

# Genetic diversity of clinical strains of *Salmonella enterica* serovar Typhimurium

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## Abstract

**Aims:** *Salmonella* spp. is one of the most common pathogenic bacteria responsible for gastroenteritis in humans. Among *Salmonella* spp., *S. typhimurium* has many hosts other than humans and its prevalence rate is high in the society. The aim of this study was to investigate the ribotype diversity of *S. typhimurium* stains isolated in some hospitals in Tehran, Iran.

**Methods:** In this descriptive study, clinical samples collected from different hospitals in Tehran were investigated. Bacterial isolation was carried out using selective culture media by standard procedures and identification was achieved through biochemical and serological methods. Total bacterial DNAs was extracted and ribotyping was used for molecular typing of the clinical isolates of *S. typhimurium*.

**Results:** The sizes of the ribotyping bands ranged from 1.4 to 16.8 kb in all ribotypes. The *S. typhimurium* isolates were divided into 7 clusters based on the diversity of their ribosomal genome areas. Most isolates (5 strains) belonged to the cluster 2b. Three isolates belonged to the 6b cluster and for the rest of isolates, each belonged to one of other clusters.

**Conclusion:** *S. typhimurium* strains circulating in the studied hospitals of Tehran, do not belong to a specific ribotype and have ribotype diversity, but other molecular typing methods should be implemented in order to make a more precise judgement about the presence of whether limited or widespread clones and the relationship among the different strains of this serotype.

**Keywords:** *Salmonella typhimurium*, Gastroenteritis, Molecular Typing

## Introduction

Classification of *Salmonella* is too much complicated. Based on the latest classification, members of *Salmonella* spp. are divided into two major species: *Salmonella enterica* and *Salmonella bongori*. *Salmonella enterica* is divided into seven sub-classes, based on biochemical and DNA homology, each of which includes different serotypes. *Salmonella* is divided into more than 2600 serovar, based on flagella and somatic antigens, most of which are infectious for human and animals and generally the transmission of the diseases is done through water and food [1, 2, 3]. Among the bacteria transmitted through the food, *Salmonella* is of particular importance [4, 5]. Gastroenteritis is the most widespread and prevalent infection due to infection with *Salmonella* spp. in humans, in particular, by *S. typhimurium* and *S. enteritidis*. Around 1.4 million cases of *non-typhi salmonellae* infection are reported in the US annually [6 and 7].

Humans get infection usually through the consumption of raw foods such as meat, egg and daily foods. Therefore, the main sources of salmonellae in human are contaminated foods. [8, 9]. *S. typhimurium* and *S. enteritidis* are the most important agents in creating bowel diseases in humans and animals which are

considered as one of the most important health problems worldwide [10, 11, 12, 13, 14].

*S. typhimurium* is among the *Salmonella* species that has many hosts in addition to the human beings and the possibility of their prevalence is high [13, 15 and 16]. Among the important features of this species is its ability to remain alive and proliferous (the ability to multiply and reproduce) for a long time in the environment and food samples [16].

Although, the members of *Salmonella* spp. are quite similar to each other in terms of genetic likeness, there are massive changes in the emergence of the disease, its virulence and the disease's pathogenicity. Obtaining or losing some of genes plays a major role in the evolution of different serotypes of *Salmonella*. Methods based on analyzing DNA remove many limitations on phenotype method and improves our knowledge about epidemiological and genetic relations involved in human infections [17, 18, 19].

Ribotyping is an appropriate method to identify and classify bacteria. Due to the repeatability and high precision of this method, it is able to classify bacteria up to the species and serotype level. Ribotyping is able to discriminate different serotypes of bacterial species according to the source of infection, irrespective of the host or geographical place [17, 20, 21].

The aim of this study was to determine the ribotype

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diversity of *S. typhimurium* stains isolated in some hospitals of Tehran, Iran.

## Methods

A total of 68 *S. enterica* strains had been isolated from clinical samples from different hospitals located in Tehran, Iran.

Each clinical isolate was transferred into enrichment Selenite F medium immediately and had been incubated for 8-12 hours at 37°C. Then these samples were sub-cultured on selective and differential media like XLD (Xylose lysine deoxycholate) agar and SS (*Salmonella* and *Shigella* agar) agar, and were incubated for 24 hours at 37°C. In the next day, suspected *Salmonella* colonies were isolated, so that every single colonies in sterile conditions was transferred on TSI (Triple Sugar Iron Agar), Urea, Lysine Iron Agar, Citrate and MRVP for further identifications. All isolates were confirmed by using antisera O and H (Staten Serum Institute, Copenhagen, Denmark) based on slide agglutination test to characteristics of O and H antigen respectively. Finally, all isolates tested positive with antisera were transferred to LB broth mixed with glycerol and kept at -80 °C. Out of all *S. enterica* isolates, 13 strains that belonged to B serogroup enrolled in this study. Total bacterial DNA was extracted [11] and digested with *Pst*I restriction enzyme under conditions recommended by the manufacturer (Roche Diagnostics, Mannheim, Germany). Digested DNA fragments were resolved on a 1% agarose gel in TBE buffer and then transferred onto nylon membrane by the alkali-blotting procedure on a vacuum blotter. Hybridization was then performed with a digoxigenin-11-dUTP (DIG) labeled oligonucleotide probe mixture. The membranes were then visualized by addition of alkaline phosphate-conjugated anti-digoxigenin antibody (Roche Diagnostic GmbH, Mannheim, Germany) and nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) as the substrate [22]. NBT/BCIP nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate color development was clearly visible between 30 min and 1 h after the start of the reaction.

## Results

The sizes of the ribotyping bands ranged from 1.4 to 16.8 kb in all ribotypes. The results showed that thirteen strains of *S. enterica* serotype Typhimurium were divided into 7 clusters (1b to 7b). Most strains

(5) were in cluster 2b. Three other strains were in the cluster 6b and there was a strain in each of the cluster 1b, 3b, 4b, 5b and 7b (Figure 3). Table 2 describes characterization of *S. typhimurium* strains.

## Discussion

In the present report, we described ribotype patterns of 13 strains of *Salmonella* serotype Typhimurium using ribotyping method.

Due to the increase number of hospital pathogens, identification of microbial strains and investigation of epidemiology relationship and genetic diversity among them is of importance. Conventional methods such as biotyping, serotyping, bacteriophage typing, bacteriocin typing, and anti-microbial profiles have been evaluated as inappropriate methods for typing of all bacterial species, however in the recent years, molecular methods have been used successfully for genotyping of many pathogenic bacteria [17, 18, 19]. Modern typing methods are based on genotype characterization; moreover, most of them appear to be suitable for studying both population genetic structure and evolutionary relationships within the genus. The most genotyping methods applied for typing of *S. typhimurium* are plasmid profile analysis (PP), insertion sequences (ISs), polymerase chain reaction (PCR), restriction analysis of the virulence plasmid, ribotyping, pulsed field gel electrophoresis (PFGE), and random amplified polymorphic DNA analysis (RAPD). [23, 24, 25]. Ribosomal sequence genes are highly conserved and probes used in this method would be hybrid with the ribosomal from many bacterial species. Since, all bacterial strains have ribosomal operons, all of them could be classified using this method [17, 18, 19, 20].

Ling et al reported that *S. enteritidis*, *S. typhimurium* and *S. derby* were the three most common *Salmonella* serotypes isolated during 15 years in Hong Kong. They studied the strains of *S. enteritidis*, *S. typhimurium* (isolated during 1997-2004), *S. derby* (isolated during 1995-2004), and *S. typhi* (isolated during 1998-2004) and found that no difference in banding patterns was observed when the strains were tested by various molecular typing methods [26]. Guerra et al have also used ribotyping method using *HincII*, *SalI*, and *PvuII* restriction enzymes for typing of 84 isolates of *S. typhimurium*. The method differentiated the strains into 19 different ribotypes. They concluded that this technique was a powerful tool for typing of *S. typhimurium* in comparison with other methods such as phage typing [27].

## Conclusion

*S. typhimurium* strains circulating in the studied hospitals of Tehran, do not belong to a specific ribotype and have ribotype diversity, but other molecular typing methods should be implemented in order to make a more precise judgment about the presence of whether limited or widespread clones and the relationship among the different strains of this serotype.

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