran.

Patients and Methods

Salmonella Isolates

A total of 68 *Salmonella* isolates were collected from Baqiyatallah Hospital (a general hospital) and Mofid Pediatric Hospital in Tehran from

April to September 2015. These isolates were obtained from the clinical samples (stool, blood, and urine) of patients. *Salmonella* Typhimurium was identified and confirmed according to the conventional standard biochemical and serological tests.^{14,15}

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed against ceftriaxone (30 µg), ceftazidime (30 µg), amikacin (30 µg), nalidixic acid (30 µg), kanamycin (30 µg), ampicillin (10 µg), amoxicillin/clavulanic acid (20/10 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), streptomycin (10 µg), tetracycline (30 µg), doxycycline (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), cefotaxime (30 µg), and imipenem (10 µg) (Mast Company, UK) for the isolates of *Salmonella* Typhimurium using the disk diffusion method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). In addition, *Escherichia coli* ATCC 25922 was used as the quality control organism.

Multilocus Sequence Typing

Salmonella Typhimurium genomic DNAs were extracted using an extraction kit (CinnaGen, Iran). Further characterization and common sequence types (STs) of the *Salmonella* Typhimurium isolates were determined using MLST. The internal fragments of 7 housekeeping genes (*aroC*, *dnaN*, *hemD*, *hisD*, *pure*, *sucA*, and *thrA*) of *S. enterica* were amplified using specific primers by referring to online MLST database <http://mlst.warwick.ac.uk/mlst/dbs/Senterica>.¹⁶ Polymerase chain reaction (PCR) cycling conditions followed this condition: 95 °C for 5 minutes, 35 cycles of 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and 72 °C for 5 minutes. The PCR products were sent to Macrogen Company in South Korea for sequencing. Allele numbers and STs were assigned based on the instructions in the online MLST database.

Results

Among the 68 collected *Salmonella* species, a total of 21 (31%) isolates were identified as *Salmonella* Typhimurium. Seven (33%) *Salmonella* Typhimurium strains were isolated from the general hospital and 14 (67%) from the pediatric hospital. In the clinical samples, 57% (n=12), 14% (n=3), and 29% (n=6) of the *Salmonella* Typhimurium strains were obtained from stool, urine, and blood specimens, respectively. The demographic data showed

that 76% (n=16) and 24% (n=5) of the isolates were obtained from male and female patients, correspondingly.

The antibiotic susceptibility test showed that all the isolates were susceptible to gentamicin, ceftriaxone, ceftazidime, cefotaxime, imipenem, and ciprofloxacin. The highest antimicrobial resistance was found against tetracycline, chloramphenicol, and amoxicillin/clavulanic acid (figure 1). Multidrug-resistant (MDR) pattern was detected in 76% (n=16) of the isolates, which showed resistance to 3 or more antibiotic families. Simultaneous resistance to chloramphenicol, streptomycin, tetracycline, doxycycline, ampicillin, and amoxicillin/clavulanic acid (C/S/TE/D/AM/AMC) was observed in 38% (n=8) of the *Salmonella* Typhimurium isolates.

The 21 *Salmonella* Typhimurium isolates were assigned to 2 different STs using MLST: ST19 (82%, n=18) was more common than ST328 (18%, n=3). The allele profiles of the *aroC*, *dnaN*, *hemD*, *hisD*, *pure*, *sucA*, and *thrA* genes were 10, 7, 12, 9, 5, 9, and 2 in ST19 and 116, 7, 12, 9, 5, 9, and 2 in ST328, respectively. ST19 and ST328 are single-locus variants at the *aroC* locus. Both STs are placed in eBURST group number 1 (eBG1) or ST complex 1. ST19 and ST328 were detected in both hospitals. ST19 was identified in urine, blood, and stool specimens, whereas ST328 was not detected in blood samples. The *Salmonella* Typhimurium isolates that harbored ST19 and ST328 exhibited different antibiotic resistance patterns. Different antibiotic resistance patterns were also observed in each of the STs. Both STs were observed in female and male patients. The antimicrobial

resistance pattern, the source of isolation, and the STs of each strain are shown in table 1.

Discussion

The role of NTS types in high-risk individuals like children and immunocompromised patients and the emergence of MDR bacteria strains such as *Salmonella* Typhimurium have recently been of growing concern in many parts of the world, including Iran.¹⁷⁻¹⁹ According to the results of the present study, *Salmonella* Typhimurium accounted for over 30% of the causes of salmonellosis among the patients admitted to the hospitals under study. The highest amount of *Salmonella* Typhimurium (64%) was recovered from the pediatric hospital, showing higher susceptibility of children to gastrointestinal infections caused by this bacterium. The presence of MDR strains with a 76% frequency among the *Salmonella* Typhimurium isolates and the dominant antibiotic resistance profile (C/S/TE/D/AM/AMC) are highly significant. Our results are consistent with the results of studies conducted in Guangdong, China, in which high levels of resistance to chloramphenicol, streptomycin, ampicillin, and tetracycline in the clinical strains of *Salmonella* Typhimurium were reported.²⁰ In the current study, cephalosporins, aminoglycosides, imipenem, and ciprofloxacin showed high levels of activity against *Salmonella* Typhimurium and can, accordingly, be suggested as the first choice for the treatment of infections due to this bacterium in our area.

Previous studies which have used PFGE or PCR have shown that invasive *Salmonella*

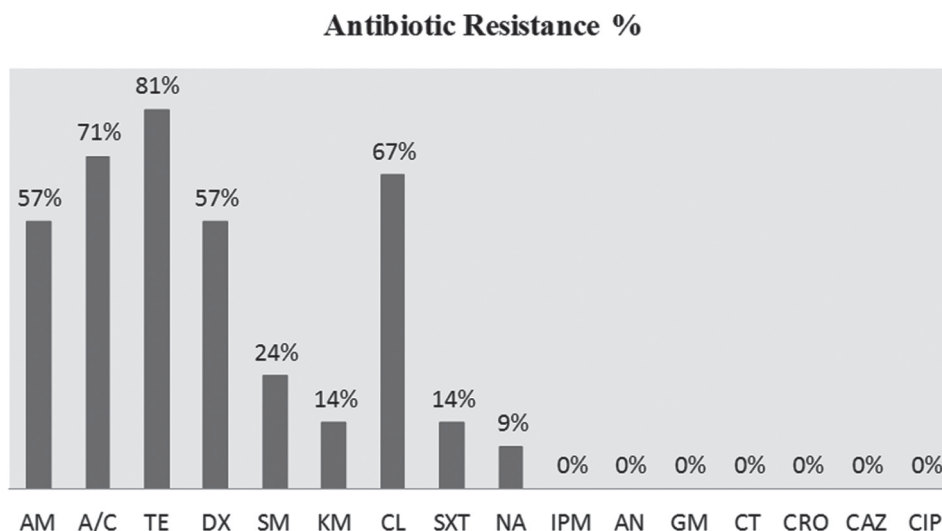


Figure 1: Rate (%) of antibiotic resistance in *Salmonella* Typhimurium strains isolated from clinical samples from Tehran hospitals. AM: Ampicillin; A/C: Amoxicillin/clavulanic acid; TE: Tetracycline; DX: Doxycycline; SM: Streptomycin; KM: Kanamycin; CL: Chloramphenicol; SXT: Trimethoprim/sulfamethoxazole; NA: Nalidixic acid; IPM: Imipenem; AN: Amikacin; GM: Gentamicin; CT: Cefotaxime; CRO: Ceftriaxone; CAZ: Ceftazidime; CIP: Ciprofloxacin.

Table 1: Comparison of the 21 clinical isolates of *Salmonella Typhimurium* according to the hospital, samples, sex of the patients, sequence type (ST), and antibiotic resistance patterns

Isolate	Hospital	Sample	Sex	ST	AM	SM	CI	TE	DX	A/C	K	NA	SXT	KM
1	Pediatric	Stool	M	19	S	R	S	R	R	S	S	S	S	S
2	Pediatric	Stool	M	19	S	R	R	R	S	R	S	S	S	S
3	General	Urine	M	19	S	S	S	S	S	S	S	S	S	S
4	General	Urine	M	328	R	R	R	R	S	R	R	S	R	R
5	General	Stool	M	19	S	R	S	S	R	S	S	S	R	S
6	Pediatric	Blood	F	19	R	S	R	R	R	R	S	S	S	S
7	Pediatric	Stool	M	328	S	S	R	R	R	R	R	R	R	R
8	General	Stool	F	19	R	S	R	R	R	R	S	S	S	S
9	Pediatric	Stool	M	19	R	R	R	R	S	R	S	S	S	S
10	Pediatric	Stool	M	19	S	S	S	S	S	S	S	S	S	S
11	Pediatric	Blood	M	19	S	S	S	S	S	R	S	S	S	S
12	General	Stool	F	19	R	S	R	R	R	R	S	S	S	S
13	Pediatric	Blood	M	19	R	S	R	R	R	R	S	S	S	S
14	Pediatric	Blood	M	19	S	S	R	R	R	R	S	S	S	S
15	General	Stool	F	328	R	S	R	R	R	S	R	R	S	R
16	General	Blood	F	19	R	S	S	R	R	R	S	S	S	S
17	Pediatric	Stool	M	19	R	S	R	R	S	R	S	S	S	S
18	Pediatric	Stool	M	19	R	S	R	R	R	S	S	S	S	S
19	Pediatric	Stool	M	19	R	S	R	R	S	R	S	S	S	S
20	Pediatric	Urine	M	19	S	S	S	R	S	R	S	S	S	S
21	Pediatric	Blood	M	19	R	S	R	R	R	R	S	S	S	S

All the isolates were susceptible to gentamicin, amikacin, ceftriaxone, ceftazidime, cefotaxime, imipenem, and ciprofloxacin

Typhimurium strains are genetically related.²¹⁻²⁴ Among the available techniques for typing and analyzing the genetic links of bacteria, we employed MLST because a) it clearly determines the phylogenetic links and the evolution trend of pathogens, b) its results can easily be exchanged between research centers and hospitals, and c) few studies have so far used this method to analyze bacteria such as the different types of *Salmonella* in Iran. In contrast to Iran, nevertheless, epidemiological studies have addressed the characteristics of *Salmonella Typhimurium* in China, Thailand, Japan, Hong Kong, and Taiwan via molecular typing methods like MLST and introduced the genetic features of *Salmonella Typhimurium* clones.²³⁻²⁵

Our MLST-based study also showed close genetic relationships between the *Salmonella Typhimurium* clones. The presence of a dominant clone in the samples of both hospitals showed the presence of a shared infection source or similar eating habits among the patients.²² The results of our study also demonstrated that ST19 and ST328 were found in both female and male patients, with the presence of different antibiotic resistance patterns among the isolates. These results revealed that there was no relatedness between ST, antibiotic resistance profiles, and sex of the patients.

In the present study, most (86%) of the *Salmonella Typhimurium* isolates were ST19.

There is some evidence showing that ST19 is also globally distributed in Asia.^{20,26} A similar study evaluated the molecular epidemiology and genetic characteristics of 294 endemic *Salmonella Typhimurium* clinical isolates, which were collected from 1977 to 2011 in Guangdong, China. These isolates were assigned to 13 STs by MLST. The results of that study are in agreement with those of the present study insofar as the former revealed that ST19 was one of the most common STs and that the eBG1 was the major group endemic in Guangdong. In that study, each of the STs showed a specific antibiotic resistance profile, while different antibiotic resistance profiles for the STs of *Salmonella Typhimurium* were observed.²⁰ Another common ST reported as one of the most prevalent STs of *Salmonella Typhimurium* in Asia is ST34. Be that as it may, ST34 was not found in our research, whereas Wong et al.²² and Achtman et al.²⁷ showed that ST34 was also the major MDR ST of *Salmonella Typhimurium* in Hong Kong and China and reported it as the major endemic *Salmonella Typhimurium* ST in Asia. However, ST19 seems to be globally distributed and ST313 is known as the predominant ST in Africa.²⁸ A study conducted in Africa revealed that a large number of its invasive *Salmonella Typhimurium* isolates belonged to ST313 and were obtained from cerebrospinal fluid and blood, while a small portion of the isolates had ST19 and were

all obtained from the patients' blood.²⁶ In our study, ST19 was obtained from stool and blood samples too. In contrast to our study, however, ST328 was reported as the dominant ST of *Salmonella* Typhimurium isolates in Taiwan.²⁴

Conclusion

The genotypic characterization of 21 *Salmonella* Typhimurium isolates recovered from 2 hospitals in Tehran, Iran, was reported here by MLST. These 21 isolates were assigned to ST19 and ST328, with ST19 being the more common ST. There was no resistant profile specific for a particular ST. A thorough assessment of the comprehensive relationships between the clinical strains of *Salmonella* Typhimurium in Iran requires more clinical samples from different sources.

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Conflict of Interest: None declared.

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