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Prevalence, antibiotic resistance, virulence and antimicrobial resistance gene profiles of *Salmonella* species recovered from retail beef and poultry processing environments

Zahra Osivand[1], Ebrahim Rahimi[1*], Amir Shakerian[2] and Faham Khamesipour[3]

Abstract

Background This study investigates the prevalence, antimicrobial resistance, genotypic resistance profiles, and virulence gene distribution of *Salmonella* isolates from poultry and beef processing environments in Shahrekord, Iran.

Method A total of 680 samples were collected from poultry ($n=300$) and beef ($n=380$) products between January and December 2023.

Results *Salmonella* was detected in 21% (63/300) of poultry samples and 15.8% (60/380) of beef samples, with non-typhoidal *Salmonella* (NTS) being the predominant serovar. High antimicrobial resistance (AMR) rates were observed across both food types, with the most common resistances found in ciprofloxacin (48%), tetracycline (44%), and ampicillin (39%). Genotypic analysis revealed the presence of key resistance genes, including bla_{TEM} (35%), *tetA* (29%), and *sul1* (23%). Virulence gene analysis identified *invA* (92%), *agfA* (80%), and *hilA* (76%) as the most prevalent genes. Comparative analysis of resistance patterns between poultry and beef samples revealed higher resistance in poultry isolates to ciprofloxacin and tetracycline.

Conclusion This study highlights significant antimicrobial resistance and the presence of virulence factors in *Salmonella* isolates from retail beef and poultry, suggesting a potential risk to public health and the need for enhanced surveillance and control measures in food processing environments.

Clinical trial number Not applicable.

Keywords *Salmonella*, Prevalence, Antimicrobial resistance, Virulence, Beef, Poultry, Processing environment

*Correspondence:
Ebrahim Rahimi
ebrahimrahimi55@yahoo.com
[1]Department of Food Hygiene, Faculty of Veterinary Medicine, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[2]Research Center of Nutrition and Organic Products, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
[3]Halal Research Center of IRI, Food and Drug Administration, Ministry of Health and Medical Education, Tehran, Iran

Background

Salmonella species are among the leading causes of foodborne illness worldwide, posing significant public health challenges due to their widespread distribution and ability to cause severe gastrointestinal infections [1–4]. These bacteria possess various virulence factors that enable colonization, invasion, and survival in host organisms. The contamination of retail beef and poultry products with *Salmonella* is particularly concerning, as these food products serve as primary transmission vehicles to humans [5, 6].

A critical concern associated with *Salmonella* is its ability to develop resistance to antibiotics. The misuse and overuse of antibiotics in animal husbandry and food production have contributed to the emergence of multidrug-resistant (MDR) *Salmonella* strains, complicating treatment options and increasing the risk of severe disease outcomes [7, 8]. Furthermore, the presence of antimicrobial resistance (AMR) genes in *Salmonella* facilitates the horizontal transfer of resistance traits to other bacteria, further exacerbating the public health burden [7, 8].

In addition to antibiotic resistance, *Salmonella* virulence factors significantly impact its pathogenic potential. These include adhesins, invasins, and toxins encoded by specific genes such as *invA*, *stn*, and *spiC*. Identifying and characterizing these virulence genes provide insights into the pathogenic potential of *Salmonella* strains isolated from food products [5–9].

In Iran, like many other countries, the retail meat industry plays a crucial role in providing consumers with a variety of meat products, including beef and poultry [9]. However, inadequate hygiene practices, improper handling, and suboptimal processing conditions in retail meat establishments can facilitate the contamination of meat products with pathogenic bacteria such as *Salmonella* [10, 11]. Shahrekord, located in Chaharmahal and Bakhtiari Province, Iran, has a notable retail meat market. However, limited studies have comprehensively examined the prevalence, resistance patterns, and virulence profiles of Salmonella isolates from retail meat products and processing environments in this region. This study aimed to investigate the prevalence, antimicrobial resistance patterns, genotypic resistance profiles, and virulence gene distributions of Salmonella isolates recovered from retail beef and poultry processing environments in Shahrekord.

Methods

Study design and sample collection

A cross-sectional study was conducted from January to December 2023 to investigate the prevalence, antimicrobial resistance, genotypic resistance profiles, and virulence gene distributions of *Salmonella* isolates in retail beef and poultry processing environments in Shahrekord, Iran. A total of 300 samples were collected from various poultry processing environments, including carcass dressing water ($n = 100$), chopping board ($n = 100$), and knife swabs ($n = 100$), obtained from randomly selected chicken markets around Shahrekord city. Additionally, 380 samples were obtained from retail beef products, comprising raw intact cuts ($n = 150$), raw beef organ meats ($n = 100$), raw processed beef products ($n = 80$), and ready-to-eat beef products ($n = 50$). Sampling was conducted monthly to account for seasonal variations.

Environmental samples were collected using sterile swabs moistened with buffered peptone water, while meat samples were aseptically placed in sterile containers. Samples were transported under cold chain conditions (4 °C) and processed within 24 h of collection.

Salmonella isolation and identification

Samples were processed using standard microbiological methods as outlined by the International Organization for Standardization (ISO 6579-1:2017). Pre-enrichment was performed in buffered peptone water, followed by selective enrichment in Rappaport-Vassiliadis broth. Suspected *Salmonella* colonies were isolated on Xylose Lysine Deoxycholate (XLD) agar and Brilliant Green agar. Biochemical identification included triple sugar iron agar (TSI), urease, and citrate utilization tests. Confirmatory identification was performed using polymerase chain reaction (PCR) targeting the *invA* gene (Table 1) [3, 4].

Serotyping of *Salmonella* isolates was conducted using slide agglutination tests with commercial antisera targeting *S. Typhimurium*, *S. Enteritidis*, and other common serovars.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disc diffusion method following Clinical and Laboratory Standards Institute (CLSI) guidelines (M100, 2022). The antibiotics tested and their concentrations were as follows: amoxicillin (25 µg), ampicillin (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), gentamicin (10 µg), kanamycin (30 µg), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (25 µg). Breakpoints were interpreted according to CLSI guidelines specific to *Salmonella* spp [12].

Genotypic resistance analysis

PCR assays were performed to detect resistance genes, including bla_{TEM} (beta-lactam resistance), *tetA* (tetracycline resistance), *sul1*, *sul2*, *sul3* (sulfonamide resistance), and *strA/B* (streptomycin resistance) (Table 1). DNA extraction was conducted using a commercial kit, and PCR reactions included a master mix containing 2X Taq

Table 1 Primers used to detect Salmonella enterica serovars, resistance genes and virulence genes

Target Gene	Sequence (5′–3′)	Amplicon Size (bp)	Thermal Profile	References
invA	F-GTGAAATTATCGCCACGTTCGGGCAA R-TCATCGCACCGTCAAAGGAACC	284	95˚C for 1 min; 38 cycles of 95˚C for 30s, 64˚C for 30s and 72˚C for 30s; elongation step at 72˚C for 4 min	[3]
Typh	F-TTGTTCACTTTTTACCCCTGAA R-CCCTGACAGCCGTTAGATATT	401	95˚C for 2 min; 30 cycles of 95˚C for 1 min, 57˚C for 1 min and 72˚C for 2 min; elongation at 72˚C for 5 min	[4]
sdf-1	F-TGTGTTTTATCTGATGCAAGAGG R-CGTTCTTCTGGTACTTACGATGAC	293	95˚C for 2 min; 30 cycles of 95˚C for 1 min, 57˚C for 1 min and 72˚C for 2 min; elongation at 72˚C for 5 min	[4]
bla_{TEM}	F-CCGTTCATCCATAGTTGCCTGAC R-TTTCCGTGTCGCCCTTATTC	800	94˚C for 10 min; 30 cycles of 94˚C for 40s, 60˚C for 40s and 72˚C for 1 min; elongation step at 72˚C for 7 min	[4]
sul1	F-CGG CGT GGG CTA CCT GAA CG R-GCC GAT CGC GTG AAG TTC CG	433	95˚C for 15 min; 30 cycles of 95˚C for 1 min, 66˚C for 1 min and 72˚C for 1 min; elongation step at 72˚C for 10 min	[4]
sul2	F-CGG CGT GGG CTA CCT GAA CG R-GCC GAT CGC GTG AAG TTC CG	721	95˚C for 15 min; 30 cycles of 95˚C for 1 min, 66˚C for 1 min and 72˚C for 1 min; elongation step at 72˚C for 10 min	[4]
sul3	F-CAACGGAAGTGG GCGTTG TGGA R-GCT GCA CCA ATT CGC TGAACG	244	95˚C for 15 min; 30 cycles of 95˚C for 1 min, 66˚C for 1 min and 72˚C for 1 min; elongation step at 72˚C for 10 min	[4]
tet(A)	F-GGC GGTCTT CTT CAT CATGC R-CGG CAG GCA GAG CAA GTAGA	502	94˚C for 15 min; 30 cycles of 94˚C for 1 min, 63˚C for 1 min and 72˚C for 1 min; elongation step at 72˚C for 10 min	[4]
strA/strB	F-ATGGTGGACCCTAAAACTCT R-CGTCTAGGATCGAGACAAAG	893	94˚C for 15 min; 30 cycles of 94˚C for 1 min, 63˚C for 1 min and 72˚C for 1 min; elongation step at 72˚C for 10 min	[4]
agfA	F-TCCACAATGGGGCGGCGGCG R-CCTGACGCACCATTACGCTG	350	94˚C for 1 s; 58˚C for 1 s; 74˚C 21 s	[3]
lpfA	F-CTTTCGCTGCTGAATCTGGT R-CAGTGTTAACAGAAACCAGT	250	94˚C for 1 s; 55˚C for 1 s; 74˚C 21 s	[3]
hilA	F-CTGCCGCAGTGTTAAGGATA R-CTGTCGCCTTAATCGCATGT	497	94˚C for 120 s; 62˚C for 1 min; 72˚C 1 min	[3]
sivH	F-GTATGCGAACAAGCGTAACAC R-CAGAATGCGAATCCTTCGCAC	763	94˚C for 30 s; 56˚C for 45 s; 72˚C 45 s	[4]
sefA	F-GATACTGCTGAACGTAGAAGG R-GCGTAAATCAGCATCTGCAGTAGC	488	94˚C for 1 s; 56˚C for 1 s; 74˚C 21 s	[4]
sopE	F-GGATGCCTTCTGATGTTGACTGG R-ACACACTTTCACCGAGGAAGCG	398	94˚C for 1 min; 55˚C for 1 min; 72˚C for 1 min	[4]
spvC	F-CCCAAACCCATACTTACTCTG R-CGGAAATACCATCTACAAATA	669	93˚C for 1 min; 42˚C for 1 min; 72˚C for 2 min	[4]

DNA polymerase, specific primers, and template DNA [2–4].

Virulence gene detection

PCR assays were used to detect virulence genes, including *invA*, *agfA*, *ipfA*, *hilA*, *sivH*, *sopE*, *sefA*, and *spvC* (Table 1). PCR products were analyzed via agarose gel electrophoresis (1.5% agarose, ethidium bromide staining), visualized under UV transillumination [1–4].

Data analysis

Prevalence, antimicrobial resistance patterns, genotypic resistance profiles, and virulence gene distributions were analyzed using SPSS (v.26). Descriptive statistics were reported as frequencies and percentages. Comparative analyses were performed using Chi-square or Fisher's exact tests, with $p < 0.05$ considered statistically significant.

Results

Prevalence of *Salmonella* and nontyphoidal *Salmonella* (*NTS*) serovars in poultry processing environments

In this study, the prevalence of *Salmonella* and nontyphoidal *Salmonella* (*NTS*) enterica serovars was assessed in poultry processing environments. A total of 300 samples were collected, with *Salmonella* detected in 90 samples (30.0%) and *NTS enterica* in 70 samples (23.3%). Among sample types, carcass dressing water (CDW) had 30% *Salmonella* positivity and 20% *NTS enterica* positivity. Chopping board swabs (CBS) showed the highest contamination, with 35% and 25% positivity for *Salmonella* and *NTS enterica*, respectively. Knife swabs (KS) had *Salmonella* in 25% of samples, all of which were *NTS enterica* positive.

Salmonella was detected in 30% of carcass dressing water samples (30/100), 35% of chopping board swabs (35/100), and 25% of knife swabs (25/100). The overall prevalence of *Salmonella* in poultry processing

environments was 30% (90/300), while the prevalence of NTS was 23.3% (70/300).

Prevalence of *Salmonella* in retail beef product samples

A total of 380 retail beef product samples were collected and analyzed. *Salmonella* was detected in 16% of raw intact beef cuts (8/50), 23.3% of raw beef organ meats (42/180), 20% of raw processed beef products (20/100), and 12% of ready-to-eat beef products (6/50). The overall prevalence of *Salmonella* in retail beef products was 20% (76/380), while NTS was detected in 15.3% (58/380) of samples.

Specific *Salmonella* serovars were identified in poultry processing environments

In poultry processing environments, specific *Salmonella* serovars were identified, with a total prevalence of 30.0%. *Salmonella Typhimurium* was detected in 25 samples (8.3%), while *Salmonella Enteritidis* was found in 20 samples (6.7%). Additionally, 45 samples (15.0%) contained untyped *Salmonella* serovars.

Prevalence of *Salmonella* and nontyphoidal *Salmonella* (*NTS*) serovars in retail beef products

The prevalence of *Salmonella* and nontyphoidal *Salmonella* (*NTS*) enterica serovars in retail beef products was assessed across 380 samples, with *Salmonella* detected in 76 samples (20.0%) and *NTS* in 58 samples (15.3%). Among sample types, raw beef organ meats had the highest *Salmonella* contamination (23.3%), followed by raw processed beef products (20.0%) and raw intact beef cuts (16.0%). *NTS* prevalence was highest in raw processed beef products (20.0%) and ready-to-eat beef products (16.0%).

Specific *Salmonella* serovars were identified in retail beef products

In retail beef products, specific *Salmonella* serovars were identified, with a total prevalence of 20.0%. *Salmonella Typhimurium* was detected in 18 samples (4.7%), while *Salmonella Enteritidis* was found in 16 samples (4.2%). Additionally, 42 samples (11.1%) contained untyped *Salmonella* serovars, indicating a significant presence of unidentified strains.

Summary of overall prevalence

The overall prevalence of *Salmonella* and nontyphoidal *Salmonella* (*NTS*) serovars was analyzed across 680 combined poultry and beef samples. *Salmonella* was detected in 166 samples (24.4%), while *NTS* serovars were identified in 128 samples (18.8%). Among specific serovars, *S. Typhimurium* was present in 43 samples (6.3%), and *S. Enteritidis* was found in 36 samples (5.3%). Additionally, 87 samples (12.8%) contained untyped *Salmonella* strains.

Phenotypic resistance patterns and antimicrobial resistance patterns of NTS isolates

Antimicrobial susceptibility testing revealed high levels of resistance among Salmonella isolates. The most common resistance profiles observed were against ciprofloxacin (48%), streptomycin (46%), ampicillin (39%), and tetracycline (44%). Tables 2, 3, 4, 5 and 6 presents the resistance rates for each antibiotic tested.

Genotypic resistance profiles

Genotypic analysis revealed the presence of various resistance genes among Salmonella isolates. The bla_{TEM} gene, encoding for beta-lactamase TEM, was the most prevalent (35%), followed by *tetA* (29%) and sul1 (23%). A detailed summary of genotypic resistance patterns is provided in Tables 7, 8 and 9.

PCR detection of virulence genes for NTS isolates

Tables 10, 11 and 12 are the results showcasing the distribution of virulence genes among *Salmonella* enterica serovars, including *S. Typhimurium*, *S. Enteritidis*, and untyped *Salmonella*, along with the overall resistance.

Virulence gene analysis identified *invA* (92%), *agfA* (80%), and *hilA* (76%) as the most prevalent genes. Additionally, the distribution of other virulence genes, including *sivH*, *sopE*, *sefA*, and *spvC*, varied among Salmonella serovars and sample types (Table 12).

Discussion

This study provides significant insights into the prevalence, antimicrobial resistance patterns, and virulence profiles of *Salmonella* and non-typhoidal *Salmonella* (NTS) serovars across poultry processing environments and retail beef products. These findings underscore the pervasive nature of *Salmonella* contamination in food production environments and the challenges posed by antimicrobial resistance (AMR) and virulence in these pathogens.

Prevalence of *Salmonella* in poultry and beef

The results indicate a notable prevalence of *Salmonella* in both poultry processing environments and retail beef products, with contamination rates of 30% and 20% for *Salmonella* in poultry and beef, respectively. The prevalence of *Salmonella* detected in this study aligns with previous reports from various countries, though regional variations exist. Studies conducted in Europe, North America, and Asia have reported similar prevalence rates in beef and poultry environments; however, some studies have documented higher or lower rates depending on sample sources, hygiene standards, and regulatory

Table 2 Phenotypic resistance patterns of NTS serovars

Serovar	No. of Isolates	Ciprofloxacin	Streptomycin	Ampicillin	Tetracycline	Nalidixic Acid	Gentamicin	Sulfamethoxazole-Trimethoprim	Amoxicillin-Clavulanate	Chloramphenicol	Azithromycin	Amikacin	Meropenem	Aztreonam	Ceftriaxone	Cefotaxime	Ceftazidime	Quinolones/Fluoroquinolones
S. Typhimurium	43	15 (34.9%)	20 (46.5%)	25 (58.1%)	22 (51.2%)	18 (41.9%)	10 (23.3%)	17 (39.5%)	8 (18.6%)	13 (30.2%)	9 (20.9%)	4 (9.3%)	1 (2.3%)	2 (4.7%)	5 (11.6%)	6 (13.9%)	3 (7.0%)	16 (37.2%)
S. Enteritidis	36	12 (33.3%)	18 (50.0%)	20 (55.6%)	19 (52.8%)	14 (38.9%)	8 (22.2%)	14 (38.9%)	7 (19.4%)	10 (27.8%)	7 (19.4%)	5 (13.9%)	2 (5.6%)	1 (2.8%)	4 (11.1%)	5 (13.9%)	2 (5.6%)	14 (38.9%)
Untyped Salmonella	87	30 (34.5%)	38 (43.7%)	50 (57.5%)	45 (51.7%)	35 (40.2%)	20 (23.0%)	32 (36.8%)	17 (19.5%)	28 (32.2%)	20 (23.0%)	9 (10.3%)	3 (3.4%)	4 (4.6%)	12 (13.8%)	14 (16.1%)	8 (9.2%)	34 (39.1%)
Overall Resistance	166	57 (34.3%)	76 (45.8%)	95 (57.2%)	86 (51.8%)	67 (40.4%)	38 (22.9%)	63 (37.9%)	32 (19.3%)	51 (30.7%)	36 (21.7%)	18 (10.8%)	6 (3.6%)	7 (4.2%)	21 (12.7%)	25 (15.1%)	13 (7.8%)	64 (38.6%)

Table 3 Antimicrobial resistance of NTS serovars

Antimicrobial Agent	Number Resistant Isolates ($n = 166$)	Percentage (%)
Ciprofloxacin	57	34.3%
Streptomycin	76	45.8%
Ampicillin	95	57.2%
Tetracycline	86	51.8%
Nalidixic Acid	67	40.4%
Gentamicin	38	22.9%
Sulfamethoxazole-Trimethoprim	63	37.9%
Amoxicillin-Clavulanate	32	19.3%
Chloramphenicol	51	30.7%
Azithromycin	36	21.7%
Amikacin	18	10.8%
Meropenem	6	3.6%
Aztreonam	7	4.2%
Ceftriaxone	21	12.7%
Cefotaxime	25	15.1%
Ceftazidime	13	7.8%
Quinolones/Fluoroquinolones	64	38.6%

frameworks [13–19]. For instance, a study in China found higher contamination levels in poultry compared to beef, which may be attributed to differences in processing conditions and biosecurity measures [20].

These findings are consistent with previous studies in different regions which have reported similar prevalence rates. For instance, a study by Zhao et al. (2017) [14] found a *Salmonella* prevalence of 28.8% in retail meat samples in China, and another study by Cummings et al. (2021) [17] reported a prevalence of 25.5% in poultry processing environments in the United States. Among the poultry processing samples, chopping board swabs (CBS) exhibited the highest contamination levels, highlighting the critical role of equipment surfaces in cross-contamination. The high prevalence of *Salmonella* in carcass dressing water (CDW) and knife swabs emphasizes the need for stringent hygiene protocols during poultry processing, as these sample types are likely to come into direct contact with edible portions of poultry products.

Similarly, retail beef products showed a lower, but still concerning, prevalence of *Salmonella*, especially in raw beef organ meats (23.3%) and raw processed beef products (20%). These findings suggest that certain beef products, particularly organ meats, may pose a higher risk of *Salmonella* contamination, which should prompt targeted monitoring and intervention strategies in the beef supply chain.

The identification of specific *Salmonella* serovars in both poultry and beef products is also noteworthy. *Salmonella Typhimurium* and *Salmonella Enteritidis* were among the most commonly detected serovars, which are

Table 4 Phenotypic resistance patterns of NTS serovars in poultry processing environments

Antimicrobial Agent	S. Typhimurium (*n* = 25)	S. Enteritidis (*n* = 20)	Untyped Salmonella (*n* = 45)	Overall (*n* = 90)
Ciprofloxacin	10 (40.0%)	7 (35.0%)	15 (33.3%)	32 (35.6%)
Streptomycin	12 (48.0%)	9 (45.0%)	20 (44.4%)	41 (45.6%)
Ampicillin	14 (56.0%)	11 (55.0%)	26 (57.8%)	51 (56.7%)
Tetracycline	13 (52.0%)	10 (50.0%)	22 (48.9%)	45 (50.0%)
Nalidixic Acid	11 (44.0%)	8 (40.0%)	18 (40.0%)	37 (41.1%)
Gentamicin	6 (24.0%)	4 (20.0%)	10 (22.2%)	20 (22.2%)
Sulfamethoxazole-Trimethoprim	10 (40.0%)	8 (40.0%)	17 (37.8%)	35 (38.9%)
Amoxicillin-Clavulanate	5 (20.0%)	4 (20.0%)	9 (20.0%)	18 (20.0%)
Chloramphenicol	7 (28.0%)	6 (30.0%)	13 (28.9%)	26 (28.9%)
Azithromycin	5 (20.0%)	4 (20.0%)	10 (22.2%)	19 (21.1%)
Amikacin	2 (8.0%)	2 (10.0%)	5 (11.1%)	9 (10.0%)
Meropenem	1 (4.0%)	1 (5.0%)	2 (4.4%)	4 (4.4%)
Aztreonam	1 (4.0%)	1 (5.0%)	2 (4.4%)	4 (4.4%)
Ceftriaxone	3 (12.0%)	2 (10.0%)	5 (11.1%)	10 (11.1%)
Cefotaxime	3 (12.0%)	2 (10.0%)	6 (13.3%)	11 (12.2%)
Ceftazidime	2 (8.0%)	1 (5.0%)	3 (6.7%)	6 (6.7%)
Quinolones/Fluoroquinolones	9 (36.0%)	7 (35.0%)	16 (35.6%)	32 (35.6%)

Table 5 Phenotypic resistance patterns of NTS serovars in retail beef products

Antimicrobial Agent	S. Typhimurium (*n* = 18)	S. Enteritidis (*n* = 16)	Untyped Salmonella (*n* = 42)	Overall (*n* = 76)
Ciprofloxacin	5 (27.8%)	5 (31.3%)	15 (35.7%)	25 (32.9%)
Streptomycin	8 (44.4%)	9 (56.3%)	18 (42.9%)	35 (46.1%)
Ampicillin	11 (61.1%)	9 (56.3%)	27 (64.3%)	47 (61.8%)
Tetracycline	9 (50.0%)	9 (56.3%)	23 (54.8%)	41 (53.9%)
Nalidixic Acid	7 (38.9%)	6 (37.5%)	17 (40.5%)	30 (39.5%)
Gentamicin	4 (22.2%)	4 (25.0%)	10 (23.8%)	18 (23.7%)
Sulfamethoxazole-Trimethoprim	7 (38.9%)	6 (37.5%)	16 (38.1%)	29 (38.2%)
Amoxicillin-Clavulanate	3 (16.7%)	3 (18.8%)	11 (26.2%)	17 (22.4%)
Chloramphenicol	6 (33.3%)	4 (25.0%)	15 (35.7%)	25 (32.9%)
Azithromycin	4 (22.2%)	3 (18.8%)	11 (26.2%)	18 (23.7%)
Amikacin	2 (11.1%)	2 (12.5%)	5 (11.9%)	9 (11.8%)
Meropenem	1 (5.6%)	1 (6.3%)	2 (4.8%)	4 (5.3%)
Aztreonam	1 (5.6%)	1 (6.3%)	2 (4.8%)	4 (5.3%)
Ceftriaxone	2 (11.1%)	2 (12.5%)	5 (11.9%)	9 (11.8%)
Cefotaxime	2 (11.1%)	2 (12.5%)	5 (11.9%)	9 (11.8%)
Ceftazidime	1 (5.6%)	1 (6.3%)	2 (4.8%)	4 (5.3%)
Quinolones/Fluoroquinolones	7 (38.9%)	6 (37.5%)	16 (38.1%)	29 (38.2%)

often associated with foodborne illnesses. The presence of untyped *Salmonella* strains in both sample types (15% in poultry and 11.1% in beef) reflects the potential for the emergence of novel strains that may complicate surveillance and control efforts.

Factors contributing to *Salmonella* contamination may include inadequate sanitation practices, improper handling procedures, and cross-contamination during processing and transportation [18].

Antimicrobial resistance patterns

The high rates of antimicrobial resistance observed in this study, particularly against commonly used antibiotics such as ampicillin, tetracycline, and streptomycin, are of great concern. Resistance to ciprofloxacin and nalidixic acid, which are fluoroquinolones often used to treat severe *Salmonella* infections, further emphasizes the challenge posed by resistant strains in both poultry and beef products. The finding that 48% of *Salmonella* isolates from poultry processing environments and 61.8% of isolates from retail beef products were resistant to ampicillin is particularly troubling, given the widespread use of this antibiotic in both human and veterinary medicine. These results align with global trends indicating a rise in antimicrobial-resistant *Salmonella* strains. For example, in a study conducted by Yang et al. (2020), *Salmonella* isolates from retail meats in the United States showed high resistance rates to tetracycline (52.3%) and ampicillin (49.1%). Similar trends in antimicrobial resistance (AMR) have been observed in studies from Europe, Asia,

Table 6 Overall phenotypic resistance patterns of NTS serovars

Antimicrobial Agent	S. Typhimurium (*n* = 43)	S. Enteritidis (*n* = 36)	Untyped Salmonella (*n* = 87)	Overall (*n* = 166)
Ciprofloxacin	15 (34.9%)	12 (33.3%)	30 (34.5%)	57 (34.3%)
Streptomycin	20 (46.5%)	18 (50.0%)	38 (43.7%)	76 (45.8%)
Ampicillin	25 (58.1%)	20 (55.6%)	50 (57.5%)	95 (57.2%)
Tetracycline	22 (51.2%)	19 (52.8%)	45 (51.7%)	86 (51.8%)
Nalidixic Acid	18 (41.9%)	14 (38.9%)	35 (40.2%)	67 (40.4%)
Gentamicin	10 (23.3%)	8 (22.2%)	20 (23.0%)	38 (22.9%)
Sulfamethoxazole-Trimethoprim	17 (39.5%)	14 (38.9%)	32 (36.8%)	63 (37.9%)
Amoxicillin-Clavulanate	8 (18.6%)	7 (19.4%)	17 (19.5%)	32 (19.3%)
Chloramphenicol	13 (30.2%)	10 (27.8%)	28 (32.2%)	51 (30.7%)
Azithromycin	9 (20.9%)	7 (19.4%)	20 (23.0%)	36 (21.7%)
Amikacin	4 (9.3%)	5 (13.9%)	9 (10.3%)	18 (10.8%)
Meropenem	1 (2.3%)	2 (5.6%)	3 (3.4%)	6 (3.6%)
Aztreonam	2 (4.7%)	1 (2.8%)	4 (4.6%)	7 (4.2%)
Ceftriaxone	5 (11.6%)	4 (11.1%)	12 (13.8%)	21 (12.7%)
Cefotaxime	6 (13.9%)	5 (13.9%)	14 (16.1%)	25 (15.1%)
Ceftazidime	3 (7.0%)	2 (5.6%)	8 (9.2%)	13 (7.8%)
Quinolones/Fluoroquinolones	16 (37.2%)	14 (38.9%)	34 (39.1%)	64 (38.6%)

Table 7 Genotypic resistance patterns of NTS serovars in poultry processing environments

Resistance Gene	S. Typhimurium (*n* = 25)	S. Enteritidis (*n* = 20)	Untyped Salmonella (*n* = 45)	Overall (*n* = 90)
bla_{TEM}	15 (60.0%)	11 (55.0%)	25 (55.6%)	51 (56.7%)
TetA	12 (48.0%)	9 (45.0%)	20 (44.4%)	41 (45.6%)
Sul1	14 (56.0%)	10 (50.0%)	23 (51.1%)	47 (52.2%)
Sul2	11 (44.0%)	8 (40.0%)	18 (40.0%)	37 (41.1%)
Sul3	8 (32.0%)	6 (30.0%)	13 (28.9%)	27 (30.0%)
StrA/B	13 (52.0%)	10 (50.0%)	22 (48.9%)	45 (50.0%)

Table 8 Genotypic resistance patterns of NTS serovars in retail beef products

Resistance Gene	S. Typhimurium (*n* = 18)	S. Enteritidis (*n* = 16)	Untyped Salmonella (*n* = 42)	Overall (*n* = 76)
bla_{TEM}	10 (55.6%)	9 (56.3%)	25 (59.5%)	44 (57.9%)
TetA	9 (50.0%)	8 (50.0%)	23 (54.8%)	40 (52.6%)
Sul1	11 (61.1%)	8 (50.0%)	24 (57.1%)	43 (56.6%)
Sul2	8 (44.4%)	6 (37.5%)	18 (42.9%)	32 (42.1%)
Sul3	6 (33.3%)	5 (31.3%)	13 (31.0%)	24 (31.6%)
StrA/B	9 (50.0%)	8 (50.0%)	22 (52.4%)	39 (51.3%)

Table 9 Overall genotypic resistance patterns of NTS serovars

Resistance Gene	S. Typhimurium (*n* = 43)	S. Enteritidis (*n* = 36)	Untyped Salmonella (*n* = 87)	Overall (*n* = 166)
bla_{TEM}	25 (58.1%)	20 (55.6%)	50 (57.5%)	95 (57.2%)
TetA	21 (48.8%)	17 (47.2%)	43 (49.4%)	81 (48.8%)
Sul1	25 (58.1%)	18 (50.0%)	47 (54.0%)	90 (54.2%)
Sul2	19 (44.2%)	14 (38.9%)	36 (41.4%)	69 (41.6%)
Sul3	14 (32.6%)	11 (30.6%)	26 (29.9%)	51 (30.7%)
StrA/B	22 (51.2%)	18 (50.0%)	44 (50.6%)	84 (50.6%)

Table 10 Distribution of virulence genes in *Salmonella* serovars from poultry processing environments

Virulence Gene	S. Typhimurium (*n* = 25)	S. Enteritidis (*n* = 20)	Untyped Salmonella (*n* = 45)	Overall (*n* = 90)
InvA	20 (80.0%)	15 (75.0%)	35 (77.8%)	70 (77.8%)
AgfA	18 (72.0%)	12 (60.0%)	30 (66.7%)	60 (66.7%)
IpfA	15 (60.0%)	10 (50.0%)	25 (55.6%)	50 (55.6%)
HilA	12 (48.0%)	8 (40.0%)	20 (44.4%)	40 (44.4%)
SivH	10 (40.0%)	7 (35.0%)	15 (33.3%)	32 (35.6%)
SopE	8 (32.0%)	6 (30.0%)	13 (28.9%)	27 (30.0%)
SefA	6 (24.0%)	4 (20.0%)	10 (22.2%)	20 (22.2%)
SpvC	5 (20.0%)	3 (15.0%)	8 (17.8%)	16 (17.8%)

Table 11 Distribution of virulence genes in *Salmonella* serovars from retail beef products

Virulence Gene	S. Typhimurium (*n* = 18)	S. Enteritidis (*n* = 16)	Untyped Salmonella (*n* = 42)	Overall (*n* = 76)
InvA	15 (83.3%)	13 (81.3%)	35 (83.3%)	63 (82.9%)
AgfA	12 (66.7%)	11 (68.8%)	28 (66.7%)	51 (67.1%)
IpfA	10 (55.6%)	9 (56.3%)	22 (52.4%)	41 (53.9%)
HilA	8 (44.4%)	8 (50.0%)	20 (47.6%)	36 (47.4%)
SivH	7 (38.9%)	6 (37.5%)	16 (38.1%)	29 (38.2%)
SopE	6 (33.3%)	5 (31.3%)	13 (31.0%)	24 (31.6%)
SefA	4 (22.2%)	4 (25.0%)	11 (26.2%)	19 (25.0%)
SpvC	3 (16.7%)	2 (12.5%)	8 (19.0%)	13 (17.1%)

and North America [21, 22]. For instance, Sodagari et al. (2023) reported comparable resistance rates in poultry isolates [23], while Geresu et al. (2021) identified different resistance patterns in beef samples [24]. These variations highlight the role of regional antibiotic policies and management practices in shaping resistance profiles.

Table 12 Overall distribution of virulence genes in *Salmonella* serovars

Virulence Gene	S. Typhimurium (*n* = 43)	S. Enteritidis (*n* = 36)	Untyped Salmonella (*n* = 87)	Total (*n* = 166)
InvA	35 (81.4%)	28 (77.8%)	70 (80.5%)	133 (80.1%)
AgfA	30 (69.8%)	23 (63.9%)	59 (67.8%)	112 (67.5%)
IpfA	25 (58.1%)	19 (52.8%)	44 (50.6%)	88 (53.0%)
HilA	20 (46.5%)	16 (44.4%)	40 (46.0%)	76 (45.8%)
SivH	16 (37.2%)	13 (36.1%)	32 (36.8%)	61 (36.7%)
SopE	13 (30.2%)	11 (30.6%)	27 (31.0%)	51 (30.7%)
SefA	10 (23.3%)	8 (22.2%)	20 (23.0%)	38 (22.9%)
SpvC	8 (18.6%)	6 (16.7%)	16 (18.4%)	30 (18.1%)

The presence of multi-drug-resistant *Salmonella* strains poses significant public health risks, as these strains are harder to treat and may lead to longer, more severe infections. The genotypic analysis revealed that common resistance genes such as *blaTEM*, *tetA*, and *sul1* are prevalent across both poultry and beef isolates, suggesting a consistent pattern of resistance that may be due to the overuse or misuse of antibiotics in food animal production. The widespread presence of these resistance genes calls for a more stringent approach to antimicrobial stewardship in both poultry and beef industries, with emphasis on reducing unnecessary antibiotic use and enhancing surveillance systems. The emergence of multidrug-resistant *Salmonella* strains poses significant challenges for clinical management and public health interventions [19].

Antibiotic use as growth promoters in food-producing animals has been associated with increased AMR [25]. While some countries have banned this practice, it remains partially permitted in Iran. Stricter regulations and surveillance are needed to mitigate the risk of AMR development.

Genotypic resistance profiles

Genotypic analysis revealed the presence of various resistance genes, with bla_{TEM} (57.2%), *TetA* (48.8%), and *Sul1* (54.2%) being the most prevalent. These findings are consistent with other studies that have identified these genes as common determinants of antibiotic resistance in *Salmonella*. A study by Guerra et al. (2016) [13] found similar prevalence rates of these resistance genes in *Salmonella* isolates from retail meat in Italy. The presence of these genes in high proportions underscores the importance of monitoring genetic markers for resistance to understand the mechanisms driving antimicrobial resistance and to develop effective control measures.

The detection of various resistance genes, including bla_{TEM}, *TetA*, *Sul1*, *Sul2*, *Sul3*, and *StrA/B*, highlights the genetic diversity of antimicrobial resistance mechanisms among *Salmonella* isolates. The presence of these resistance genes underscores the role of horizontal gene transfer and genetic adaptation in conferring resistance to antimicrobial agents. Understanding the genetic basis of antimicrobial resistance is essential for developing targeted strategies to combat resistance and preserve the effectiveness of antimicrobial therapies [26].

A significant finding in our study is the 3.6% resistance rate to meropenem among *Salmonella* isolates. This result is critical, as meropenem is a last-resort antibiotic for severe human infections and is prohibited for use in animals in the EU and other regions [27]. The presence of meropenem-resistant *Salmonella* suggests the possible acquisition of carbapenemase genes, such as *bla_KPC* or *bla_NDM*, through horizontal gene transfer. Studies from Taiwan [28] and China [29] have similarly reported carbapenem-resistant *Salmonella*, with plasmid-mediated resistance being a key factor. Further molecular analysis is needed to confirm the genetic basis of resistance in our isolates.

Virulence profiles of salmonella strains

Virulence gene analysis revealed that the majority of *Salmonella* isolates from both poultry and beef products carried the *invA* gene, which is associated with the ability to invade host cells. The *agfA* gene, which encodes for biofilm formation, was also commonly detected, indicating the potential for these strains to persist in the food production environment. The presence of other virulence factors, such as *hilA*, *sopE*, and *spvC*, further highlights the pathogenic potential of these strains.

In poultry processing environments, the high prevalence of virulence genes such as *invA* (77.8%) and *agfA* (66.7%) suggests that these strains are well-equipped to colonize and persist in the gastrointestinal tract of animals, potentially leading to foodborne illnesses in humans. Similar trends were observed in retail beef products, where *invA* was detected in 82.9% of isolates and *agfA* in 67.1%. These findings underscore the importance of controlling *Salmonella* contamination at multiple stages in the food supply chain, from farm to table.

The distribution of virulence genes was similar across both poultry and beef sources, with some variations in prevalence rates. This suggests that while environmental factors may influence gene distribution, the overall virulence potential of *Salmonella* strains in these food products remains a significant concern for public health.

The most prevalent virulence genes were *invA* and *agfA*. The *invA* gene, crucial for the invasion of host cells, was detected in a significant proportion of isolates, corroborating findings from other studies. For example, a study by Rahman et al. (2018) [15] identified *invA* in 95% of *Salmonella* isolates from poultry in Bangladesh.

Implications for food safety and public health

The findings from this study have important implications for food safety practices in poultry and beef industries. The high prevalence of *Salmonella* contamination, especially in high-risk products such as raw beef organ meats and poultry processing environments, calls for enhanced surveillance, better hygiene practices, and more rigorous implementation of food safety regulations. Effective cleaning and sanitization of surfaces, equipment, and water sources in poultry processing plants are essential to minimize the risk of cross-contamination.

Additionally, the widespread antimicrobial resistance observed in this study highlights the urgent need for improved antimicrobial stewardship programs in both poultry and beef production. Reducing the unnecessary use of antibiotics in animal husbandry, coupled with increased surveillance of resistance patterns, will be crucial in controlling the spread of resistant *Salmonella* strains. Implementing these measures could also help mitigate the risk of antimicrobial resistance transferring to humans through the consumption of contaminated food.

Limitations and future directions

It is important to acknowledge the limitations of this study, including its cross-sectional design, limited sample size, and potential selection bias. Future research should focus on longitudinal studies to monitor trends in *Salmonella* prevalence and antimicrobial resistance over time. Additionally, molecular epidemiological studies, such as whole-genome sequencing, can provide valuable insights into the genetic diversity and transmission dynamics of *Salmonella* strains in the meat supply chain.

Conclusions

This study underscores the need for enhanced monitoring, better control strategies, and stricter antimicrobial stewardship practices in the poultry and beef industries to mitigate the risks posed by *Salmonella* contamination and antimicrobial resistance. The widespread presence of virulence factors in *Salmonella* strains further emphasizes the importance of controlling contamination at all stages of food production. Given the public health risks associated with *Salmonella* and antimicrobial-resistant pathogens, these findings serve as a call to action for improved food safety and public health measures.

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Author contributions

Ebrahim Rahimi, and Zahra Osivand carried out the molecular genetic studies, participated in the primers sequence alignment and drafted the manuscript. Zahra Osivand, Amir Shakerian and Ebrahim Rahimi carried out the sampling and culture method. Ebrahim Rahimi and Amir Shakerian, Faham Khamesipour participated in the design of the study, performed the statistical analysis and wrote the manuscript. All authors read and approved the final manuscript.

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Data availability

The data supporting this study's findings are available on request from the corresponding author upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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