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Original Article

# Detection of Zoonotic Antibiotic Resistant *Salmonella* spp. Carrying Virulence Genes in Rural *Mus musculus*, Golestan Province, North of Iran

**Somayeh Namroodi**[1*]

[1]Department of Environmental sciences, Faculty of fisheries and environmental Sciences, Gorgan University of Agricultural Sciences & Natural Resources, Gorgan, Iran

**Abstract**

**Background and aims:** *Salmonella* spp. infect cold-blooded and warm-blooded animals and may cause a worldwide zoonotic disease, salmonellosis, in infected animals**.** Rodents can be *Salmonella* carriers without any signs of salmonellosis**.** The frequency of salmonella contamination, the presence of virulence genes (*SpvR* and *SpvB*) and antibiotic resistance pattern of isolated *Salmonella* were studied in rural *Mus musculus* to reveal the possible role of them in *Salmonella* dissemination in Golestan province.

**Methods:** A total of 190 wet rectal swabs of *M. musculus* were obtained from rural areas of Golestan province with different climate conditions. The swabs were cultured and positive samples were serotyped and their antibiotic resistance patterns were studied. The presence of *Salmonella* and virulence genes was analyzed by SpvR and SpvB genes primers, respectively.

**Results:** *Salmonella* spp. were detected in 15 (7%) of 190 fecal samples by bacterial culture and PCR. *S. enteritidis* (n=9) and *S. typhimurium* (n=6) were identified. The highest and lowest frequencies were detected in humid (13.1%) and arid areas (3.1%), respectively. SpvR and SpvB genes were diagnosed in 10 of 15 (66.6%) isolated *Salmonella*. The highest resistance of detected *Salmonella* spp. was observed against streptomycin (53%). All the isolates were sensitive to chloramphenicol, gentamicin and trimethoprim.

**Conclusion:** The *Salmonella* contamination in sampled house mice indicates that native people should be made aware of the risk of *Salmonella* infection and possible ways of salmonella transmission through rodents. In addition, the application of appropriate therapeutic approaches to prevent the spread of antibiotic resistant *Salmonella* is recommended.

**Keywords:** Salmonella, *Mus musculus*, Golestan, Virulence gene

*Corresponding Author:
Somayeh Namroodi,
Tel: +981732427040
Email:
snamroodi2000@yahoo.com

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## Introduction

*Salmonella* is a genus of rod-shaped gram-negative bacteria from the Family *Enterobacteriaceae* with over 2500 serotypes. These bacteria infect a wide range of cold-blooded and warm-blooded animals and may cause salmonellosis in infected animals.[1] Because of the wide diversity of animal reservoirs of these bacteria, *Salmonella* spp. are one of the most commonly transmitted bacteria from animals to humans. Generally, *Salmonella* spp. are transmitted to humans through contaminated food and water.[1]

The gastrointestinal tract is the main site of the presence of *Salmonella* in the body. Therefore, the main source of environmental *Salmonella* contamination is the feces of the infected hosts. The symptoms of *Salmonella* contamination depend on its pathogenicity which is under control of specific virulence genes (SpvB and SpvR). These genes also play an important role in generating antibiotic resistance and dissemination of the bacteria in the bodies of hosts (humans and animals).[2]

While *Salmonella* species lacking the virulence genes may not cause any symptoms in the host, *Salmonella* spp, especially *S. typhimurium* and *S. enteritidis*, that carry plasmids containing virulence genes, can cause systemic disease in infected hosts.[3]

The abundant use of antibiotics induces antimicrobial resistance in *Salmonella* spp. and causes extensive problems in the treatment of salmonellosis.[3,4]

The abundance and wide variety of plant species in Golestan province created suitable habitats for the presence of a wide variety of animals.[5]

Rodents are the largest group of mammals that have

always been considered as one of the main reservoirs of some zoonotic diseases.[6] Considering the importance of salmonellosis in animals and humans, comprehensive information about the frequency of salmonella contamination in its carriers and also control of bacteria is required.

Given that rodents can be *Salmonella* carriers without any sign of salmonellosis, and also due to the abundance of rodents in rural areas of Golestan province, it seems that rodents can play an important role in *Salmonella* spp. dissemination.[7] Having enough information about the frequency of salmonella contamination in rodents is not only vital because of the general health of villagers in rural areas and domestic animals, but also is important for the control of *Salmonella* dissemination in poultry farms, which are usually built around rural areas in Golestan province.

Considering the importance of salmonellosis in humans and animals (domestic and wild) and the abundance of house mouse (*Mus musculus*) as the dominant species of rodents in the northern regions of Iran, the frequency of *Salmonella* contamination in house mice, the presence of virulence genes (SpvR and SpvB) and antibiotic resistance pattern of isolated salmonella were analyzed in rural areas (with different climates) of Golestan province to determine the role of house mouse in *Salmonella* dissemination.[8]

## Materials and Methods

### Sampling

Golestan province consists of regions with three kinds of climatic condition: arid, semi-arid and humid.[5] Using handmade wooden mousetrap, 190 house mice were captured in areas with arid, semi-arid and humid weather conditions and the data including time and region of sampling were written in special forms (Table 1). Then, fecal samples were taken by wet rectal swabs. The fecal swab was transferred to Eppendorf containing selenite F medium. After the incubation of samples at 37°C for 18 hours, they were inoculated on Brilliant Green (BG) agar and Salmonella-Shigella agar (SS) plates. Each plate was incubated at 37°C for 24 hours. The suspected *Salmonella* colonies were biochemically examined using triple sugar iron (TSI) agar plate, lysine-iron agar (LIA) medium, urea agar plate, SIM agar plate, Simmon's citrate agar plate, and lactose, sucrose, maltose, and mannitol broth media.[9]
To define the serotype of *Salmonella* isolates, each positive sample was cultured on TSI slant medium and grown at 37°C for 24 hours. Then, the colonies were mixed with 9% physiologic serum and tested using antisera O (B, D, E, C) and H based on slide and tube agglutination tests to determine O and H antigens, respectively.[10]

**Table 1.** Sampled Areas Based on Climate Condition

| Climate Condition | City or Village |
|---|---|
| Humid | Gorgan |
| | Kurdkuy |
| | Ramian |
| | Bandargaz |
| | Nodehmalek |
| | Zangian |
| Semi-arid | Ataabad |
| | West Gharahgol |
| | Radkan |
| | Ziarat |
| | Gomishan |
| Arid | Maravehtappeh |
| | Gharah achagh |
| | Aghghala |
| | Gildagh |
| | Gookdarreh |

### DNA Extraction and PCR

Before DNA extraction, positive samples were transferred to Luria Bertani agar (LB) plates and kept at 37°C for 24 hours in an incubator. DNA sample was extracted from positive colonies by boiling them according to the method used by Hyeon et al.[11] Primers specific to ST 11 and ST 15, and SpvR and SpvB genes, were used, respectively, to detect *Salmonella* and virulence genes (Table 2).[12-14] Positive (*S. typhymurium*: ATCC14028) and negative (deionized water) samples were included in each cycle.

### Antibiotic Susceptibility Test

The resistance of *Salmonella* spp. isolates to the 9 antibiotics (gentamicin 10 µg, ampicillin 10 µg, cephalothin 30 µg, tetracycline 30 µg, streptomycin 10 µg, nalidixic acid, 30 µg), kanamycin 30 µg, chloramphenicol 30 µg, and trimethoprim 23.25 µg) was diagnosed by the disc diffusion method according to the Clinical & Laboratory Standards Institute (CLSI) standards, which classify the isolates based on their zone sizes as resistant, intermediate, or susceptible.[15]

### Statistical Analysis

SPSS software version 13.0 and chi-square test were used to analyze the obtained data.

## Results

*Salmonella* spp. were detected in 15 (7%) (6 (7.5%) males and 9 females (8.1%)) of 190 (80 males and 110 females) fecal samples by bacterial culture and PCR. The serotyping of the positive samples identified *S. enteritidis* (n=9) and *S. typhimurium* (n=6). The highest and the lowest prevalence of positive samples were detected in humid (13.1%) and arid areas (3.1%), respectively. *Salmonella* contamination was detected to be higher in mice from the humid area than those from the arid and semi-arid areas (Table 3).

**Table 2.** Primers Sequence Used in the Current Study

| Target Gene | Sequence (5´–3´) | Product Size (bp) |
|---|---|---|
| SpvR | 5′ CAGGTTCCTTCAGTATCGCA3′ | 310 |
| SpvB | 5′ CTATCAGCCCCGCACGGAGAGCAGTTTTTA3′ | 717 |
| Random sequence (ST11, ST15) | 5′GCCAACCATTGCTAAATTGGCGCA 3′<br>5′GTAGAAATTCCCAGCGGGTACTGC3′ | 429 |

SpvR and SpvB genes were diagnosed in 10 of 15 (66.6%) isolated *Salmonella* serovars by PCR.

Antibiotic resistance patterns of detected *Salmonella* spp. were different. The highest and the lowest resistances of detected *Salmonella* spp. were observed against streptomycin (53%) and ampicillin (40%), respectively. All the isolates were sensitive to chloramphenicol, gentamicin and trimethoprim (Table 4).

## Discussion

The *Salmonella* contamination rate of 7% in house mice in the current study indicates the presence of this bacterium in the wildlife and rural ecosystem of Golestan province as well as dissemination of the bacteria in these ecosystems through the sampled house mice.

Due to periodic salmonella excretion through feces of *Salmonella* infected mice, it is necessary to take several samples at appropriate intervals for accurate detection of the carriers of the salmonella.[14] Therefore, considering that domestic mice were sampled only for one time, the precise level of salmonella contamination of sampled house mice should be higher than 7%.

As seen in the results, the frequency of *Salmonella* contamination in house mice was in relation to weather conditions of sampled areas, and the highest rate of salmonella contamination was detected in humid areas. Suitable condition for *Salmonella* growth in humid areas can be one of the explanations for obtaining such a result.[16]

This study is the first one on salmonella contamination in rural house mice in Golestan province and also Iran. Due to the importance of rodents in the spread of *Salmonella*, similar studies have been conducted on rodents in other countries.

In 1992, the *Salmonella* contamination rate in rodents was reported to be 1-15% worldwide.[17] In a similar study, Nkogwe et al reported salmonella contamination in 2% of apparently healthy wild rats in Trinidad and Tobago.[18]

*Salmonell*a contaminations of rats without clinical signs were 16% and 0% in the United States and Trinidad, respectively.[19]

In 1996, Oboegbulem et al reported the highest rate of salmonella contamination (32%) in *Thryonomys swinderianus* from Nigeria.[20] Hilton et al documented that 10% of brown rats in England were contaminated with *Salmonella*.[21]

The observed differences in the results of similar studies may be due to the differences in the ecosystem condition (e.g., urban, rural and wild) of sampled rodents' habitat, the contact of rodents with other host species of *Salmonella* (such as birds), the sensitivity of applied diagnosis tests, the sample size of investigated rodents and the climatic conditions of the studied areas.[15] In line with most of the mentioned similar studies, this study revealed that none of the sampled house mice had symptoms of salmonellosis.

**Table 3.** Frequency of Contamination in Sampled Mus Musculus Based on Sampling Areas

| Climate Condition | City or Village | Frequency of *Salmonella* Contamination |
|---|---|---|
| Humid (14.5%) | Gorgan | 1/8 (12.5%) |
| | Kurdkuy | 1/7 (14.2%) |
| | Ramian | 1/8 (12.5%) |
| | Bandargaz | 1/10 (10%) |
| | Nodehmalek | 2/8 (16.6%) |
| | Zangian | 2/14 (14.2%) |
| Semi-humid (8.6%) | Ataabad | 2/14 (14.2%) |
| | West Gharahgol | 0% |
| | Radkan | 1/10 (10%) |
| | Ziarat | 1/12 (8.3%) |
| | Gomishan | 1/12 (8.3%) |
| Arid (3.1%) | Maravehtappeh | 0% |
| | Gharah achagh | 0% |
| | Aghghala | 1/18 (5.5%) |
| | Gildagh | 1/15 (6%) |
| | Gookdarreh | 0% |

**Table 4.** Antibiotic Resistance Patterns of Isolated *Salmonella* spp.

| Antibiotic | Resistant | Semi-resistant | Sensitive |
|---|---|---|---|
| | No. (%) | No. (%) | No. (%) |
| Streptomycin | 8 (53.3) | 6 (40) | 1 (6.6) |
| Cephalothin | 0 (0) | 1 (6.6) | 14 (93.3) |
| Chloramphenicol | 0 (0) | 0 (0) | 15 (100) |
| Nalidixic acid | 5 (33.3) | 0 (0) | 10 (66.6) |
| Trimethoprim | 0 (0) | 0 (0) | 15 (100) |
| Tetracycline | 2 (13.3) | 1 (6.6) | 12 (80) |
| Kanamycin | 3 (20) | 9 (60) | 3 (20) |
| Ampicillin | 6 (40) | 2 (13.3) | 7 (46.6) |
| Gentamicin | 0 (0) | 0 (0) | 15 (100) |

Such a result can highlight the dangerous role of house mice in unsuccessful control of salmonellosis in rural areas of Golestan province.[16,21]

Two isolated *Salmonella* spp. from house mice in this study, *S. typhimurium* and *S. enteritidis*, usually contaminate humans by food sources. This indicates the possible dangerous role of house mice in the occurrence of human salmonellosis in the *Salmonella* contaminated villages of Golestan province.[17,22]

A limited number of studies have been conducted on the identification of *Salmonella* serotypes in rodents. The results of previous studies indicate that rodents have been infected with *S. thompson*, *S. poona*, *S. paratyphi A* and *S. infantis*.[23]

Henzler et al reported 24 % salmonella contamination in apparently healthy rats in broiler breeding farms. They also said that rats could play an important role in the transmission of *S. enteritidis* to avicultures.[17]

Similar to this study, previous studies on the other animal species (e.g., cats, dogs, turtles, sheep, and jackals) with different nutritional and behavioral patterns in Golestan province indicate that *S. typhimurium* and *S. enteritidis* are the two most common *Salmonella* spp., which affect animals in Golestan province.[24-28]

Generally, dominant serotypes may vary in different regions and in different periods of time depending on the main carrier species, the rate of antibiotics usage and the host population density.[1,29]

The main sources of *S. typhimurium* and *S. enteritidis,* which had contaminated sampled house mice, were not determined in this study. Considering that these 2 serotypes have been isolated from fecal samples of sampled cats, dogs, sheep, jackals and turtles in Golestan province, all of these species may transmit the bacteria to the sampled rural house mice. Moreover, given the fact that rodents usually live together in densely populated areas, salmonella contamination in sampled mice could have been occurred due to contact with Salmonella-contaminated feces of other rodents.[20] Obviously, molecular studies are needed to verify such hypotheses.

Despite the lack of observation of clinical signs of salmonellosis in sampled house mice, the presence of SpvR and SpvB genes in 66% of isolated *Salmonella* spp. reveals the spreading risk of highly pathogenic *Salmonella* spp. through sampled house mice in rural areas of Golestan province. There is not any similar study on the presence of these genes in *Salmonella* spp. isolated from rodents. However, the presence of these genes in *Salmonella* spp. isolated from rural cats in Golestan province has been documented.[27]

Based on the results of this study, all detected *Salmonella* spp. (100%) were susceptible to gentamicin, trimethoprim, and chloramphenicol. Given that these antibiotics are not commonly used in livestock and poultry industry, it seems that they can be used as an effective treatment for salmonellosis in house mice in sampled areas.

The highest rates of antibiotic resistance were to streptomycin (53%) and ampicillin (40%). The abundant use of streptomycin and ampicillin in the treatment of livestock and breeding birds in Golestan province can be one of the reasons for the detection of such antibiotic resistance pattern in isolated *Salmonella* spp.

Antibiotic resistance patterns in *Salmonella spp.* isolated from other animal species (e.g., sheep, cats, dogs, turtles, and jackals) in Golestan province have shown a similar trend. This can indicate *Salmonella* transmission chain in these animals.[24-28]

However, the results of various studies indicate that the antibiotic resistance pattern of bacteria varies in different regions and times depending on *Salmonella* serotype, animal host species, the category and dosage of common antibiotics.[1]

## Conclusion

The *Salmonella* contamination of house mice in Golestan province indicates the risk of salmonella transmission from house mice to humans, livestock and other hosts through contamination of water, food and environment by the feces of house mice. Therefore, it seems that the natives of the Golestan province, especially in areas where the *Salmonella* contamination in rodents was high, should be made aware of the risk of *Salmonella* infection and possible ways of *Salmonella* transmission through rodents. Moreover, the application of appropriate therapeutic approaches to prevent the spread of antibiotic resistant strains of *Salmonella* seems necessary.

## Ethical Approval

Not applicable.

## Conflict of Interest Disclosures

None.

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