



Original Article

# Prevalence of *Salmonella* in Poultry Slaughterhouses of Kerman, Iran

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## Abstract

**Aim:** *Salmonella* is a prevalent infectious agent that infects several animals. Chicken is a main meal for humans, and the infectivity of the animal by bacteria threatens both human health and economic conditions. Improving our knowledge regarding the prevalence of *Salmonella* in chicken can help us organize new strategies to increase the quality of food in Iran. This project aimed to explore the prevalence of *Salmonella* infection among chickens from poultry slaughterhouses in Kerman, Iran.

**Methods:** In this cross-sectional study, 100 samples of chicken meat from poultry slaughterhouses supplied to shopping centers in Kerman were collected for investigation. To confirm the *Salmonella* infection, tissues were homogenized under sterile conditions and then either cultured in differentiated media, or their bacterial DNA was extracted and tested by real-time polymerase chain reaction (RT-PCR). The infected chicken underwent a PCR test to determine the *Salmonella* species. The isolates of *Salmonella* were subjected to antibiotic susceptibility testing by the disc-diffusion method against imipenem (10 µg), and the presence of the *bla*NDM gene was detected by PCR.

**Results:** The findings revealed that 50 out of 100 samples were infected by *Salmonella*, which was confirmed by both microbial culture and RT-PCR. The PCR test demonstrated that three samples were *Salmonella* Enteritidis, and two samples were *Salmonella* Typhimurium. Finally, 17 (34%) *Salmonella* isolates were resistant to imipenem, and the frequency of the *bla*NDM gene was 38 (76%) out of 50 samples.

**Conclusion:** The isolation of *Salmonella* from the chicken's meat may indicate a chicken's systemic infection and failure to control the most important microbe for public health. Thus, the control measures have to be revised, and a national *Salmonella* control program should be put in place urgently.

**Keywords:** Chicken, *Salmonella typhimurium*, *Salmonella enteritidis*



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## Introduction

*Salmonella* is a major pathogen impacting the poultry industry, leading to economic repercussions and public health issues (1,2). As one of the most common pathogens associated with poultry, *Salmonella* poses risks not only to consumer health but also to the poultry industry, affecting food safety and economic stability (3). The complexity of the poultry supply chain, from farm to table, creates multiple opportunities for contamination, necessitating thorough evaluation and monitoring of *Salmonella* prevalence in chicken meat (4). The relationship between *Salmonella* and chickens is intricate, with numerous molecular processes playing a role in the onset of illness

and inflammation (5,6). Understanding the dynamics of *Salmonella* prevalence in poultry is essential for developing effective interventions to mitigate the risks associated with this pathogen. Previous studies have indicated varying prevalence rates, with some regions reporting levels as high as 50% in retail chicken samples, underscoring the need for ongoing surveillance and intervention strategies (7). However, the exact prevalence of the bacteria and their pathogenic species in the Iranian chicken meat, in Kerman province, is yet to be clarified. Reports indicate that the prevalence of various serotypes of *Salmonella* species isolated from chickens is notably high in several countries (8,9). However, some serotypes,



such as *Salmonella* Typhimurium and *Salmonella* Enteritidis, have repeatedly been reported from other countries (10,11). Metallo-beta-lactamases (MBLs) are a type of enzyme that plays a significant role in antibiotic resistance, particularly in Gram-negative bacteria, including *Salmonella*. MBLs are capable of hydrolyzing beta-lactam antibiotics, rendering them ineffective. Although *Salmonella* species are primarily associated with foodborne illnesses, antibiotic resistance in these bacteria is becoming an increasing public health concern. The presence of MBLs in *Salmonella* can complicate the treatment of infections, especially when they carry genes encoding MBLs that confer resistance to beta-lactam antibiotics. These genes, such as *bla*NDM, can be acquired through horizontal gene transfer from other resistant bacteria (12).

This research paper aims to evaluate the prevalence of *Salmonella* infection in Iranian chicken meat products, examining the pathogenic species, *Salmonella* Enteritidis and Typhimurium, distribution. By analyzing data from multiple studies and conducting new assessments, this study seeks to provide a comprehensive understanding of *Salmonella* contamination in chicken meat, contributing to improved food safety measures and public health strategies.

## Materials and Methods

### Ethics Committee Approval and Consent of the Owners of Cottage Processor Outlets

Prior to the completion of the questionnaires and the procurement of chickens for the study, consent was obtained from the owners of the Cottage Processor outlets. Additionally, the research protocol received approval from the Ethics Committee of Islamic Azad University, Kazerun Branch, ensuring that all ethical guidelines were adhered to before the study began.

### Retail Outlets for Broiler Chickens in Kerman, Iran

The retail outlets involved in this study primarily consisted of cottage poultry processors, commonly referred to as “chicken and fish shopping centers”. In Iran, cottage poultry processors are outside city businesses that slaughter and process chickens on demand from chicken and fish shopping centers in fresh whole birds or cut-up chicken parts. There is a cottage poultry processor in Kerman, which provides services for the chicken and fish shopping center. Chickens are slaughtered, plastic or galvanized cones are used for holding the birds during and after the severing of the jugular vein, large pots or vessels are filled with hot water for scalding prior to feathering, and machines or drums are utilized for the feathering process. Iran’s Health Ministry is the responsible organization for maintaining the health of the chickens through their supervision and analysis. This cross-sectional study included a total of fifty poultry meat samples, which were collected from fifty chicken and fish shopping centers in Kerman, Sirjan, and Bardsir, three

cities in Kerman province, Iran. Accordingly, the samples were randomly collected from fresh chicken meats (25 samples of whole, cold-packed chicken carcasses and 25 samples of cut chicken breast), which were slaughtered for less than 24 hours.

### Salmonella Detection

*Salmonella* infection was evaluated using microbial culture methods and real-time polymerase chain reaction (RT-PCR). To facilitate this process, three types of chicken meat were collected and transported to the molecular laboratory in sterile media. All samples were analyzed within 4–6 hours post-collection. For pre-enrichment, 25 g of each sample were homogenized with 225 mL of 2% buffered peptone water for 2 minutes at maximum speed using a stomacher. The homogenized samples were then placed into 10 mL of selective enrichment media, specifically tetrathionate broth (Merck, Germany), and incubated at 37 °C for 24 hours. The following day, two loopfuls of the broth were streaked onto bismuth sulfite agar and xylose lysine deoxycholate agar (Merck, Germany), which were then incubated at 37 °C for 24–48 hours to isolate visible colonies of *Salmonella* species. Pink colonies with a black center on xylose lysine deoxycholate agar and brown, gray, or black colonies exhibiting a metallic sheen on bismuth sulfite agar were initially identified as presumptive *Salmonella* isolates. The identification process for these bacterial isolates included biochemical tests such as catalase, oxidase, indole production, citrate utilization, triple sugar iron, lysine iron agar, and methyl red-Voges Proskauer tests (10). Subsequently, the bacteria were preserved in brain heart infusion broth (Merck, Germany) supplemented with glycerol and stored at -70 °C for future studies. The *Salmonella*-positive isolates underwent additional culturing and molecular confirmation to ensure accurate identification. For RT-PCR amplification, the genomic DNA extraction kit (Karmania Pars Gene, Iran) was employed to purify DNA templates following the manufacturer’s instructions. Universal primers were utilized to detect the *Salmonella* genus, with the list of primers provided in Table 1. The RT-PCR was conducted in a 20 µL volume. The temperature protocol consisted of an initial step at 95 °C for 15 minutes, followed by 40 cycles of 95 °C for 20 seconds, annealing at 64 °C for 20 seconds, extension at 72 °C for 1 minute, and a final step to generate a melt curve from 50 °C to 95 °C. After the confirmation of the *Salmonella* genus by RT-PCR, a conventional PCR was used for detecting two serotypes of *Salmonella*, *Salmonella* enteritidis and *Salmonella* Typhimurium, using specific primers (Table 1). The conventional PCR temperature protocol consisted of an initial step at 95 °C for 2 minutes, followed by 35 cycles of 95 °C for 20 seconds, annealing at 64 °C for 20 seconds, and extension at 72 °C for 30 seconds, followed by 72 °C for 5 minutes as the final extension. The PCR products were run on a 1% agarose gel in parallel with a 100 bp

**Table 1.** Primer Sequences

Genes	Primer Sequences (5'-3')	Product Size (bp)
Universal <i>Salmonella</i> forward	ATTACTTGTGCCGAAGAGCC	145
Universal <i>Salmonella</i> reverse	GATGCTGTTATCGTCCAGGC	
<i>Salmonella</i> Typhimurium forward	ACGACTGGGATATGAACGGGGAA	107
<i>Salmonella</i> Typhimurium reverse	TCGTTGTACTTGATGCTGCGGAG	
<i>Salmonella</i> Enteritidis forward	AGTGCCATACTTTTAATGAC	316
<i>Salmonella</i> Enteritidis reverse	ACTATGTCGATACGGTGGG	
<i>bla</i> NDM forward	TCTCGACATGCCGGGTTT	472
<i>bla</i> NDM reverse	GAGATTGCCGAGCGACTT	

ladder, which was pre-treated with a safe stain (Karmania Pars Gene, Iran). PCR products for *Salmonella* Enteritidis and *Salmonella* Typhimurium were 316 bp and 107 bp, respectively.

### Determination of Antimicrobial Susceptibility and Frequency of the *bla*NDM Gene

The antibiotic susceptibility pattern for bacterial isolates was determined using the disk diffusion method according to the Clinical and Laboratory Standards Institute recommendation. The tested antibiotic disk was imipenem (10 µg) from Padtanteb Company, Iran. All the isolates were screened for the presence of the *bla*NDM gene by the PCR assay. The conventional PCR for the detection of the *bla*NDM gene was performed using a commercial master mix from Karmania Pars Gene Company, Iran, and specific primers (Table 1). The temperature protocol consisted of an initial step at 95 °C for 2 minutes, followed by 35 cycles of 95 °C for 20 seconds, annealing at 62 °C for 20 seconds, and extension at 72 °C for 30 seconds, followed by 72 °C for 5 minutes. The PCR products were run on a 1% agarose gel in parallel with a 1 kb ladder, which was pre-treated with a safe stain (Karmania Pars Gene, Iran). The PCR product for the *bla*NDM gene was 472 bp (12).

### Statistical Analysis

SPSS software (version 21) was used to calculate the raw data. The Kolmogorov-Smirnov test revealed that the raw data had no normal distribution. Accordingly, the chi-square test was used to analyze the data, which are presented as percentages.

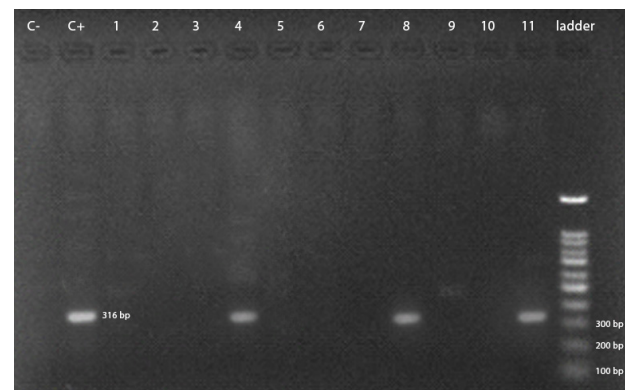
### Results

Out of the 20 samples tested, 50 (100%) were found to be contaminated with *Salmonella* species. This contamination was initially identified through biochemical tests and further confirmed using molecular detection methods.

The bacteria culture proved that the isolated bacteria were *Salmonella*, which was confirmed by the RT-PCR test.

The Chi-square test showed that the *Salmonella* infection is significantly high among the Iranian chickens ( $P < 0.001$ ).

As illustrated in Figure 1, three out of fifty samples were positive for *Salmonella* Enteritidis. The positive samples



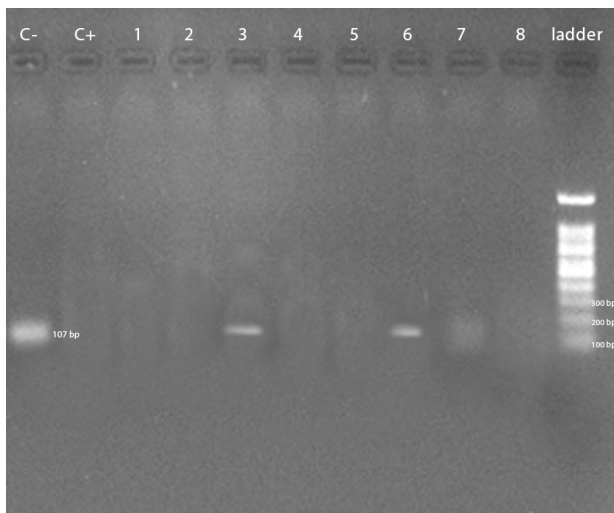
**Figure 1.** PCR Product Gel Electrophoresis for the Detection of *Salmonella* Enteritidis. Note. PCR: Polymerase chain reaction. Three out of 50 samples, which were infected by *Salmonella*, were *Salmonella* enteritidis.

of *Salmonella* Enteritidis were from Kerman, Sirjan, and Bardsir cities, where the first two samples were from whole packaged chicken carcasses and the third sample was from chicken breast samples. Moreover, two out of 50 samples were positive for *Salmonella* Typhimurium (Figure 2). The positive samples of *Salmonella* Typhimurium were all from Bardsir and whole chicken carcasses. The antibiogram tests revealed that 17 (34%) *Salmonella* isolates were resistant to imipenem, and the PCR results demonstrated that the frequencies of the *bla*NDM gene were 38 (76%) out of 50 samples. Figure 3 displays the PCR product of the *bla*NDM gene.

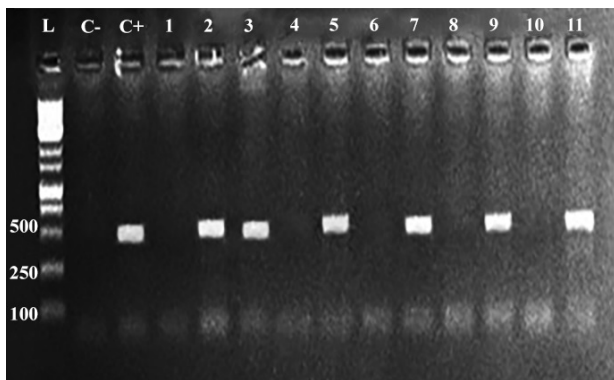
### Discussion

*Salmonella* infection in chicken meat is a critical public health concern due to its prevalence and the severe foodborne illnesses it can cause (13). *Salmonella* species, particularly *Salmonella enterica* serotypes, such as Typhimurium and Enteritidis, are commonly found in poultry and are significant contributors to global salmonellosis outbreaks (14). The contamination can occur at various stages, including production, processing, distribution, and preparation, making it a complex issue to address (15). The results confirmed that 100% of the poultry meat was infected by *Salmonella* species. Among them, 5 samples were infected by pathological species of *Salmonella*, including three *Salmonella* Enteritidis and two *Salmonella* Typhimurium. Considering that poultry meat needs to be clear of pathological bacteria, it needs





**Figure 2.** PCR Product Gel Electrophoresis for the Detection of *Salmonella* Typhimurium. Note. PCR: Polymerase chain reaction. Two out of 50 samples, which were infected by *Salmonella*, were *Salmonella* Typhimurium.



**Figure 3.** Agarose Gel Electrophoresis of the PCR-Amplified *blaNDM* Gene. Note. PCR: Polymerase chain reaction. The *blaNDM* gene appears at 472 bp. L: Ladder; C-: Negative control; C+: Positive control.

to be checked by the Iranian Health Organization using molecular testing. Several studies have reported the prevalence of *Salmonella* infections in poultry in Iran. According to various studies conducted over the last ten years, the prevalence of *Salmonella* infections in broiler chicken farms in Iran ranges from 22.5% to 64.2% (16). In a cross-sectional study, 7.9% of broiler breeder farms in Iran were found to be infected with *Salmonella*, with older flocks and farms with more houses at greater risk (16). These findings highlight the widespread nature of *Salmonella* infections in poultry, which can pose risks to human health through the food supply and direct contact. However, there are no reports regarding the prevalence of *Salmonella* infections in chickens in the Kerman province, Iran. Continued surveillance and control measures are needed to mitigate the public health impact of salmonellosis. It may be announced that although the infection of chicken meat is high regarding *Salmonella* species, the infection is not high via eating chicken meat in Iran. It appears that cooking the chicken leads to killing the bacteria. However, direct exposure to raw chicken meat may increase the risk of transmission of *Salmonella*

species to humans via infected chicken meat. Research indicates that improper cooking and handling of chicken meat are primary factors that lead to *Salmonella* infections in humans (17). For instance, undercooked poultry can harbor viable *Salmonella* cells, posing a risk for consumers (17). Studies have shown that proper cooking methods significantly reduce the risk of infection; nonetheless, many consumers do not adhere to recommended cooking temperatures, leading to potential outbreaks. Moreover, the emergence of antibiotic-resistant *Salmonella* strains complicates treatment options for infected individuals, highlighting the need for stringent control measures throughout the poultry production chain (18). Effective interventions, such as biosecurity measures on farms, improved processing techniques, and public education on safe food handling practices, are essential to mitigate the risks associated with *Salmonella* in chicken meat (19). Further, tackling *Salmonella* infections in chicken meat necessitates a comprehensive strategy that includes improved food safety practices, consumer education, and continuous research aimed at developing effective control measures to safeguard public health. The research represents that *Salmonella* infections in chicken meat can indeed influence epigenetic factors in humans that affect several molecules, including pro-inflammatory factors, although the specific mechanisms and effects require further investigation (20). The isolation of *Salmonella* from chicken meat suggests not only the presence of systemic infection in chickens but also highlights significant gaps in the current food safety and public health measures. This finding raises serious concerns about the potential risks to consumers, as *Salmonella* is a leading cause of foodborne illness worldwide. The failure to effectively control this pathogen in poultry production confirms an urgent need for a comprehensive reassessment of existing control measures (21). Furthermore, several investigations proved that Iranian chicken meat is infected by *Salmonella* serotypes that are resistant to antibiotics. For example, Mir et al reported that 100% of the infected chicken meat in Zahedan, a southeastern province of Iran, was resistant to penicillin (22). The results were also confirmed by other Iranian investigators. Thus, it is essential to evaluate and update current biosecurity protocols and hygiene practices in poultry farms (23,24). This includes improving sanitation, monitoring flock health, and implementing strict measures to prevent cross-contamination during processing. In addition, establishing a coordinated national program focusing on the surveillance, prevention, and control of *Salmonella* in poultry is crucial. This program should involve collaboration between government agencies, poultry producers, and public health organizations. Additionally, it is essential to establish strong surveillance systems to monitor the prevalence of *Salmonella* in poultry throughout all stages, from farm to table. Regular testing and reporting will facilitate the early identification of outbreaks and guide necessary interventions. By implementing these

suggestions, it is possible to enhance the safety of chicken meat, protect public health, and reduce the burden of Salmonella-related illnesses. Immediate action is essential to address the current challenges and ensure a safer food supply. Overall, 34% of Salmonella isolates were resistant to imipenem, and 76% tested positive for the *bla*NDM gene, which are concerning findings. Imipenem is an important antibiotic used to treat serious bacterial infections, and resistance to it among Salmonella isolates can pose a significant public health threat. The presence of the *bla*NDM gene, which confers resistance to carbapenem antibiotics such as imipenem, suggests the potential for the spread of multidrug-resistant strains of Salmonella. These results highlight the issue of antibiotic resistance in chicken meat, which can serve as a source of transmission of resistant bacteria to humans through food consumption. If these antibiotic-resistant strains enter the food chain and cause infections in humans, it can lead to difficult-to-treat or even untreatable infections, compromising the effectiveness of antibiotic therapy and posing a serious risk to public health (25). Given the high prevalence of antibiotic resistance found in this study, Iranian health authorities must take immediate action to address this issue. Strategies such as having surveillance regulations of antibiotic use in livestock, implementing strict regulations on antibiotic usage in poultry farming, promoting responsible antibiotic prescribing practices, and increasing awareness about the dangers of antibiotic resistance are essential to mitigate the impact of antibiotic resistance on public health in Iran. Additionally, further research and monitoring of antibiotic resistance patterns in food-producing animals and their products are necessary to better understand and combat this emerging threat (26).

## Conclusion

The study found a significant prevalence of Salmonella infections, with 50% of the samples testing positive for Salmonella. Among these, specific serotypes, Salmonella Enteritidis and Salmonella Typhimurium, were identified. Additionally, a concerning level of antibiotic resistance was observed, with 34% of the Salmonella isolates showing resistance to Imipenem. Furthermore, a high frequency of the *bla*NDM gene, which is associated with antibiotic resistance, was detected in 76% of the samples. This suggests not only a notable presence of Salmonella in the samples but also raises alarms about the potential for antibiotic-resistant strains, highlighting the need for ongoing monitoring and effective management strategies.

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## Authors' Contribution

**Conceptualization:** Zahra Mirafzali.

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**Investigation:** Zahra Mirafzali.

**Project administration:** Mohammad Hossein Marhamatizadeh.

**Supervision:** Ashraf Kariminik and Mohammad Hossein Marhamatizadeh.

**Validation:** Gholam Hossein Habibi.

**Writing—original draft:** Ashraf Kariminik.

## Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Ethical Approval

The project protocol was approved by the local ethical committee (IR.IAU.KAU.REC.1402.073).

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