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Antimicrobial resistance of *Salmonella* spp. recovered from raw chicken meat at abattoirs and retail markets in Jordan

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Abstract

Salmonella is a major causative factor of bacterial foodborne diseases in humans. This study aimed to investigate the antimicrobial vulnerability profiles and the presence of genes of antimicrobial resistance (AMR) among *Salmonella* spp. recovered from chilled raw chicken meat at chicken abattoirs and local markets in Jordan. Out of 700 samples, a total of 106 (15.14%) isolates tested were positive for *Salmonella*-(*invA*) gene: chicken carcasses collected from abattoirs (14.5%, 29/200) and retail markets (16.25%, 65/400) as well as chicken breast collected from retail markets (12.0%, 12/100). More than 98% of the isolates exhibited resistance to at least one antibiotic. Notably, the isolates showed high resistance toward ampicillin (75.5%) and amoxicillin/clavulanic acid (45.3%), sulfafurazole (66.0%), tetracycline (61.3%), and ciprofloxacin (42.5%). There was a lower level of resistance to cefotaxime (24.5%), chloramphenicol (19.8%), imipenem (11.3%), ceftazidime (9.4%), and gentamicin (7.5%). AMR profiling showed that 41.5% (n = 44) of isolates were multidrug resistant (MDR). The occurrence of bla_{TEM} resistance gene (RG) was mostly prominent (92.5% (74/80)) among isolates resistant to ampicillin and those resistant to amoxicillin/clavulanic acid (52.1% (25/48)). Among 65 tetracycline-resistant *Salmonella* isolates, 89.2% (n = 58) harbored the *tet(B)* RG, and among 70 sulfonamide-resistant *Salmonella* spp., 38.6% (27/70) and 90.0% (63/70) of resistant isolates harbored *sul1* and *sul2* RG, respectively. The obtained results demonstrate the necessity to strictly control the usage of antibiotics in poultry to manage MDR *Salmonella* infections.

Keywords: Chicken meat; *Salmonella*; antimicrobial resistance; multidrug resistance; resistance genes; abattoirs

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Introduction

Salmonella is one of the most common causes of bacterial foodborne illness in humans (Galán-Relaño *et al.*, 2023). The occurrence of *Salmonella* contamination is a major challenge to the poultry industry and regulatory authorities since it is considered the leading cause for foodborne infections worldwide (CDC, 2024; Pavelquesi *et al.*, 2023). Nontyphoidal *Salmonella* spp. are frequently detected in food of animal sources, mainly poultry meat and eggs (Al-Qadiri *et al.*, 2008; Castro-Vargas *et al.*, 2020). In the United States, it was reported that nontyphoidal *Salmonella* infection is the most prevalent bacterial foodborne disease (Akil and Ahmad, 2019). In another study in the United States, the organism was isolated from 8.6% of chicken breast samples; more than 73.1% of the isolated *Salmonella* spp. exhibited resistance to more than one antibiotic, and 48.1% exhibited resistance to more than three types of antibiotics (multi drug resistance (MDR)) (Mujahid *et al.*, 2023).

Foodborne infection by *Salmonella* can be severe and poses an additional public health burden when the detected isolates are resistant to antibiotics (NARMS, 2024). Chicken meat is the principal foodborne mode of transmission for *Salmonella* spp. and the cause of most salmonellosis cases (Dewey-Mattia *et al.*, 2018). Several studies indicated that approximately 25% of the salmonellosis cases are transmitted through contaminated raw poultry (Chai *et al.*, 2017; Smadi and Sargeant, 2013). Transmission routes of *Salmonella* spp. are contaminated feed, unhygienic farming, slaughtering, processing, and packing practices, and the risk for infection is exacerbated through transportation, storage, retailing, and handling or preparation by consumers. Nevertheless, poultry may act as a primary *Salmonella* reservoir contaminating chicken meat and eggs (Chai *et al.*, 2017).

As a public health concern, emerging antibiotic resistance by foodborne pathogens is a global challenge since AMR bacteria may elicit a life-threatening illness. Globally, it is estimated that *Salmonella* is associated with 700,000 deaths. Of these, 23,000 mortalities take place annually in the United States (Dadgostar, 2019; Nair *et al.*, 2018). Many recent studies reported a progressive increase in the occurrence of resistance to commonly used antibiotics of *Salmonella* spp. isolated from humans or animals (Borges *et al.*, 2019; Pavelquesi *et al.*, 2023; Perin *et al.*, 2020). Isolates of *Salmonella* spp. showing MDR involved in infections contain genes that code for antibiotic resistance such as bla_{TEM} (beta-lactam-resistance gene (RG)), *sul1* and *sul2* (sulfonamide RGs), and *tetB* (tetracycline RG) (Cunha-Neto *et al.*, 2018).

It was reported that nontyphoidal *Salmonella* isolates that are resistant to widely used antibiotics such as ampicillin, ceftriaxone, ciprofloxacin, fluoroquinolones, cephalosporin, sulfonamides, and tetracyclines were responsible for most of the hospitalizations and mortalities (Li *et al.*, 2013; Mukherjee *et al.*, 2019; Pavelquesi *et al.*, 2023, Zhu *et al.*, 2017). Since it is difficult to eliminate *Salmonella* from its reservoir, it is essential to target *Salmonella* spp. in the animal food supply chain, apply strict precautionary measures, and develop new treatment strategies to reduce public health risk.

This study is one of few studies to detect the occurrence of *Salmonella* in the slaughterhouse and retail market in Jordan. Therefore, the study aimed to investigate the occurrence and the antibiotic resistance profiles of *Salmonella* spp. in chilled raw chicken meat collected from retail markets and from poultry slaughter facilities.

Materials and Methods

Sample collection

Between October 2022 and August 2024, a total of 700 chilled raw chicken meat samples were collected under aseptic conditions (International Standard method (ISO) 17604:2015; ISO/TS 17728:2015) as follows: (i) eviscerated chicken carcasses at the main broiler chicken abattoirs (n= 200); (ii) eviscerated chicken carcasses from the retail markets (n= 400), and (iii) chicken breasts (n = 100) from the retail markets (supermarkets, small grocery stores, and butcher shops). Samples were kept refrigerated during transportation (<8°C) and were microbiologically examined within 4 h.

Bacterial isolation and serotyping

Chicken meat samples of 25 g were diluted in 225 mL of sterile buffered peptone water (BPW) (Oxoid, CM1049), then 10-fold sequentially diluted and examined for aerobic plate count (APC) by spread plating 0.1 mL of the serially diluted sample in duplicate on tryptone soy agar (TSA) plates (Oxoid, CM0131). Plates were enumerated after being incubated at 30°C for 72 h, and the counts were determined and reported as Log_{10} CFU/g of the sample (ISO 4833-2:2013).

The process of detecting, isolating, and identifying *Salmonella* spp. was conducted according to the ISO method (ISO 6579-1:2017). Pre-enrichment in a nonselective medium was conducted in triplicate with a 25-g chicken sample being homogenized in 225 mL of sterile

BPW. The sample with BPW was aseptically placed in a stomacher sterile strainer-filter bag and pounded using a stomacher for 2 min, and then incubated at 37°C for 18 h. Thereafter, aliquots of 0.1 mL of the pre-enrichment BPW were transferred into 10 mL of Rappaport-Vassiliadis soya peptone (RVS broth) (Oxoid, CM0866) and Muller-Kauffmann tetrathionate-novobiocin broth (MKTTn broth) (Oxoid, CM1048), respectively. The inoculated RVS and MKTTn broths were incubated at 41 and 37°C for 24 h, respectively. Then, 1 mL of the incubated RVS and MKTTn broths were consecutively diluted (10-fold) and a 100 µl aliquot of each 10^{-2} dilution was then sub-cultured by streaking onto xylose lysine deoxycholate (XLD) agar (Oxoid, CM0469) and *Salmonella Shigella* (SS) agar (Oxoid, CM0099) as differential selective media. Eventually, the plates were incubated at 37°C for 24 h. Presumptive *Salmonella* isolates were further confirmed by sub-culturing onto lysine iron (LI) agar (Oxoid, CM0381) and triple sugar iron (TSI) agar (Oxoid, CM0277). Positive cultures were then serotyped by agglutination test for *Salmonella* antigens (Oxoid *Salmonella* latex test, FT0203). Serotyping screening to identify *Salmonella* Typhimurium and *Salmonella* Enteritidis was carried out using agglutination test for O and H antigens (Thermo Fisher Scientific-Remel R30953101, R30952901).

Antimicrobial susceptibility testing

Salmonella inocula (*invA* gene-positive strains) were prepared as a suspension from Mueller-Hinton broth (Oxoid, CM0405) with turbidity equivalent to a 0.5 McFarland standard (10^8 CFU/mL), adjusted using a spectrophotometer set at 625 nm. Ten classes of antibiotics were used to test for the susceptibility of the isolated *Salmonella* spp. for the antibiotics used in salmonellosis infection (Clinical and Laboratory Standards Institute (CLSI), 2020; Hudzicki, 2009). The abbreviations and concentrations of these antimicrobial agents (AGs) according to Thermo Fisher Scientific-Oxoid are beta-lactam antibiotics: ampicillin (AMP, 10 µg) and amoxicillin/clavulanic acid (AMC, 20/10 µg); beta-lactam cephalosporin antibiotics: ceftazidime (CAZ, 30 µg) and cefotaxime (CTX, 30 µg); aminoglycoside gentamicin (CN, 10 µg); protein synthesis inhibitors: chloramphenicol (C, 30 µg) and tetracycline (TE, 30 µg); beta-lactam carbapenem imipenem (IPM, 10 µg); sulfonamide sulfafurazole (SF, 300 µg); and fluoroquinolone ciprofloxacin (CIP, 5 µg). All the antimicrobial disks were obtained from Thermo Fisher Scientific-Oxoid (CT0003B, CT0223B; CT0412B; CT0166B; CT0024B; CT0013B; CT0054B; CT0455B; CT0075B; and CT0425B, respectively). For comparison purposes (control), *Escherichia coli* ATCC 25922 was used. *Salmonella* isolates' vulnerability to the action of antibiotics was depicted as resistant (R), intermediate (I), or susceptible (S), according to the CLSI (2020) guidelines. *Salmonella* spp. that were resistant to three or more antimicrobials were defined as MDR (NARMS, 2017).

Molecular verification and detection of antimicrobial resistance genes

Salmonella total genomic DNA was extracted from the recovered isolates cultivated in Mueller-Hinton broth at 37°C for 18 h using PureLink™ Genomic DNA Mini Kit (K182002) (Thermo Fisher Scientific) following the manufacturer's instructions. The extracted DNA was qualitatively and quantitatively analyzed using 1.5% (w/v) agarose gel electrophoresis and Nano Drop One Microvolume UV-Vis Spectrophotometer (Thermo Scientific, Asheville, NC), respectively. PCR amplification was performed using SimpliAmp™ Thermal Cycler (Thermo Fisher Scientific). The amplified PCR products were examined through 1.5% (w/v) agarose gel electrophoresis. Ultraviolet (UV) light was used to visualize the separated DNA fragments on the gel with a 100 bp marker.

Molecular verification of *Salmonella* positive isolates was carried out by detection of *Salmonella*-specific invasive encoding gene (*invA*). PCR amplification targeting the *invA* gene, known for its role in bacterial invasion, was conducted following the method described by Rahn *et al.* (1992). Molecular confirmation of *Salmonella* spp. was achieved using specific primers that produced a 284 bp amplicon: F139 forward primer (5′-GTG AAATTATCGCCACGTTCGGGCAA-3′) and R141 reverse primer (5′-TCATCGCACCGTCAAAGGAACC-3′).

Salmonella isolates that elicited resistance to a class of AGs were examined for the presence of RGs using a PCR technique. The presence of RGs associated with bla_{TEM} for beta-lactams, *tetB* for tetracycline, and *sul1* and *sul2* for sulfonamide was detected by PCR amplification. The primer sequences, predicted sizes, and thermocycling conditions for PCR amplification of the studied RGs are depicted in Table 1.

Statistical analysis

The Statistical Analytical System (SAS, 2011) (SAS Inst. Inc., Cary, N.C., USA) package (version 8.2) was used to determine statistically significant differences ($P < 0.05$) between the prevalence rate of *Salmonella* spp. in correlation with (i) verification method (latex agglutination vs. *invA* gene) and (ii) different source and type of chicken meat using Chi-square (χ^2) test. Pairwise comparisons of means (post hoc test) was performed to determine significant differences ($P < 0.05$) between the mean of APCs with the Tukey-Kramer adjustment.

Table 1. Primers used for the detection of genes encoding resistance to different antimicrobials and the PCR thermocycling conditions.

Antimicrobial	Target gene	Forward primer (5′→3′) Reverse primer (5′→3′)	Amplicon (bp)	PCR conditions	Source
Beta-lactams	bla_{TEM}	CAGCGGTAAGATCCTTGAGA TTCATCCATAGTTGCCTGACT	661	Denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 50 s, with final extension at 72°C for 7 min	Li *et al.* (2013)
Tetracycline	*tet(B)*	GAGACGCAATCGAATTCGG TTTAGTGGCTATTCTTCCTGCC	228	Denaturation at 95°C for 10 min, followed by 35 cycles of 94°C for 45 s, 55–70°C for 50 s, 72°C for 50 s, with final extension at 72°C for 10 min	Zhu *et al.* (2017)
Sulfonamide	*Sul1*	CTTCGATGAGAGCCGGCGGC GCAAGGCGGAAACCCGCGCC	437		
	Sul2	GCGCTCAAGGCAGATGGCATT GCGTTTGATACCGGCACCCGT	285		

Results and Discussion

Prevalence of *Salmonella* spp. isolates

This is one of few studies in Jordan that intended to demarcate the incidence of *Salmonella* and to examine the resistance of the isolates to commonly used antibiotics from chilled raw chicken meat samples obtained from broiler chicken abattoirs and retail markets. Out of 700 samples, a total of 106 (15.14%) isolates tested were positive for *Salmonella*-(*invA*) gene (Table 2). All the isolated *Salmonella* were identified by biochemical methods and confirmed by discerning the *Salmonella*-invasive specific gene (*invA*). Several previous studies used this gene to confirm the presence of *Salmonella* in poultry meat (Ahaduzzamana *et al.*, 2021; Boubendir *et al.*, 2021; Li *et al.*, 2013). There was a significant difference ($P<0.05$) in the prevalence rate of isolates verified by latex agglutination as compared with PCR techniques. The *Salmonella*-specific (*invA*) gene was detected in 14.5% (29/200) of chicken carcass samples collected from abattoirs, 16.25% (65/400) of chicken carcasses from retail markets, and 12.0% (12/100) of chicken breast samples collected from retail markets (Table 2). Serotyping indicated that a total of less than 4% of the recovered isolates were identified as *S.* Typhimurium and *S.* Enteritidis.

In a recent study that aimed to examine the occurrence of *Salmonella* spp. in a poultry slaughterhouse in Jordan, *Salmonella* spp. were confirmed in 15.47% out of 635 samples collected, in which 50 (56.2%), 2 (2.2%), and 1 (1.1%) of the isolates were identified as *Salmonella* Infantis, *S.* Enteritidis, and *S.* Typhimurium, respectively (Gharaibeh, *et al.*, 2024). This study pointed out that *Salmonella* spp. were recoverable at the highest rate (37.8 %) from the post-chiller and the lowest (3%) when using the farm drag swab method.

In the current study, chicken carcass samples collected from retail markets showed the highest degree of contamination, while the skinless chicken breast samples had the lowest. The reasons behind the lower prevalence in chicken breast could be because of the removal of skin that harbors *Salmonella* as well as the use of food sanitizers and UV light at various stages of processing and packaging that provides bactericidal and bacteriostatic activity. In a study by Golden and Mishra (2020), the prevalence rate of *Salmonella* spp. increased from 14.3% in broiler chickens to 19.0–23.0% in retail chicken. In another study in China, the overall occurrence of *Salmonella* in chicken meat during the slaughtering process at an abattoir was 30.14%, where the organism was isolated at the rate of 48.0%, 18.8%, 31.3%, and 14.0% from cecal content, chicken carcasses, chicken meat cuts, and frozen chicken meat, respectively. The predominant serotypes of *Salmonella* were *S.* Enteritidis and *S.* Typhimurium. In comparison, the occurrence of *Salmonella* in chicken meat after the slaughtering process and before transportation and distribution was 18.0% in 2017, 12.6% in 2018, and 15.1% in 2019 (Pavelquesi *et al.*, 2023). In the United States, samples of raw chicken breast taken from retail markets were reported to be contaminated by *Salmonella* (4.2%) and *Campylobacter* (8.6%), in which a total of 10.82% of samples contained one or both bacterial species (Mujahid *et al.*, 2023). The variations in the prevalence rates of *Salmonella* spp. in different countries and between versatile studies that addressed the same issue could be attributed to the variations in poultry farming practices, method of sampling and sampling seasons, and the differences in detection and isolation methods applied. According to microbiological criteria for the Near East issued by Food and Agriculture Organization of the United Nations & World Health Organization (FAO/WHO, 2002), *Salmonella* should not be detected even in 1 g sample of raw poultry meat. To some extent, it is impractical to avoid contamination by *Salmonella*, since multiple contamination sources are possible throughout the farming, slaughtering, processing, and distribution supply chain. Nevertheless, it is important to confirm that *S.* Enteritidis and *S.* Typhimurium are not detected in chicken meat (FAO/WHO, 2002), and the target approach is to minimize cross contamination

Table 2. Prevalence rate of *Salmonella* spp. isolated from chilled raw chicken meat.

Chicken meat samples	Number of samples (%)	Number (%) of isolates positive for *Salmonella* spp.[1,2]		Mean viable aerobic plate count (log_{10} CFU/g)[3]
		Latex agglutination-positive strains	*invA* gene-positive strains	
Eviscerated chicken carcasses (abattoirs)	200 (28.57)	25 (12.5)^{A,a}	29 (14.5)^{A,b}	4.11 ± 0.12^{a}
Eviscerated chicken carcasses (retail markets)	400 (57.14)	58 (14.5)^{B,a}	65 (16.25)^{B,b}	5.06 ± 0.10^{b}
Chicken breasts (retail markets)	100 (14.28)	10 (10.0)^{C,a}	12 (12.0)^{C,b}	4.49 ± 0.08^{c}
Total	700 (100.00)	93 (13.29)^{a}	106 (15.14)^{b}	

[1]Within the same column, values without shared uppercase letter are significantly different ($P < 0.05$).
[2]Within the same row, values without shared lowercase letters are significantly different ($P < 0.05$).
[3]Values are the means ± standard deviation. Means without shared superscripts are significantly different (Tukey's HSD test, $P < 0.05$)

using a preventive control strategy based on target risk promoted by global food safety management systems such as HACCP/HARPC and ISO 22000.

In the current study, APC provided a general indication of microbial contamination and the hygienic conditions applied during processing, distribution, and marketing. A total microbial count of less than 6.0 Log_{10} CFU/g (Table 2) was observed for chicken meat in this study, which was within the acceptable microbiological criteria for chilled poultry meat (FAO/WHO, 2002). However, as evident in Table 2, the occurrence of *Salmonella* in raw chicken exceeds the reference standards, making it necessary to reduce the level of raw chicken contamination with *Salmonella.*

Antimicrobial susceptibility testing

Table 3 and Figure 1 show the susceptibility of 106 *Salmonella* isolates to 10 classes of AGs, revealing that more than 98% of the isolates were resistant to one or more of the tested AGs, and only 1.9% were susceptible to all tested drugs. High resistance rates were observed against beta-lactams group of ampicillin (75.5%) and amoxicillin/clavulanic acid (45.3%); sulfonamide-sulfafurazole (66.0%); tetracycline (61.3%); and ciprofloxacin (42.5%). However, the isolates exhibited lower level of resistance toward cefotaxime (24.5%), chloramphenicol (19.8%), imipenem (11.3%), ceftazidime (9.4%), and gentamicin (7.5%). Gentamicin showed the highest efficacy against all isolated *Salmonella*, where 80.2% of the isolates exhibited sensitivity to the antibiotic. Gharaibeh *et al.* (2024) reported that *Salmonella* detected in chicken samples collected from an abattoir in Jordan showed high degrees of resistance against ciprofloxacin, tetracycline, sulfamethoxazole/trimethoprim, and ampicillin. A lower level of resistance was elicited against cefotaxime, gentamicin, and ceftazidime. Several studies reported high resistance rate among *Salmonella* isolates detected in chicken meat against beta-lactams (ampicillin), sulfonamide, and tetracycline (Gharaibeh *et al.*, 2024; Li *et al.*, 2013; Mujahid *et al.*, 2023; Pavelquesi *et al.*, 2023; Punchihewage-Don *et al.*, 2024; Zhu *et al.*, 2017); amoxicillin/clavulanic acid (Pavelquesi *et al.*, 2023); and ciprofloxacin and quinolone-nalidixic acid (Gharaibeh *et al.*, 2024; Vinueza-Burgos *et al.*, 2016; Voss-Rech *et al.*, 2017; Zhu *et al.*, 2017), whereas *Salmonella* isolates showed lower level of resistance with a variation in sensitivity to gentamicin, cefotaxime, chloramphenicol, imipenem, and ceftazidime according to the same studies.

A growing tendency of resistance to extended spectrum beta-lactam cephalosporins (ESBLs) was reported in *Enterobacteriaceae* worldwide (Dierikx *et al.*, 2010). Bacteria resistant to ESBLs are often isolated from the intestinal tract of animals and have been isolated from poultry, turkey, cattle, swine, cats, dogs, horses, and wild animals (Costa *et al.*, 2006; Hasman *et al.*, 2005;; Li *et al.*, 2007; Nair *et al.*, 2018; Vo *et al.*, 2006). According to the Food and Drug Administration (FDA), *Salmonella* spp. isolated from ground beef showed remarkable level (38.5%) of resistance to ceftriaxone (a broad-spectrum cephalosporin antibiotic) and were MDR (FDA, 2017).

ESBLs producing *Salmonella* act to cleave the beta-lactam ring to inactivate such antimicrobials (Chon *et al.*, 2015). This may limit medical treatment of salmonellosis using cephalosporins as drugs of choice. The decrease in susceptibility of *Salmonella* to antibiotics commonly used to treat salmonellosis could be because of the use of these drugs extensively in both human and veterinary medicine (CDC, 2019; Lai *et al.*, 2014; Voss-Rech *et al.*, 2017). A considerable surge in resistance of *Salmonella* spp. to protein synthesis inhibitors, tetracyclines, and bacteriostatic sulfonamides, which are used as broad-spectrum agents was reported in several studies (Nair *et al.*, 2018; Pavelquesi *et al.*, 2021). Antibiotics

Table 3. Antimicrobial resistance patterns and susceptibility profile of 106 *Salmonella* spp. (*invA* gene-positive) isolates.

Antimicrobials[1]	Resistant (R) No. (%)	Intermediate (I) No. (%)	(R + I) No. (%)	Susceptible (S) No. (%)	Zone diameter breakpoints (nearest whole mm)[2]		
					R	I	S
Beta-lactams							
Ampicillin AMP	80 (75.5)	16 (15.1)	96 (90.6)	10 (9.4)	<13	14–16	>17
Amoxicillin/clavulanic acid AMC	48 (45.3)	37 (34.9)	85 (80.2)	21 (19.8)	<13	14–17	>18
Beta-lactam cephalosporin							
Ceftazidime CAZ	10 (9.4)	15 (14.2)	25 (23.6)	81 (76.4)	<17	18–20	>21
Cefotaxime CTX	26 (24.5)	19 (17.9)	45 (42.5)	61 (57.5)	<22	23–25	>26
Aminoglycoside							
Gentamicin CN	8 (7.5)	13 (12.3)	21 (19.8)	85 (80.2)	<12	13–14	>15
Protein synthesis inhibitors							
Chloramphenicol C	21 (19.8)	30 (28.3)	51 (48.1)	55 (51.9)	<12	13–17	>18
Tetracycline TE	65 (61.3)	17 (16.0)	82 (77.4)	24 (22.6)	<11	12–14	>15
Beta-lactam carbapenem							
Imipenem IPM	12 (11.3)	14 (13.2)	26 (24.5)	80 (75.5)	<19	20–22	>23
Sulfonamide							
Sulfafurazole SF	70 (66.0)	15 (14.2)	85 (80.2)	21 (19.8)	<12	13–16	>17
Fluoroquinolone							
Ciprofloxacin CIP	45 (42.5)	32 (30.2)	77 (72.6)	29 (27.4)	<20	21–30	>31

[1]Thermo Fisher Scientific-Oxoid.
[2]Antimicrobial resistant (R), intermediate (I), and susceptible (S) categories (CLSI, 2020).

Figure 1. Prevalence of antibiotic resistance among *Salmonella* isolates (%) recovered from chilled raw chicken meat at chicken abattoirs and local markets in Jordan.

are sometimes illegally or irresponsibly used as growth promoter additives in animal feed. Also, antibiotics are broadly used in veterinary medicine. These factors may have contributed to the progressive increase in antibiotic resistance, which may pose a serious public health threat. Hence, it is necessary to preclude potential causes of antibiotic resistance and control the transfer of antibiotic-resistant microbes from livestock to the human population. One study reported that resistance of *Salmonella* spp. to antibiotics was more prevalent in conventional poultry farms compared to nonconventional (antibiotic-free) farms (Gebreyes *et al.*, 2006).

As shown in Figure 1, 41.5% (n = 44) of recovered *Salmonella* spp. were resistant to three or more of the studied antimicrobials and are considered to be MDR isolates. Resistance to up to seven categories of antimicrobials was found in 2.8% of isolates, presenting a health concern considering the cross connection between broiler farms, slaughtering environments, and distribution and sale of human food. Several studies from different geographical areas have confirmed high rates of MDR (>50% of isolates) in recovered *Salmonella* from broiler and layer farms, abattoirs, and in poultry meat (Furukawa *et al.*, 2017; Gharaibeh *et al.*, 2024; Moe *et al.*, 2017; Mujahid *et al.*, 2023; Pavelquesi *et al.*, 2023; Perin *et al.*, 2020; Sodagari *et al.*, 2015; Zhu *et al.*, 2017).

The emergence of MDR foodborne bacteria is a global health problem, and there is a need to control the overuse or misuse of antimicrobials in animal husbandry, which could be a major factor driving the increase in the incidence of MDR bacteria. Strict application of good agricultural practices and systems of food safety were proven to be effective in controlling MDR *Salmonella* food infection, otherwise higher morbidity and mortality rates may occur from microbial foodborne illnesses. It is also beneficial to review the 2016–2017 NARMS Integrated Summary, which provides consolidated antimicrobial susceptibility testing (AST) data collected in the United States. Data were grouped from clinical isolates of human origin, intestinal isolates from food-producing animals at slaughterhouses, samples collected at slaughter as part of (PR/HACCP) testing, and from different types of raw meats collected at retail markets in 18 states (Mujahid *et al.*, 2023; NARMS, 2017).

Detection of antimicrobial RGs

As shown in Table 4, the occurrence rate of bla_{TEM} RG was 92.5% and 52.1% among *Salmonella* isolates found phenotypically resistant to ampicillin and amoxicillin/clavulanic acid, respectively. The genotypic results were in agreement with those of phenotypic AST. As previously discussed, the increasing resistance to ESBLs has been observed in *Enterobacteriaceae* because of the effect of ESBL enzymes, in which bla_{TEM} is one of the most prevalent ESBL genes (Chon *et al.*, 2015; Li *et al.*, 2013; Zhu *et al.*, 2017). It was indicated that *Salmonella* spp. recovered from chicken that are resistant to beta-lactams exhibited more than 96% bla_{TEM} prevalence (Moawad *et al.*, 2017). A study carried out in Jordan indicated that ESBL genes have been identified in the recovered *Salmonella* isolates for samples collected from an integrated poultry abattoir, in which bla_{TEM} was identified as the most prevalent gene in 88.8% of the isolates (Gharaibeh *et al.*, 2024). In another study, it was possible to identify bla_{TEM} in 93.4% of *Salmonella* isolated from broiler chickens at different slaughtering processes. bla_{TEM} was present in 93.4% of

Table 4. Occurrence of antimicrobial-resistance genes for the isolated *Salmonella* spp.

Resistance genes	Antimicrobials	No. (%) of confirmed isolates for the presence of antimicrobial-resistance genes		
		R	I	S
	Ampicillin	(80 isolates)[1]	(16 isolates)[1]	(10 isolates)[1]
bla_{TEM}		74 (92.5)[2]	9 (56.3)[2]	1 (10.0)[2]
	Amoxicillin/clavulanic acid	(48 isolates)	(37 isolates)	(21 isolates)
bla_{TEM}		25 (52.1)	13 (35.1)	2 (9.5)
	Tetracycline	(65 isolates)	(17 isolates)	(24 isolates)
tet(B)		58 (89.2)	8 (47.1)	3 (12.5)
	Sulfonamide	(70 isolates)	(15 isolates)	(21 isolates)
Sul1		27 (38.6)	2 (13.3)	0 (0.0)
Sul2		63 (90.0)	10 (66.7)	1 (4.8)

[1]Number of phenotypic antimicrobial resistant (R), intermediate (I), and susceptible (S) isolates.
[2]Number and percentage of confirmed isolates for the presence of antimicrobial-resistance gene.

the beta-lactam-resistant isolates, whereas $bla_{CTX\text{-}M}$ gene has been recognized in 12.7% of the isolates, and both genes were identified in 11.4% of the recovered isolates (Zhu *et al.*, 2017).

The emerging ESBL-producing *Salmonella* isolates carry beta-lactam RG, which are located in transferable genetic components, (transposons, integrons, and plasmids). Hence, horizontal gene transfer (HGT) could be one of the mechanisms through which *Salmonella* species acquire novel genetic material, with this being the most common way for bacteria to become resistant to antimicrobials (Michaelis and Grohmann, 2023). However, some difference in results regarding the prevalence of $bla_{TEM\ gene}$ among beta-lactam-resistant *Salmonella* could reflect different restrictions and strategies of using antibiotics applied in different countries (Sabry *et al.*, 2020).

In the current study, among 65 phenotypically tetracycline-resistant *Salmonella* isolates, 89.2% (n = 58) harbored the *tet(B)* RG, which is considered to be a gene specific to Gram-negative bacteria (McMillan *et al.*, 2019). The *tet* genes may confer resistance to tetracycline, doxycycline, and minocycline by encoding membrane-associated tetracycline efflux protein pumps that are associated with plasmids and transposons (Roberts and Schwarz, 2016). Among 98 tetracycline-resistant *Salmonella* spp. recovered from broiler chickens at different stages in the slaughtering process in China, 85.7% of isolates harbored at least one *tet* gene. The *tet(C)* and *tet(B)* genes were the most concurrent and prevalent (71.4 and 50%, respectively) (Zhu *et al.*, 2017). Thakur *et al.* (2007) studied the antibiotic resistance patterns of *Salmonella* isolated from swine farms. Environmental samples in the form of swabs were collected from intensive and extensive farms. High resistance profiles of *Salmonella* spp. against tetracycline and streptomycin (78.5 and 31.5%, respectively) were reported, with *S.* Typhimurium var. Copenhagen showing the highest resistance against different types of antibiotics.

Among 70 sulfonamide-resistant *Salmonella* spp., 38.6% and 90.0% of resistant isolates harbored *sul1* and *sul2* genes, respectively. The mechanism of sulfonamide resistance is attributed to the possession of the *sul* genes (*sul1*, *sul2*, and *sul3*) that results in the overproduction of an insensitive form of dihydropteroate synthetase enzyme that counteracts the action of sulfonamides (Alcaine *et al.*, 2007; Huovinen *et al.*, 1995). These genes have been detected in major *Salmonella* serogroups, including *S.* Typhimurium *S.* Enteritidis, *S.* Hadar, and *S.* Heidelberg (Alcaine *et al.*, 2007; Huovinen *et al.*, 1995). *Salmonella* isolates resistant to sulfadimethoxine and trimethoprim-sulfamethoxazole combination were commonly isolated from poultry farms, which are considered the main reservoirs of antimicrobial-resistant bacteria (Chen and Jiang, 2014; Liljebjelke *et al.*, 2017, Nair *et al.*, 2018). Zhu *et al.* (2017) found that *sul2* gene had the highest occurrence (97.8%), followed by *sul3* (50.5%) and *sul1* (50.5%) among sulfonamide-resistant *Salmonella* isolates recovered from broiler chicken samples.

In the present study, it was noticed that genes that code for antimicrobial-RGs (bla_{TEM}, *tet(B)*, *sul1* and *sul2*) were also detected in some of the recovered isolates. Nonetheless, these isolates showed intermediate and susceptible patterns to the tested antimicrobials. This might be because DNA sequences were entirely unexpressed or expressed at an inadequate level in vitro. Antibiotic RGs in the silent state have been detected in seafood-associated nontyphoidal *Salmonella* isolates in the south-west coast of India, in which 40% of chloramphenicol-susceptible strains carried the *catA1* gene (Deekshit *et al.*, 2012). RGs were also detected in susceptible and intermediately susceptible *Salmonella* spp. recovered from chilled chicken available in the local market in Brazil. Of the recovered *Salmonella*, 23.1% of the of sulfonamide-sensitive *Salmonella* harbored *sul2* RG, 19.3% of the isolates that were sensitive or showed intermediate sensitivity to tetracycline carried the *tet(B)* gene, and 6.4% of the isolates that had sensitive or intermediate reaction to β-lactams had the bla_{CTX} gene (Pavelquesi *et al.*, 2023). Since some antimicrobial RGs are considered to be silent in vitro, these genes could transfer and spread through horizontal gene or become active in vivo, particularly under the selective effect of antimicrobial use, and could become active after being conveyed to a new host (Adesiji *et al.*, 2014; Deekshit *et al.*, 2012; Stasiak *et al.*, 2021; Yaqoob *et al.*, 2007).

Conclusion

This study revealed different prevalence patterns of *Salmonella* spp. recovered from raw chicken meat. Contamination of chicken with *Salmonella* could be unavoidable, since multiple contamination sources may harbor the microbe during farming, slaughtering, processing, and marketing. Nevertheless, the strategy of achieving *Salmonella*-free raw chicken meat should become a high priority because of the emergence of antimicrobial-resistant strains. *Salmonella* isolates exhibited a phenotypic resistance against beta-lactams, sulfonamide, tetracycline, and ciprofloxacin. The antimicrobial-RGs bla_{TEM}, *tet(B)*, *sul1*, and *sul2* were detected among *Salmonella* isolates with the genotypic results tracking phenotypic AST. As it was evident in the current study, raw broiler chicken could serve as a major source of MDR *Salmonella*. Strict control of the use of antibiotics is prudent, since the overuse or misuse of antimicrobials in animal husbandry may trigger a recurrent occurrence of MDR bacteria that could otherwise

result in higher morbidity and mortality rates because of *Salmonella* foodborne infections.

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Data Availability

Data will be provided upon request.

Author Contributions

Hamzah M. Al-Qadiri was responsible for conceptualization, data curation, investigation, methodology, project administration, and writing of the original draft. Murad A. Al-Holy was concerned with conceptualization, methodology, validation, and writing. Ayed M. Al-Abdallat was in charge of methodology and PCR verification. Amin N. Olaaimat supervised data analysis and drafting of the manuscript. Mohammed Saleh did data curation and formal analysis. Faris Ghalib Bakri was responsible for investigation, methodology, and resources. Sameeh M. Abutarbush monitored methodology and validation. Barbara A. Rasco was concerned with investigation and writing–review and editing. All authors have approved the content, fulfilled the authors' criteria, and contributed significantly to this research.

Conflict of Interest

The authors declare that they have no conflict of interest.

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