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RESEARCH ARTICLE

# Investigation and Molecular Assessment of Integron-Mediated Resistance Determinants in *Salmonella* Species from Broiler Chickens in Jordan

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**ABSTRACT**

PCR mapping of integrons is an effective epidemiological tool for studying the plasmids and transposons that mobilize antibiotic resistance determinants in *Salmonella*. Integrons play a crucial role in the dissemination of antimicrobial resistance among *Salmonella* species. This study investigated the occurrence and diversity of integron classes in isolates from broiler chickens in Jordan. A total of 185 isolates were screened by PCR and sequenced for integron-associated genes. The overall prevalence of the three integron classes was 87.56%, and only 23 out of 185 samples were negative for all three tested classes. Class 1 was detected in 81.1% of samples, while classes 2 and 3 were found at lower frequencies. Gene cassette arrays of class 1 integrons were detected in nearly all integron-positive isolates (93.8%). Sequence analysis identified several resistance cassettes, including *aadA1*, *aadA15*, *(aadA2–dfrA12)*, *dfrA5*, and *dfrA15*. Class 2 integrons were less frequent, found in only three isolates carrying cassette combinations of *(dfrA1+sat2+aadA1)* and *(dfrA1+sat2)*. Phenotypically, the highest resistance rates were observed against tetracycline, ampicillin, amoxicillin, chloramphenicol, and nalidixic acid. PCR screening also revealed a high prevalence of resistance genes, particularly *aadA*, *tet(A)*, and *floR*. Overall, these findings indicate an alarming level of multidrug resistance among *Salmonella* isolates from broiler chickens in Jordan, underscoring the widespread presence of integrons and resistance gene cassettes, and highlighting the urgent need to regulate antimicrobial use in poultry production.

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## INTRODUCTION

Poultry products represent an important transmission route for multidrug-resistant (MDR) bacteria to humans. Consumers can be exposed through contaminated meat, cross-contamination during food preparation, and environmental pathways such as contaminated water and soil. In addition, workers in farms, slaughterhouses, and processing facilities face direct occupational exposure (Syed Abu Thahir *et al.*, 2023). These risks highlight the need to monitor AMR determinants, such as integrons, in *Salmonella* circulating in broiler production systems.

Integrons are found in a wide diversity of clinical bacterial strains and environmental isolates, and can occur on chromosomes (Gillings, 2013), but most integrons that confer antibiotic resistance are found embedded within mobile elements such as plasmids and transposons. Such mobile integrons are grouped into classes based on the amino acid homologies of their integron-integrase gene, a tyrosine recombinase that catalyzes the acquisition and movement of gene cassettes within the integron. Of these, the Class 1 integron is the most predominant among *Enterobacteriaceae,* followed by Class 2 and Class 3, respectively (Ke *et al.*, 2011, Deng *et al.*, 2015).

Class 1 integrons contain a 5′ conserved segment (5′CS) that includes the gene for the site-specific integrase (*intI1*), a recombination site (*attI1*), and a 3′ conserved segment (3′CS), which includes *qacE*Δ1 that confers resistance to quaternary ammonium compounds and *sul1* that confers resistance to sulfamethoxazole (Carattoli, 2003). Integrons with this canonical structure are referred to as sul1-type class 1 integrons. However, sometimes class 1 integrons lack the 3′-CS, which are classified as atypical or preclinical class 1 integrons (Sáenz *et al.*, 2010). Class 2 integrons have an integrase gene (*intI2)* and a recombination site (*attI2*) (Rodríguez-

Minguela *et al.*, 2009) and are often associated with the *Tn7* family of transposons. Integron classes 1 and 2 contain an internal variable region that includes one or more resistance gene cassettes (CAS) with variable lengths and sequences that usually consist of a recombination site (attC) and a single-promoter-less gene. Under its 2018–2022 National Action Plan, Jordan achieved partial implementation of AMR surveillance (Momani *et al.*, 2025), connecting 42 laboratories across all governorates. National AMR reports revealed persistently high resistance rates. Jordan's poultry industry represents a major source of animal protein and economic activity, relying predominantly on intensive production systems. Previous reports have indicated frequent use of broad-spectrum antibiotics for disease prevention and growth promotion, often without strict regulatory oversight (Bazzi *et al.*, 2022). Such practices may contribute to the emergence and dissemination of antimicrobial-resistant bacteria within poultry farms. Although Jordan's animal health sector lacks specific infection-control actions in its AMR plan, the Ministry of Agriculture (MOA) has launched several important initiatives. These include stricter monitoring of veterinary drug providers, requiring prescriptions for antimicrobials, reviewing disease-control protocols, and establishing systems to track antibiotic use and residues in poultry farms and slaughterhouses (Momani *et al.*, 2025). In alignment with Jordan's NAP for combating AMR 2023-2025 under the objective of enhancing investment in AMR research, understanding the distribution of integrons and their associated resistance genes in *Salmonella* from broiler chickens is essential.

## MATERIALS AND METHODS

**Sample collection and isolation:** Two hundred samples were collected from chicken integrated companies and farms in northern Jordan between June and November 2023. Samples were obtained from multiple production points, including hatcheries, broiler farms, parent stock flocks, slaughterhouses, and retail markets. Samples were transported on ice and processed within 24 h to preserve viability. Isolation followed standard culture methods using selective enrichment and subculturing. Black colonies were further confirmed by PCR targeting the invasive encoding gene (*invA*) (Upadhyay *et al.*, 2010). The pure confirmed isolates were stored at - 80°C in Tryptic Soy Broth with 15% glycerol for long-term storage. Template DNA was prepared using 500µL of TSB culture by the boiling-freezing method (De Medici *et al.*, 2003).

**Antimicrobial susceptibility tests:** Antimicrobial susceptibility testing was performed to determine the resistance profiles of the *Salmonella* isolates and to assess the burden of multidrug resistance in broiler-associated strains.

*Salmonella* isolates were tested against 14 different antimicrobials according to the CLSI guidelines (Lewis and James, 2022), including drugs commonly used in poultry production and those critical for human medicine, enabling assessment of resistance patterns with both veterinary and public health relevance.

**Detection of Antimicrobial Resistance genes (ARGs):** The selected ARG panel targeted resistance determinants that are most associated with *Salmonella* circulating in poultry production systems and that confer resistance to antibiotics widely used in both veterinary practices. Which included ampicillin and amoxicillin resistance genes (*blaTEM* and *blaOXA*)(Olesen *et al.*, 2004, Dallenne *et al.*, 2010); chloramphenicol/ florfenicol-resistance genes (*catA1* and *floR*)(Aarestrup *et al.*, 2003, Doublet *et al.*, 2004); tetracycline-resistance genes [*tet(A), tet(B), tet(C), tet(D), tet(E) and tet(G)*](Sáenz *et al.*, 2004, Adesiji *et al.*, 2014, Dessie *et al.*, 2013) because tetracyclines remain one of the most heavily used antimicrobials in poultry production globally, streptomycin-resistance genes (*aadA, strA, and strB*)(Scholz *et al.*, 1989, Doosti *et al.*, 2017, Vuthy *et al.*, 2017), gentamicin-resistance genes [*aac (3)-IIa, aph (3) IIa,* and *aac6*](Ma *et al.*, 2007), neomycin-resistance gene (*aph (3)-I)*(Melano *et al.*, 2003), and kanamycin resistance gene *(aph(3)-Ia*) (Sandvang and Aarestrup, 2000).

Screening was performed on all samples to identify the genetic determinants underlying phenotypic resistance and to assess whether integron carriage correlated with the presence of specific resistance determinants, strengthening the interpretation of AMR patterns.

**Detection of integron classes 1, 2 and 3:** To determine the prevalence of class 1–3 integrons among the isolates, *a* PCR amplification targeting integron-integrase genes (*intI1, intI2, intI3)* was made (Shibata *et al.,* 2003). In addition to integron class detection, PCR screening targeted sulfonamide resistance genes (*sul1, sul2, sul3*) and the quaternary ammonium compound tolerance marker (*qacEΔ1*, commonly found adjacent to *sul1*). These genes were selected because *sul1* often co-locates with the 3′ conserved segment of class 1 integrons, and *qacEΔ1-sul1* indicates potential co-selection by disinfectants. PCR essays were performed using published primer sets (Sandvang *et al.*, 1997, Peirano *et al.*, 2005, Perreten and Boerlin, 2003). Presence/absence data were recorded for each isolate and used in downstream correlation analyses with AMR, ARG, and integron class.

**Identification of gene cassettes:** To identify the gene cassettes circulating in broiler-associated *Salmonella* in Jordan. The presence of gene cassettes was assessed through PCR using the gene cassette array of class 1 primers (CAS1) (Lévesque *et al.*, 1995) and the gene cassette array of class 2 primers (CAS2) (White *et al.*, 2000). The PCR products of gene cassettes were extracted from the gel and sequenced. Identification was made using NCBI Blast and the Staramr tool inside the IntegronFinder program that scans genome assemblies against ResFinder, PlasmidFinder, and PointFinder databases (Néron *et al.*, 2022, Bharat *et al.*, 2022). Phylogenetic analysis was performed in MEGA12 (Kumar *et al.*, 2024) using the Maximum Likelihood method with the Kimura 2-parameter model on 59 nucleotide sequences. The best initial tree was chosen by comparing Neighbor-Joining and Maximum Parsimony topologies. The final dataset contained 1,962 aligned positions.

## RESULTS

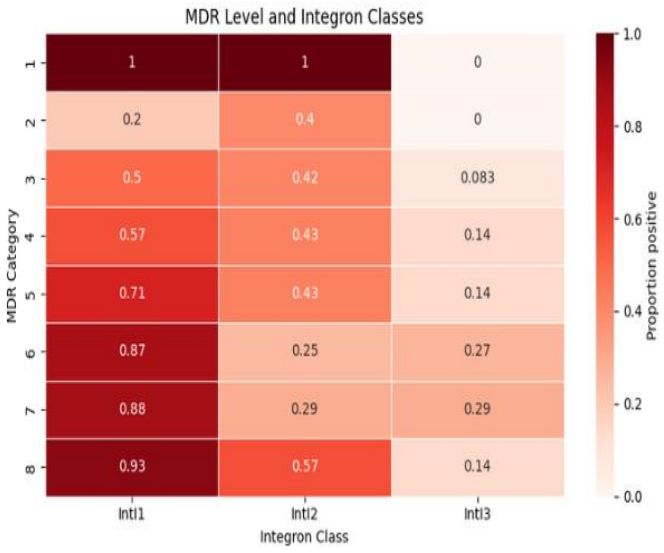
**Percentage of *Salmonella* isolates in broiler chicken samples:** A total of 185 samples out of 200 (92.5%) were positive for *Salmonella*. Fifteen samples were negative after the *invA* PCR. All PCR amplifications included negative and positive controls. By chance, all identified-source samples were *Salmonella* positive, 74 broiler farms, 7 hatcheries, 10 parent stock, 12 retail markets, and 47 slaughterhouses. Thus, no association could be built between the occurrence of *Salmonella* and food production points. The remaining 35 positive samples were from unidentified sources.

**Antimicrobial susceptibility test:** Disc diffusion tests were done for 185 *Salmonella* isolates. The highest resistance was against tetracycline (90.3%), ampicillin (85.9%), amoxicillin, chloramphenicol (73.5%), and nalidixic acid (71.4%), respectively (Table 1). Most isolates were susceptible to norfloxacin (87.6%), trimethoprim-sulfamethoxazole (77.8%), and ceftriaxone (67.0%). Antimicrobials were classified into 8 classes, including Penicilins (AMP, AML), Cephalosporins (CTX, CRO, CAZ), Tetracyclines (T), Phenicol (C), Fluoroquinolones (CIP, NOR), Quinolones (NA), Aminoglycosides (S, CN, TOB), and Folate-pathway inhibitors (SXT). By analyzing MDR isolates, samples resistant to 1 class: one sample, 2 classes: 5 samples, 3 classes: 12 samples, 4 classes: 7 samples, 5 classes: 14 samples, 6 classes: 60 samples, 7 classes: 72 samples, 8 classes: 14 samples. Only six samples were not MDR (Table 2).

**Antimicrobial resistance genes profile:** All positive samples were screened for 18 genes encoding different antimicrobial resistance that are not related to integrons (Table 3). A high percentage of strains exhibited resistance genes *aadA* (79.5%), *tet(A)* (78.4%), and *floR* (67%). In all cases, the presence of the resistance gene was higher in phenotypically resistant isolates than in susceptible isolates, and the absence of the resistance gene was greater in susceptible isolates than in resistant isolates (Table 4). The strongest co-occurrence (P>0.5) of ARG was between *catA1* and *strA*, between *strA* and *strB*, between *strB* and *aph-3-III*, and between *aph-3-IIa* and *aph-3-III* (Data not shown).

**Integron classes and genes:** The overall occurrence of the three classes was 87.6%. Only 23 samples (12.4%) were negative for all three classes of integrons (Table 5). Class 1 had the highest rate (*intI1*: 81.1%), followed by class 2 (*intI2*: 33%) and class 3 (*intI3*: 23.2%), respectively. Rates of occurrence for *sul 1*, 2, 3, and *qacEΔ1-sul1* were 73.5%, 49.5%, 32.4% and 68.6% respectively. The majority of *int1* positive samples fall into MDR categories, being resistant to 6, 7, and 8 classes (MDR ≥6: 129/150 isolates (86%)), and negative isolates rarely show high MDR (MDR ≥6**:** 10/35 isolates (28.5%)). Class 2 and 3 integrons did not show a positive association with MDR (Table 1). In fact, higher MDR levels were predominantly observed in IntI2- and IntI3-negative isolates. In all cases, Integron presence was higher within resistant isolates than within susceptible isolates, and Integron absence within susceptible isolates was higher than within resistant isolates (Table 6). The *qacEΔ1-sul1* gene was found in 106/150 positive *intI1* samples, and in 98/136 *sul1* positive samples.

a




b

| | | IntI1 | | IntI2 | | IntI3 | | Co-occurrence | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | (-) | (+) | (-) | (+) | (-) | (+) | One | Two | Three |
| AMR classes | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| | 2 | 4 | 1 | 3 | 2 | 5 | 0 | 1 | 1 | 0 |
| | 3 | 6 | 6 | 7 | 5 | 11 | 1 | 1 | 4 | 1 |
| | 4 | 3 | 4 | 4 | 3 | 6 | 1 | 2 | 3 | 0 |
| | 5 | 4 | 10 | 8 | 6 | 12 | 2 | 5 | 5 | 1 |
| | 6 | 8 | 52 | 45 | 15 | 44 | 16 | 29 | 21 | 4 |
| | 7 | 9 | 63 | 51 | 21 | 51 | 21 | 39 | 24 | 6 |
| | 8 | 1 | 13 | 6 | 8 | 12 | 2 | 6 | 7 | 1 |
| Total | | 35 | 150 | 124 | 61 | 142 | 43 | 83 | 66 | 13 |

**Fig. 1:** Integron classes detected by PCR targeting *intI1, intI2, and intI3* association with multi-drug resistance (MDR) phenotype detected by disc diffusion test in *Salmonella* isolates. Most class 1 samples fall into high MDR categories, being resistant to 6, 7, and 8 classes, while class 2 and class 3 were more correlated with lower degrees of MDR. a. Correlation heat maps made by Python in Google Colab, using pandas, seaborn, and matplotlib.plot libraries. A darker red color resembles a stronger correlation. IntI1 has stronger correlations with MDR degrees of 6 and 7. While Int2 and IntI3 have weaker correlations and lower degrees of MDR. b. A table showing positive and negative samples of each integron class, and the co-occurrence of integron classes with MDR level (1-8). One: one class, either 1, 2, or 3. Two: co-occurrence of two classes together. Three: co-occurrence of all three classes. More resistance and higher MDR level of IntI1 positive samples in comparison to negative samples. While InI2 and IntI3 don't show this association. Co-occurrence of two integron classes has relatively higher MDR than three classes, while only one class shows the highest MDR level and count.

**Table 1:** Antimicrobial susceptibility test of disc diffusion test, compared between integron-positive and integron-negative samples. Integron-positive isolates are positive for at least one of the three integron classes detected by PCR.

| Antimicrobial | Integron-positive (Total:162) | | | Integron-negative (Total:23) | | | Total Resistance |
|---|---|---|---|---|---|---|---|
| | I | R | S | I | R | S | (Total:185) |
| NA (30 µg) | (13.58%) 22 | (74.07%) 120 | (12.34%) 20 | (17.39%) 4 | (52.17%) 12 | (30.43%) 7 | (71.35%) 132 |
| CIP (5 µg) | (66.66%) 108 | (26.54%) 43 | (06.79%) 11 | (60.86%) 14 | (13.04%) 3 | (26.08%) 6 | (24.86%) 46 |
| SXT (23.75+1.75 µg) | (01.23%) 2 | (23.45%) 38 | (75.30%) 122 | (00.00%) 0 | (04.34%) 1 | (95.65%) 22 | (21.08%) 39 |
| NOR (5 µg) | (03.08%) 5 | (11.11%) 18 | (85.80%) 139 | (00.00%) 0 | (00.00%) 0 | (100%) 23 | (09.72%) 18 |
| AML (20 µg) | (05.55%) 9 | (76.54%) 124 | (17.90%) 29 | (04.34%) 1 | (52.17%) 12 | (43.47%) 10 | (73.51%) 136 |
| S (10 µg) | (12.34%) 20 | (84.56%) 137 | (03.08%) 5 | (17.39%) 4 | (56.52%) 13 | (26.08%) 6 | (81.08%) 150 |
| CTX (30 µg) | (08.64%) 14 | (37.65%) 61 | (53.70%) 87 | (08.69%) 2 | (21.73%) 5 | (69.56%) 16 | (35.67%) 66 |
| CRO (30 µg) | (05.55%) 9 | (30.24%) 49 | (64.19%) 104 | (04.34%) 1 | (08.69%) 2 | (86.95%) 20 | (27.56%) 51 |
| C (30 µg) | (00.61%) 1 | (77.16%) 125 | (22.22%) 36 | (00.00%) 0 | (47.82%) 11 | (52.17%) 12 | (73.51%) 136 |
| CAZ (30 µg) | (14.19%) 23 | (27.16%) 4 | (58.64%) 95 | (08.69%) 2 | (26.08%) 6 | (65.21%) 15 | (27.02%) 50 |
| T (30 µg) | (00.61%) 1 | (95.06%) 154 | (04.32%) 7 | (00.00%) 0 | (56.52%) 13 | (43.47%) 10 | (90.27%) 167 |
| CN (10 µg) | (05.55%) 9 | (36.41%) 59 | (58.02%) 94 | (00.00%) 0 | (21.73%) 5 | (78.26%) 18 | (34.59%) 64 |
| TOB (10 µg) | (16.04%) 26 | (35.18%) 57 | (48.76%) 79 | (21.73%) 5 | (26.08%) 6 | (52.17%) 12 | (34.05%) 3 |
| AMP (10 µg) | (00.61%) 1 | (88.88%) 144 | (10.49%) 17 | (04.34%) 1 | (65.21%) 15 | (30.43%) 7 | (85.94%) 159 |

R: resistant isolates, I: intermediate isolates, S: sensitive isolates.

**Table 2:** Profiling of the six samples that were not MDR. Showing the source of each sample, antimicrobial resistance genes (ARG) tested positive by PCR, antimicrobial resistance phenotype (AMR) tested by Disc-diffusion test, integron class, and integron-related genes detected by PCR.

| Sample | Source | ARG | AMR | Integrons | Integron-related |
|---|---|---|---|---|---|
| 5 | Drag swab, Parent stock | *blaOXA, tet(D)* | NA, TOB | None | None |
| 167 | Internal organ, Broiler farms | *blaTEM, aph-3-I* | AML, CN, TOB | IntI1, IntI2 | *qacE1-sul1, sul3* |
| 175 | Internal organ, Broiler farm | None | AML, S | None | *sul1, sul2, sul3* |
| 177 | Fresh carcass, slaughterhouse | None | AMP, S | None | *sul3* |
| 188 | Drag swab, Parent stock | *blaOXA, tet(A), tet(D), aadA, aac3-IIa* | T, S | IntI2 | *qacE1-sul1, sul3* |
| 195 | Drag swab, Broiler farm | *aph-3-IIa* | S, CN, TOB | IntI1. IntI2 | *qacE1-sul1, sul2, sul3* |

**Table 3:** Antimicrobial resistance genes (ARG) detected by PCR amplification and their percentage out of the total sample count

| Encoding for | ARG | Presence (Total=185) | Percent |
|---|---|---|---|
| Ampicillin | *blaTEM* | 72 | 38.9% |
| | *blaOXA* | 9 | 4.9% |
| Chloramphenicol | *catA1* | 7 | 3.8% |
| | *floR* | 124 | 67% |
| Tetracycline | *tet(A)* | 145 | 78.4% |
| | *tet(B)* | 5 | 2.7% |
| | *tet(C)* | 25 | 59.5% |
| | *tet(D)* | 110 | 59.5% |
| | *tet(G)* | 3 | 1.6% |
| | *tet(E)* | 33 | 17.8% |
| Streptomycin | *strA* | 24 | 13% |
| | *strB* | 19 | 10.3% |
| | *aadA* | 147 | 79.5% |
| Gentamicin | *aac-3-IIa* | 40 | 21.6% |
| | *aph-3-IIa* | 61 | 33% |
| | *aac6* | 0 | 0 % |
| Neomycin | *aph-3-I* | 64 | 34.6% |
| Kanamycin | *aph-3-Ia* | 30 | 16.2% |

ARG: antimicrobial resistance gene

**Integron gene cassettes:** Potential integron cassettes were found in 152 samples out of the 162 integron-positive isolates (93.82%), and cassette 2 was found in only 4 samples (2.46%). Some samples showed an approximate size larger than 1500 bp, between 1000 bp and 1500 bp, 700 bp, 600 bp, and 500 bp. To determine these cassettes' identity, 52 representative samples were extracted from the gel and sent for sequencing (Macrogen). Sequence analysis revealed *aadA1, (aadA2-dfrA12)*, *dfrA5,* and *dfrA15* gene cassettes, which encode for aminoglycoside adenylyl transferases and dihydrofolate reductases. Gene cassette arrays of class 2 integrons were detected in only three samples. Two samples, approximately 1500 bp, were found to have dfrA1+sat2+aadA1 and dfrA1+sat2, respectively. One sample, approximately 1000 bp, contained estX+sat2+aadA1, resembling putative esterase, streptothricin acetyltransferase, and aminoglycoside adenyltransferase, respectively (Fig. 2). Samples from the NCBI database that were used as references for *aadA1*: GQ924774.1 *Enterobacter cloacae* strain, FJ855126.1 *Escherichia coli* from beef cattle, KR028105.1 *Klebsiella pneumoniae*, MG757587.1 *Pseudomonas aeruginosa* from a Human rectal swab. References for *aadA2 & dfrA12*: ON206977.1 *E. coli* from Duck feces, JN108889.1 *Klebsiella pneumonia*. For *dfrA15*: AB21935.1 *Vibrio cholerae*, MW295858.1 *Enterobacter cloacae*. For *dfrA5*: JN651401.1 *Shigella flexneri*, DQ133160.1 *Salmonella enterica* Subsp. Enterica. Two representative samples of *aadA1* were submitted to NCBI GenBank, sample 37 (PV254830.1) and sample 128 (PV254831.1).

**Table 4**: Statistical analysis of cross-tabulation between genotypic and related phenotypic antimicrobial resistance tested by PCR and disc diffusion test, respectively, using Chi-Square, 95% Confidence interval

| *Antimicrobial* | *ARG* | *R, (+)* | *R, (-)* | *S, (+)* | S, (-) | P-value** |
|---|---|---|---|---|---|---|
| Amoxicillin | *blaTEM* | (47.26%) 69/146 | (52.73%) 77/146 | (07.69%) 3/39 | (92.30%) 36/39 | <0.001 b*** |
| Ampicillin | *blaTEM* | (42.85%) 69/161 | (57.14%) 92/161 | (12.5%) 3/24 | (87.5%) 21/24 | 0.004 b** |
| Chloramphenicol | *floR* | (86.86%) 119/137 | (13.13%) 18/137 | (10.41%) 5/48 | (89.58%) 43/48 | <0.001 a*** |
| Tetracycline | *tet(A)* | (85.11%) 143/168 | (14.88%) 25/168 | (11.76%) 2/17 | (88.23%) 15/17 | <0.001 b*** |
| | *tet(D)* | (62.50%) 105/168 | (37.5%) 63/168 | (29.41%) 5/17 | (70.58%) 12/17 | 0.008 a** |
| Streptomycin | *aadA* | (83.33%) 145/174 | (16.66%) 29/174 | (18.18%) 2/11 | (81.81%) 9/11 | <0.001 b*** |
| Gentamycin | *aac (3)-IIa* | (34.24%) 25/73 | (65.75%) 48/73 | (13.39%) 15/112 | (86.60%) 97/112 | <0.001 a*** |
| | *aph (3)-IIa* | (53.42%) 39/73 | (46.57%) 34/73 | (19.64%) 22/112 | (80.35%) 90/112 | <0.001 a*** |

ARG: antimicrobial resistance gene, R: resistant isolates, S: susceptible isolates, **P-value: Chi-square value for the difference between genotypic (one gene) and phenotypic (with the specifically related phenotype) antimicrobial resistance (R+I), a: chi-square (two-sided), b: Fischer-Exact test was performed instead of when variables had expected count less than 5 in one or more cells, *P≤0.05, **P≤0.01, ***P≤0.001.


**Fig. 2:** Maximum Likelihood method and Kimura's (1980) for 43 *aadA1*, 2 *dfrA12+aadA2*, 3 *dfrA5*, and 1 *dfrA15* from this study, which are *Salmonella* from Broiler Chicken, and 10 references from NCBI GenBank. Evolutionary analyses were conducted in MEGA12.

**Table 5:** Integron classes and related genes detected by PCR amplification and their percentage out of the total sample count

| Class | Gene | Encode for | Prevalence (Total=185) | Negative | Percent |
|---|---|---|---|---|---|
| Class 1 | *intI1* | integrase gene | 150 | 35 | 81.1% |
| | *sul1* | sulphonamide resistance | 136 | 49 | 73.5% |
| | *qacEΔ1-sul1* | The entire 3′CS contains quaternary ammonium resistance | 127 | 58 | 68.6% |
| Class 2 | *intI2* | integrase | 61 | 124 | 33% |
| and transposon Tn7 | *sul2* | sulphonamide resistance | 92 | 93 | 49.7% |
| Class 3 | *intI3* | integrase | 43 | 142 | 23.2% |
| And Tn*5053*-family transposon | *sul3* | sulphonamide resistance | 60 | 125 | 32.4% |

**Table 6:** Statistical analysis of the association between integron classes and antimicrobial susceptibility detected by the Disc-diffusion test using Chi-Square, 95% Confidence interval

| Integron class | Antimicrobial | Int (+), R | Int (-), R | Int (+), S | Int (-), S | **P-value |
|---|---|---|---|---|---|---|
| IntI1 | Ciprofloxacin (CIP) | (83.33%) 140/168 | (16.66%) 28/168 | (58.82%) 10/17 | (41.17%) 7/17 | 0.014 a* |
| | Trimethoprim- sulfamethoxazole (SXT) | (92.68%) 38/41 | (07.31%) 3/41 | (77.77%) 112/144 | (22.22%) 32/144 | 0.040 b* |
| | Norfloxacin (NOR) | (100%) 23/23 | (00.00%) 0/23 | (78.39%) 127/162 | (21.60%) 35/162 | 0.006 b** |
| | Amoxicillin (AML) | (84.93%) 124/146 | (15.06%) 22/146 | (66.66%) 26/39 | (33.33%) 13/39 | 0.010 a** |
| | Streptomycin (S) | (83.90%) 146/174 | (16.09%) 28/174 | (36.36%) 4/11 | (63.63%) 7/11 | <0.001 b*** |
| | Chloramphenicol (C) | (86.86%) 119/137 | (13.13%) 18/137 | (64.58%) 31/48 | (35.41%) 17/48 | <0.001 a*** |
| | Tetracycline (T) | (85.11%) 143/168 | (14.88%) 25/168 | (41.17%) 7/17 | (58.82%) 10/17 | <0.001 a*** |
| | Ampicillin (AMP) | (83.85%) 35/161 | (16.14%) 26/161 | (62.50%) 15/24 | (37.50%) 9/24 | 0.013 a* |
| IntI2 | Trimethoprim- sulfamethoxazole (SXT) | (70.73%) 29/41 | (29.26%) 12/41 | (22.22%) 32/144 | (77.77%) 112/144 | <0.001 a*** |
| | Amoxicillin (AML) | (27.39%) 40/146 | (72.60%) 106/146 | (53.84%) 21/39 | (46.15%) 18/39 | 0.002 a** |
| | Chloramphenicol (C) | (25.54%) 35/137 | (74.45%) 102/137 | (54.16%) 26/48 | (45.83%) 22/48 | <0.001 a*** |
| IntI3 | Cefotaxime (CTX) | (31.70%) 6/82 | (68.29%) 56/82 | (16.50%) 17/103 | (83.49%) 86/103 | 0.015 a* |
| | Ceftriaxone (CRO) | (32.78%) 20/61 | (67.21%) 41/61 | (18.54%) 23/124 | (81.45%) 101/124 | 0.031 a* |
| | Chloramphenicol (C) | (27.00%) 37/137 | (72.99%) 100/137 | (12.50%) 6/48 | (87.50%) 42/48 | 0.041 a* |
| | Ceftazidime (CAZ) | (34.66%) 26/75 | (65.33%) 49/75 | (15.45%) 17/110 | (84.54%) 93/110 | 0.002 a** |

Int(+): integron positive isolates, Int(-): integron negative isolates, R: resistant isolates, S: susceptible isolates, **P-value: Chi-square value for the difference between having detected as positive for one of the three classes of integrons and phenotypic antimicrobial resistance (R+I) tested by disc diffusion test, a: chi-square (two-sided), b: Fischer-Exact test was performed instead when variables had expected count less than 5 in one or more cells, *P≤0.05, **P≤0.01, ***P≤0.001.

## DISCUSSION

Jordan's poultry industry represents one of the country's fastest-growing livestock sectors, supplying most of the domestic meat consumption. However, recent studies in Jordan have reported high levels of antimicrobial residues in poultry meat (Awaisheh *et al.*, 2019). Where extensive multidrug resistance is strongly associated with the prophylactic and therapeutic application of antimicrobials without veterinary guidance, the use of groundwater and the proximity of farms to neighboring poultry operations were found as two risk factors. (Ibrahim *et al.*, 2019). Jordan's National Action Plan on Antimicrobial Resistance (2018–2022) aligned with the Global Action Plan on Antimicrobial Resistance, employing a One Health framework (Momani *et al.*, 2025), highlighted the limited engagement of animal and environmental sectors, and the lack of molecular AMR research. This study addresses a critical knowledge gap by characterizing the prevalence of integrons and their associated gene cassettes in *Salmonella* isolates from Jordanian broilers, thereby improving our understanding of local drivers of antimicrobial resistance.

There has been a significant increase in MDR *Salmonella* recovered from retail chickens over time (Zhao *et al.*, 2020). Our results for antibiotic susceptibility showed that only six samples were resistant to less than three classes of antibiotics. All other 179 samples were MDR. Our isolates exhibited very high resistance to tetracycline (90%), ampicillin (86%), amoxicillin (74%), chloramphenicol (74%), and nalidixic acid (71%), which is similar to the results in Saudi Arabia (Alzahrani *et al.*, 2023) but exceeding levels reported in Kenya (Langata *et al.*, 2019), China (Chen *et al.*, 2019), and Senegal (Fall-Niang *et al.*, 2019). In contrast, resistance to ciprofloxacin (25%) and gentamicin (35%) remained lower than rates reported in other regional studies (Samia *et al.*, 2021, Xu *et al.*, 2020). Overall, our findings indicate a more extensive AMR burden compared with many previously published datasets.

Significant associations were also observed between genotypes and phenotypes of antimicrobial resistance. In this study, *Salmonella* isolates had a close percentage of *tet(A)* (78.4%), a higher percentage of *tet(C)* (59.5%), and a lower percentage of *tet(B)* (2.7%) and *blaTEM* (38.9%) compared with findings in Bangladesh (Das *et al.*, 2022) and *floR* gene was observed in 67% of our tested samples. While *floR*, *tet(A)*, *tet(B)*, and *tet(G)* genes were absent in pathogenic *Salmonella* isolated from the chicken droppings in Nigeria (Shittu *et al.*, 2022). None of our samples were positive for *aac(6')*, similar to other previous findings (Samia *et al.*, 2021). There was a relatively high percentage of *aadA* (79.5%), which is similar results in Indonesia (Takaichi *et al.*, 2022).

Class 1 integron negative samples showed lower MDR for 6 classed or more 10/35 isolates (28.5%), while class 1 positive showed higher MDR 129/150 isolates (86%) $P<0.001$, suggesting that class 1 integrons are a key driver of accumulating resistance across many antimicrobial classes and integron-mediated gene acquisition plays a major role in shaping resistance patterns in broiler-associated *Salmonella*. Class 2 and class 3 integrons showed no positive correlation with MDR, and in our data, MDR was mainly detected among isolates lacking IntI2 and IntI3. The MDR patterns observed in our isolates are consistent with the review's conclusion that excessive antimicrobial use in poultry production exerts strong selective pressure, facilitating the emergence of MDR strains (Al-Tammemi *et al.*, 2025). This pattern is consistent with previous reports showing that class 2 integrons typically carry a conserved cassette (dfrA1–sat2–aadA1) with a limited resistance range, which is similar to the gene cassettes found in the three samples sequenced for CAS2, while class 3 integrons are rare in *Salmonella* (Tayh *et al*., 2025), even though our rate of classes 2 and 3 is considered relatively higher than rates found in other studies. Therefore, MDR in our isolates is likely driven by class 1 integrons and additional plasmid-borne or chromosomal AMR determinants rather than class 2 or 3 integrons.

The frequent detection of *sul1* among our *Salmonella* isolates, particularly those positive for class 1 integrons, suggests integron-mediated dissemination of sulfonamide resistance in the broiler sector. The presence of *qacEΔ1* (68.6%) is noteworthy because it confers tolerance to quaternary ammonium disinfectants (Chen *et al.*, 2023) and can promote co-selection of linked antibiotic resistance genes, facilitate ARG transfer, and it's widely present in environmental samples (Han *et al.*, 2019), *qacEΔ1*was significantly associated with *blaOXA, aadA, aph-3-IIaand aph-3-I* in our samples; this finding underscores the need to review farm disinfection practices and evaluate whether routine biocide use may unintentionally select for MDR strains. PCR screening identifies gene presence but not genomic location or expression; confirmation of physical linkage and assessment of biocide tolerance would require WGS and phenotypic assays, respectively.

A very high proportion of integron-positive *Salmonella* isolates (93.8%) carried amplifiable gene cassette regions, indicating that integrons are widespread and actively contributing to resistance gene acquisition. This aligns with reports showing that class 1 integrons commonly harbor cassette arrays in food-animal *Salmonella* (Li *et al.*, 2021). The dominant cassettes identified *aadA1*, *aadA2–dfrA12*, *dfrA5*, and *dfrA15*, consistent with well-established cassette families globally enriched in poultry-associated *Salmonella* (Meng *et al.*, 2017). Importantly, all CAS2 carried *dfrA1–sat2–aadA1* or variants thereof, which is characteristic of Tn7-associated class 2 integrons known for having a fixed, limited cassette repertoire (Tayh *et al.*, 2025). Overall, the dominance of class 1 integron cassettes and the limited, conserved nature of class 2 cassettes reflect a resistance landscape driven primarily by class 1 integron diversity, aligning with global observations in poultry *Salmonella* populations.

**Conclusions:** Our findings reinforce the urgent need for enhanced antimicrobial stewardship and routine surveillance in Jordan's poultry sector, AMU regulation, vaccination programs, and integrated One Health surveillance that links poultry farms, veterinary practices, food processing facilities, and human health data in line with global recommendations highlighted in recent One Health AMR evaluations. Investments in laboratory

capacity, genomic surveillance, and food safety monitoring will further support early detection and control of resistant strains.

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