

Detection of common clones of *Salmonella enterica* serotype Infantis from human sources in Tehran hospitals

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ABSTRACT

Aim: The aims of this study were to investigate antibiotic resistance pattern and molecular characterization of *Salmonella* Infantis strains, isolated from human sources in Tehran hospitals from 2008 to 2010.

Background: Infection caused by *Salmonella* is one of the major public health problems. Despite the clinical importance of *Salmonella enterica* subsp. *enterica* serovar *Infantis* in humans, there is no information available about the common clones of *Salmonella* *Infantis* in clinical isolates in Iran.

Methods: *S. Infantis* strains were identified by conventional microbiological and serological testing. The antimicrobial susceptibility of the *S. Infantis* isolates was determined using the disk diffusion method. The genetic relatedness and the dominant clones of *S. Infantis* strains were detected by the Multi Locus Sequence Typing (MLST) and pulsed-field gel electrophoresis (PFGE) techniques.

Results: More than 80% of the *S. Infantis* isolates represented multidrug-resistant patterns. PFGE revealed high genetic similarity among *S. Infantis* strains. While, MLST indicated high-clonal similarity among strains, where all *S. Infantis* strains were assigned to the ST32 sequence type.

Conclusion: This is the first study in Iran conducted to determine the sequence types of *S. Infantis* in clinical isolates using MLST. The genetically closed MDR *S. Infantis* clones were responsible for the apparent endemic occurrence of salmonellosis, caused by this *Salmonella* serovar, in Tehran.

Keywords: *Salmonella* *Infantis*, Multi Locus Sequence Typing, Iran.

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Introduction

Gastrointestinal tract infection is still one of the most serious public health issues in many geographic areas and is endemic in most countries including Iran (1).

Salmonella is an important cause of gastrointestinal tract and food-borne infections worldwide. Infections caused by multi-drug resistant *Salmonella* spp. are increasing in many countries, including Iran (2,3).

Most of the *Salmonella* serotypes are potential pathogens for humans and animals. *Salmonella* Typhi, *Salmonella* *Infantis*, and *Salmonella* *Enteritidis* are known as the

most frequent serovars of *Salmonella* in humans, worldwide (4,5).

S. Infantis has been one of the most frequent serovars in many countries, including Asian countries. *S. Infantis* has been isolated from humans, animals and vegetables, meats (e.g. broiler and chicken), *S. Infantis* is more prevalent in poultry than in other animals (5,7).

In Iran, *S. Infantis* has been isolated from broilers and human sources (10, 11,13). Poultry is known as one of the major putative reservoirs for *Salmonella* in Iran. Dissemination of multidrug-resistant (MDR) *S. Infantis* has been reported in Iran and other countries. This has caused problems for the clinical and veterinary sectors (2, 3, 10, 11, 13).

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Different molecular typing methods of *S. Infantis* collected from food and clinical sources may increase the understanding of the epidemiology and evolution of *S. Infantis* strains in Iran. An important aspect of molecular typing of bacteria strains is determining the clonal and strain distributions among various environments. Molecular typing methods are proved to be helpful for this purpose. Therefore, several molecular typing methods have been developed to investigate the molecular epidemiology of microbial pathogens (14). Molecular typing approaches such as Multilocus Sequence Typing (MLST), Multiple-Locus Variable number tandem repeat Analysis (MLVA), pulsed-field gel electrophoresis (PFGE), repetitive sequence-based PCR (rep-PCR), ERIC-PCR, and ribotyping have been effectively used in phylogenetic and epidemiological studies of *S. Infantis*. MLST, known as a typing method based on PCR and sequencing, helps to explore the clonal lineages and evolutionary pathways of bacteria (5, 11, 15-19). There is only one instance of common clones of *Salmonella* Typhimurium reported from Iran, which was detected by MLST (20).

There is no phylogenetic study of *S. Infantis* by MLST in Iran. Therefore, there is no information of the sequence types or clones of *S. Infantis* in food or human sources. We aimed to report the common sequence types of *S. Infantis* isolated from clinical samples for the first time in Iran hospitals. The aim of this study is to investigate the antibiotic resistance patterns, genetic linkage and dominant clones of *S. Infantis* strains isolated from human sources in Tehran hospitals which was done by MLST and PFGE.

Methods

Salmonella Infantis Isolates

The study included all *Salmonella* strains isolated from all cases of enteritis in patients hospitalized in three major hospitals; Baqiyatallah, Mofid Children's Hospital and Children's Medical Center, in Tehran, during 2008–2010. These strains were isolated from clinical samples, including blood, urine, and stool. *S. Infantis* isolates were identified and confirmed according to the conventional standard of biochemical and serological tests (21).

Antimicrobial susceptibility test

Antimicrobial susceptibility of *S. Infantis* to the following was detected, based on the CLSI criteria

(22): Ampicillin (AMP 10µg), ceftriaxone (CRO 30µg), ceftazidime (CAZ 30µg), amikacin (AN 30µg), nalidixic acid (NA 30µg), kanamycin (K 30µg), amoxicillin/ clavulanic acid (AMC 20/ 10µg), trimethoprim/ sulfamethoxazole (SXT 1.25/ 23.75µg), streptomycin (S 10µg), tetracycline (TE 30µg), chloramphenicol (CHL 30µg), ciprofloxacin (CIP 5µg), gentamicin (10µg), cefotaxime (CTX 30µg), and imipenem (IPM 10µg) (Mast Company, UK).

Pulsed-Field gel electrophoresis

The clonal relatedness of *S. Infantis* isolates was analyzed by the PFGE method with the help of *XbaI* enzyme, using a CHEF-DRIII apparatus (Bio-Rad, USA). It was conducted in accordance with the CDC (Centers for Disease Control and Prevention) Pulse Net protocol (www.cdc.gov/pulse.net). The restriction patterns were compared using Dice-coefficient online program and grouped by the Unweighted Pair-Group Method with Arithmetic Mean (UPGMA). *S. Infantis* isolates were clustered based on the similarity with a coefficient higher than 90%.

Multilocus sequence typing

A total of 15 *S. Infantis* strains were selected for the MLST analysis. These isolates were chosen based on PFGE patterns, antibiotic resistance profiles, location of sampling, and type of specimens. *S. Infantis* genomic DNAs were extracted using a commercial extraction kit (CinnaGen, Iran). The internal fragments of seven housekeeping genes; *aroC*, *dnaN*, *hemD*, *hisD*, *pure*, *sucA*, and *thrA* of *S. enterica* were amplified using specific primers as described in the online MLST database (<http://mlst.warwick.ac.uk/mlst/dbs/Senterica>). All the PCR products were subjected to sequencing (Macrogen, South Korea) and the sequencing data were recorded in salmonella MLST database. Phylogenetic analysis was performed by eBURST.

Results

Salmonella Infantis isolates

The study included 6050 patients who had been admitted with enteritis symptoms to Baqiyatallah, Mofid, and Children's Medical Center in Tehran, during 2008–2010. Of these, 110 patients of which 49 (44.5%) were females and 61 (55.5%) were males showed symptoms of salmonellosis. *Salmonella*

56 Detection of common clones of *Salmonella enterica serotype* *Infantis*

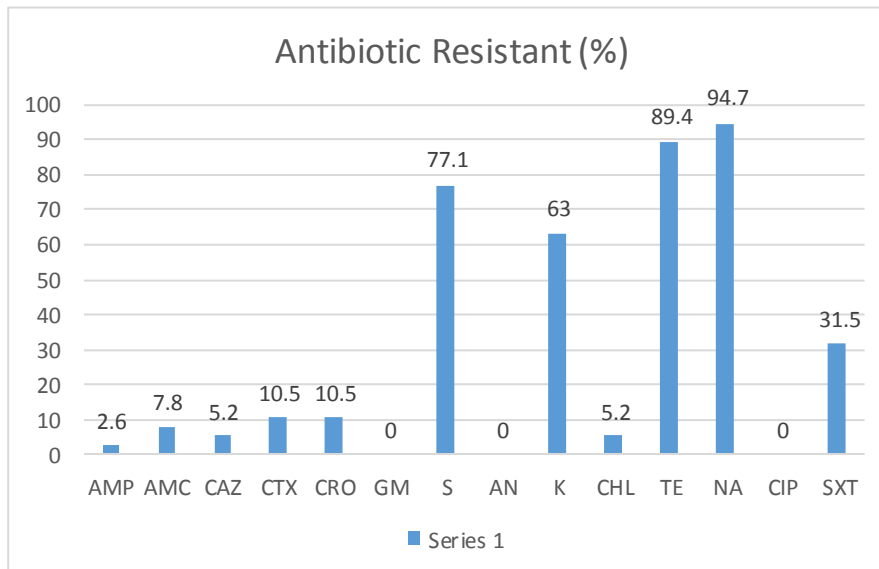


Figure 1. Rate (%) of antibiotic resistance in *Salmonella* *Infantis* strains isolated from the clinical samples collected from the hospitals in Tehran.

infection was diagnosed based on clinical presentations and laboratory confirmation. The majority of the patients (80%) were less than 12 years of age. Most of the *Salmonella* strains (94 isolates) were recovered from stools, whereas the remaining strains were isolated from urine, blood or other biological fluids.

Of 110 *Salmonella* isolates, 34.5% (38 strains) were identified as *Salmonella* *Infantis*. Thirty one *S. Infantis* strains (89%) were recovered from pediatric patients under 12 years and seven strains were isolated from patients over 12 years.

Antimicrobial susceptibility testing

The results of the antimicrobial susceptibility testing were shown in Figure 1. All the isolates were susceptible to gentamicin, amikacin, cefotaxime, imipenem, and ciprofloxacin. The high-level resistance to nalidixic acid, tetracycline, and kanamycin were detected in 94.7% (n = 36), 89.4% (n = 34), and 63% (n = 24) of the isolates, respectively. Multidrug-resistant (MDR) pattern was detected in 80% (n=25) of the isolates, which showed resistance to three or more antibiotic classes. Simultaneous resistance to streptomycin, tetracycline, nalidixic acid, and kanamycin (S/TE/NA/K) as well as streptomycin, tetracycline, nalidixic acid, kanamycin, ceftriaxone, cefotaxime, ceftazidime, and ampicillin (S/TE/D/N/NA/K/CRO/CTX/CAZ/AM) was observed

in 45% (n = 14) and 13% (n = 4) of *S. Infantis* isolates, respectively. Antibiotic resistance patterns of isolates are present in Table 1.

Pulsed-Field gel electrophoresis

According to the PFGE analysis, high-level similarity ($\geq 90\%$) was detected among *S. Infantis* strains isolated from Tehran hospitals. The isolates were divided into three different PFGE types or pulsotypes—A, B, and C (as shown in figure 2). The

Table 1. The frequency of antibiotic resistance patterns (%) in *Salmonella* *Infantis* isolates.

| Antibiotic resistance patterns | Frequency (%) |
|------------------------------------|---------------|
| SXT/ S/TE/N/CRO/CTX /AM/ CAZ/NA/AM | 2 (6.4%) |
| CF/S/TE/ N/CRO/CTX/ /AM/ CAZ/NA/AM | 1 (3.2%) |
| S/TE/ N/CRO/CTX/ AM /CAZ/NA/AM/K | 1(3.2%) |
| SXT/S/TE /N/NA/K | 2(6.4%) |
| SXT/S/TE /NA/K | 1(3.2%) |
| S/TE/ NA/K | 15 (48.3%) |
| S/TE/ NA | 3 (9.6%) |
| SXT/TE /NA | 1(3.2%) |
| S/TE | 1(3.2%) |
| TE/NA | 1(3.2%) |
| S | 3(9.6%) |

main PFGE profile or pulsotype was type A, which included 28 strains. There was no relationship between antibiotic resistant profiles, type of specimens, location of sampling, and PFGE patterns.

Multi-Locus sequence typing

All the *S. Infantis* isolates were assigned to sequence type 32 (ST32), using MLST. The allele profiles of the *aroC*, *dnaN*, *hemD*, *hisD*, *pure*, *sucA*, and *thrA* genes were 17, 18, 22, 17, 5, 21, and 19 in ST32, respectively. ST32 was located in the eBURST group number 31 (eBG1) or clonal complex 31.

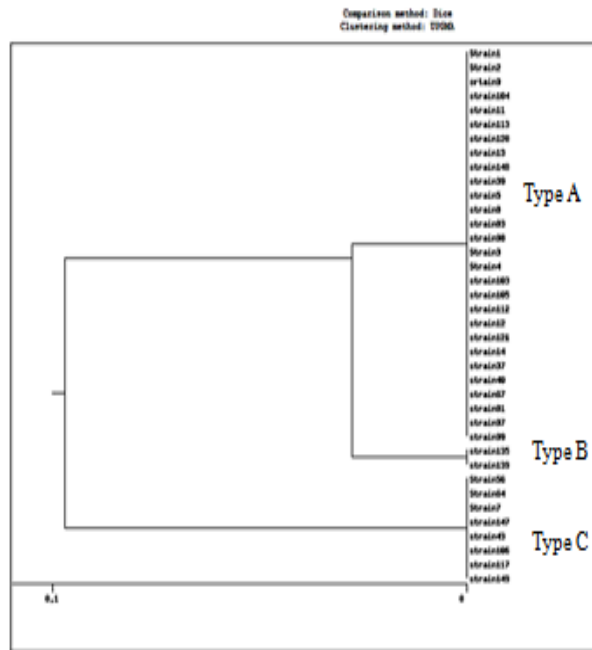


Figure 2. Dendrogram showing genetic relationships among 39 *Salmonella Infantis* strains based on PFGE patterns. The strains were compared using Dice index and clustered by UPGMA method.

Discussion

The present study provides evidence of the presence of high frequency of MDR, observed in *S. Infantis* clinical samples collected from the hospitals in Iran. The genetic relationship between these strains was also elucidated by PFGE and MLST techniques.

According to our results, the frequency of *S. Infantis* in human samples was 28%, which is higher than the rates reported earlier from Tehran, Iran by Tajbakhsh *et al.* (8%) and Hamidian *et al.* (5.4%) (10,13). However, Rahamni *et al.* reported higher prevalence (75%) of *S.*

Infantis in broiler farms located in three northern provinces of Iran (11).

In Italy, the surveillance system reported that the rate of isolation of *S. Infantis* from human infections ranged from 2-7% between the years 1980 and 2009—several years even represented the third- or fourth-most prevalent serotype (12, 23).

Our results showed high prevalence (80%) of MDR *S. Infantis* isolates, which is higher than the other studies in Iran (10, 13). The high frequency of MDR among *S. Infantis* is in agreement with the results of several studies from different countries such as Japan, Hungary, Italy, Brazil, and Germany. These studies identified healthy poultry as a potential reservoir of *S. Infantis* (9, 14, 15, 24, 25). In a study from Iran, partly similar antibiotic resistant patterns were detected in *S. Infantis* isolated from broilers. All the 27 *S. Infantis* isolates were resistant to ciprofloxacin, nalidixic acid, tetracycline, spectinomycin, streptomycin, and sulfamethoxazole (11). Furthermore, the resistance of these 27 *S. Infantis* to antibiotics was higher than the rates detected and reported by Tajbakhsh *et al.* in the *S. Infantis* isolated from the stool of patients from six hospitals in Iran. However, consistent with our results, they found that the resistance to tetracylin and nalidixic acid was more than the others antibiotics (10).

There is only one published study that used PFGE for molecular typing of *S. Infantis* strains in Iran. Rahmani *et al.* revealed highly similar PFGE patterns in *S. Infantis* strains isolated from poultry, indicating clonal relatedness across different geographical locations in Iran (11). The PFGE results of our study showed a high genotypic similarity among the strains isolated from humans admitted in different hospitals in Tehran, Iran. The PFGE analysis showed that these strains belonged to a uniquely large cluster. PFGE was used successfully for molecular typing of *S. Infantis* strains, isolated from different sources worldwide. Epidemiological evidences confirmed clonal distribution of *S. Infantis* isolates. Dionisi *et al.* showed high similarity among *S. Infantis* isolated from different sources in Italy. Cluster analysis concluded that isolates with same resistance patterns belonged to a large cluster with > 90% genetic similarity (12). Nógrády *et al.* found two large related clusters of *S. Infantis* isolates in various European countries—of which the Austrian and Polish MDR clones of a cluster are identical with, or closely related

58 Detection of common clones of *Salmonella enterica* serotype Infantis

to, the main Hungarian clone (8). Hauser *et al.* showed that two major closely related genotypes of *S. Infantis* were isolated from broiler, meat, and pork in Germany (26).

Additionally, MLST showed a high clonal similarity among all the strains assigned to the same ST32. This is consistent with the previous results found by others from different sources worldwide (5, 24, and 26-28). Our findings indicated that the majority of *S. Infantis* strains studied may have descended from a common precursor that is responsible for the contamination in humans in Tehran.

The findings of this study provide further information on the molecular epidemiology of *S. Infantis* isolated from human sources in Tehran. The genetically closed MDR *S. Infantis* clones are responsible for the apparent endemic occurrence of salmonellosis, in Tehran. Further molecular epidemiology investigations are required to assess the linkage and clonal relatedness of the *S. Infantis* strains isolated from different sources, such as humans, food and animals, in a different period of time and region in Iran.

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Conflict of interests

The authors declare that they have no conflict of interest.

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