# Detection of antimicrobial resistance genes in *Salmonella* isolates in the Region Laguna, Mexico

# Detección de genes de resistencia antimicrobiana en aislados de *Salmonella* en la Región Laguna, México

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**ABSTRACT.** The Region Laguna is known for its poultry farming. This activity carries zoonotic risks. One of these risks is *Salmonella*, the leading foodborne pathogen responsible for a significant proportion of deaths worldwide. In this study, the specific prevalence of *Salmonella* in broiler chicken feed, drinkers and chicken manure samples on farms was determined, as well as the presence of genes that confer resistance to antibiotics. A total of 240 samples were analyzed, of which 80 samples of them tested positive for *Salmonella*, and the only serotype identified was *S*. Thyphimurium. The most frequently observed genetic profile of antibiotic resistance, at 26.25%, was the tetra-resistant ACSSu. Only three of the isolates carried all six antibiotic resistance genes, so they can be considered mult-resistant. The results indicate that, in poultry environments in the Region Laguna, there are strains of *Salmonella* with patterns of multi- antibiotic resistance.

**Keywords:** Broiler chicken, foodborne pathogens, molecular methods, PCR-RFLP, *Salmonella*.

**RESUMEN.** La Región Laguna es conocida por su avicultura. Esta actividad conlleva riesgos zoonóticos. Uno de estos riesgos es la salmonela, responsable de una proporción significativa de muertes en todo el mundo. En este trabajo, se determinó la prevalencia específica de *Salmonella* en muestras de alimento para pollo, agua de bebederos y estiércol de pollo en granjas; así como la presencia de genes que confieren resistencia a antibióticos. Se analizaron un total de 240 muestras, de las cuales 80 resultaron positivas para *Salmonella*, siendo el único serotipo identificado *S*. Thyphimurium. El perfil genético de resistencia a antibióticos más frecuentemente observado, con un 26.25%, fue el tetraresistente ACSSu. Solo tres de los aislados eran portadores de los seis genes de resistencia a antibióticos, por lo que se pueden considerar multiresistentes. Los resultados indican que, en los entornos avícolas de la Región Laguna, hay cepas de *Salmonella* con patrones de multiresistencia a antibióticos.

**Palabras clave:** Pollo de engorda, patógenos trasmitidos por alimentos, métodos moleculares, PCR-RFLP, *Salmonella*.

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# INTRODUCTION

*Salmonella* is one of the most common foodborne pathogens worldwide (Zizza *et al*. 2024) and with more than 2 600 serotypes (Deng *et al*. 2025). Foodborne illnesses pose a significant risk to public health globally. *Salmonella* is one of the most common and prevalent microbial strains, responsible for zoonotic infections in both aquatic and terrestrial animals, including humans and also causing financial burdens from the costs of disease prevention and treatment (Mohammad-Maruf and Md-Saydur 2024). More than 1 000 000 non-typhoidal infections are reported annually in the US, 100 000 in Europe, and approximately 70 000 cases in Mexico (Contreras-Soto *et al*. 2019). Among *Salmonella* serotypes, those causing zoonotic diseases are considered the most important, as they are among the causes of foodborne diseases (Shaji *et al*. 2023). *Salmonella enterica* serovars Enteritidis and Typhimurium are among the most prevalent in poultry, serving as significant sources of *Salmonella*-related foodborne illnesses in humans. Poultry may contract *Salmonella* through contaminated feed, water, or environmental exposure, as well as via interaction with other infected birds or animals and through vertical transmission from breeding hens to their offspring. Consequently, effective control measures and reduction of contamination at the farm level are essential for mitigating disease impact and safeguarding public health and food safety (Ali *et al*. 2025). The poultry environment is recognized as one of the main places where *Salmonella* can be found, and the timely detection of this pathogen is crucial to avoid contamination between flocks and farms (Collineau *et al*. 2020). *Salmonella*'s importance in poultry environments is that it can be present in the intestinal tract and birds' reproductive system and excrement (Liu *et al*. 2023). Then it could be expelled in the excreta and the eggs, and therefore be found on the floor, the litter of posture and drinking fountain (Frederick and Huda 2011). Due to the risk that birds can contract pathogens, a common practice in poultry production is the indiscriminate use of antimicrobials for the prevention of diseases (Sakeena *et al*. 2018). Misuse of antibiotics causes antibiotic resistance (AR) in pathogens (Pandey *et al*. 2024). An example of such pathogens is *Salmonella* Typhimurium DT104, a multi-resistant strain (DT104 MDR) to the antibiotics ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline (ACSSuT); antibiotic resistance is on the rise globally, and if current trends continue, there could be up to 10 million annual AR-associated deaths from a variety of untreatable infections by 2050 (White and Hughes 2019). In Mexico, previous reports have mentioned the presence of multidrug-resistant *S*. Typhimurium in pig carcasses and ground beef in central Mexico (Talavera *et al.* 2011, Campos-Granados 2023). For both the public health sector and the livestock sector, multiple antibiotic resistance is considered a global problem due to the transfer of multiple resistance strains through food (Little *et al*. 2008). The *Salmonella*-based surveillance laboratory analysis (including serotypes and resistance control) is an essential component in preventing this bacterium. Because there is no information on antibiotic resistance of *Salmonella* in poultry environments of the Region Laguna in Mexico, the objectives of this cross-sectional study were to determine the specific prevalence of *Salmonella* in samples from poultry environments and the presence of genes associated with resistance to antibiotics.

## MATERIAL AND METHODS

### Sample collection and bacteriological Procedures

Two hundred and forty samples were collected from five broiler farms (A, B, C, D, E), each farm with four houses (1, 2, 3, 4) located at Region Laguna, Mexico, during the spring-summer of 2023. On each farm three types of samples were collected: Broiler litter (L), feed (F), and water of drinker (W) with four samples of each type of sample. The Broiler litter samples (100 g) were taken randomly from the collection area, poultry feed samples (100 g) were taken directly from the container and water samples (100 mL) were taken directly from the drinker's water. Enrichment and microbiological analyses were performed according to the method described in the U.S. Department of Agriculture, Food Safety and Inspection Service Microbiology Laboratory Guidebook (USDA-FSIS MLG 4.15, 2024). Briefly, 25 mL or 25 g of each sample were transferred to 225 mL of pre-enrichment buffered peptone water (BPW) (DIFCO, USA) and incubated (Incubadora, FELISA, MÉXICO) at 35 °C for 24 h. After that, 1.0 ± 0.05 mL from the BPW was transferred into 10 mL of tetrathionate broth (DIFCO, USA) and 0.1 ± 0.02 mL into 10 mL of modified Rappaport-Vassiliadis broth (DIFCO, USA), which were then incubated at 42 ± 0.5 °C for 24 ± 2 h in water bath with agitation (SHELLAB, USA). Next, both enrichments were streaked onto brilliant green sulfa (DIFCO, USA) and double-modified lysine iron agar (MCD.LAB, MÉXICO) plates and incubated at 35 ± 2 °C for 18 to 24 h. Plates were then examined for the presence of suspect colonies meeting the description of *Salmonella* colonies, which were subjected to biochemical test of Triple Sugar Iron Agar (TSI) (BIOXON, MÉXICO) and Lysine Iron Agar (LIA) (BIOXON, USA) incubated at 35 °C for 18 to 24 h.

### Confirmation of *Salmonella* genus by PCR

The molecular diagnosis for the presence of *Salmonella* was made by PCR. To increase the number of *Salmonella* cells 25 mL or 25 g of each sample was transferred to 225 mL of BPW and incubated at 35 °C for 24 h. Bacterial DNA was extracted from BPW using the PureLink™ Microbiome DNA Purification Kit (Invitrogen, USA) according to the manufacturer's protocol. For the diagnosis of *Salmonella*, PCR was performed to amplify a fragment of the *invA* gene, which is specific to the genus *Salmonella* (Rhan *et al*. 1992). All the DNA positive for *Salmonella* in the PCR for the *invA* gene were screened by PCR to identify antimicrobial resistance genes. Sequence amplification of each gene of interest was performed using a TECHNE TC512 thermal cycler (Barlo World Scientific, USA). Each PCR mix contained 1 µL (25 pmol) of each primers targeting the gene of interest (Table 1), 2.0 µL (200 µM) of the four deoxynucleoside triphosphates (Bioline Inc., Randolph, Mass., USA), 0.75 µL (1 mM) of $MgCl_2$, 2.5 µL of 1X reaction buffer (200 mM Tris-HCl pH 8, 500 mM KCl), 0.5 µL (2.5 U) *Taq* DNA polymerase (Bioline Inc., Randolph, Mass., USA), 2.0 µL (100 ng) template DNA and 15.25 µL nuclease-free water for a final volume of 25 µL. Each PCR reaction mix was subjected to the following thermal cycling conditions: denaturation at 95 °C for 1 min, then 35 cycles with denaturation at 95 °C for 30 s, primer hybridization at specific Tm for each primer pair (Table 1) for 30 s, and extension of the DNA at 72 °C for 30 s. After the last cycle, the samples were kept at 72 °C for 10 min to complete all the strands' synthesis. The PCR products were subjected to gel electrophoresis (1.5% agarose) (Promega, USA), stained with ethidium bromide (0.5 µg $ml^{-1}$) (Sigma Aldrich, USA), visualized with a UV transilluminator (Spectroline Transilluminator, Model 7C-

254R. Electronics Corp., USA), and the images were captured with the WiseDocWGD-20 gel documentation system (DAIHAN Scientific Co. Ltd. Korea). A 100-bp molecular marker (BIOLINE) was used as a molecular weight standard.

**Table 1.** Primers used in PCR tests to detect genes of interest.

| Gene | Involved function | 5´ to 3´ primer DNA sequence | Temp (°C) | Amplicon size (bp) | Reference |
|---|---|---|---|---|---|
| *invA* | Invasion | F: GTGAAATTATCGCCACGTTCGGGCAA<br>R: TCATCGCACCGTCAAAGGAACC | 58 | 287 | Rhan *et al.* 1992 |
| *fliC* | flagellin | F: CAAGTCATTAATACAAACAGCC<br>R: TTAACGCAGTAAAGAGAGGAC | 60 | Approx. 1500 | Dauga *et al.* (1998) |
| *aadA2* | streptomycin adenyl transferase | F: TGTTGGTTACTGTGGCCGTA<br>R: GATCTCGCCTTTCACAAAGC | 60 | 623 | Randall *et al.* 2004 |
| *PSE-1* | β-lactamase | F: TTTGGTTCCGCGCTATCTG<br>R: TACTCCGAGCACCAAATCC | 58 | 151 | Talavera *et al.* 2011 |
| *bla*TEM | β-lactamase | F: GCACGAGTGGGTTACATCGA<br>R: GGTCCTCCGATCGTTGTCAG | 61 | 310 | Talavera *et al.* 2011 |
| *sul1* | dihydropteroate synthase | F: TCACCGAGGACTCCTTCTTC<br>R: AATATCGGGATAGAGCGCAG | 63 | 318 | Randall *et al.* 2004 |
| *tetA*(G) | Tetracycline efflux | F: CCGGTCTTATGGGTGCTCTA<br>R: CCAGAAGAACGAAGCCAGTC | 62 | 603 | Randall *et al.* 2004 |
| *floR* | Chloramphenicol efflux | F: CGCCCTCTGGATCAAGTCAA<br>R: TCACGGGCCACGCTGTATC | 62 | 542 | Randall *et al.* 2004 |

Chi-square tests were performed to determine significant differences in *Salmonella* prevalence according to the farm of origin and the type of sample analyzed. A p-value < 0.05 was considered statistically significant. Data analysis was performed using. The SPSS statistical package (Statistical Product for Service Solutions, v 10.0, SPSS Inc., Chicago, Ill.)

## PCR-RFLP analysis

Additionally, a PCR based on the *fliC* gene was performed, whose product was digested with the *Sau*3AI enzyme to determine the serotype according to the methodology proposed by Gallegos-Robles *et al*. (2008). Briefly: the unpurified amplified PCR product of the *fliC* gene was cleaved with *Sau*3AI (Promega) according to the manufacturer’s instructions. The digestion reaction consisted of 10 µl of PCR product, 2 µl of 10X reaction buffer B (60 mM Tris-HCl [pH 7.5] 500 mM NaCl, 60 mM $MgCl_2$, 10 mM dithiothreitol), 0.2 µl of acetylated BSA (10 µg/ µl), 5 U of enzyme, and deionized water for a final volume of 20 µl. The reaction was gently vortexed and then incubated at 37 °C for 1 h. After incubation, 6 µl of sample was mixed with 3 µl of loading buffer (0.25% bromophenol blue, 0.25% xylene cyanole, 30% glycerol) and electrophoresed on a 10% acrylamide gel for 2.5 h at 100 V in 1X Tris-borate-EDTA buffer. The 100-bp Hyperladder (Bioline) was used as the molecular weight standard for determining the molecular weight of the restriction fragments. The gels were stained and captured as described above for the PCR products of the antimicrobial resistance genes. *S*. Typhimurium (ATCC 13311) was used as positive control in the PCR reactions of the *invA* gene and in the restriction patterns generated with the *Sau*3A1 enzyme.

## RESULTS AND DISCUSSION

### *Salmonella* prevalence

From a total of 240 samples collected in five broiler farms, 80 samples (33.3%) were positive for the presence of *Salmonella* identified by microbiology technique and PCR through the amplification of the *invA* gene (Li *et al*. 2012). The prevalence of *Salmonella* varied according to the farm where the sample was taken and the type of sample analyzed. Significant differences ($X^2 = 53.625$, $p < 0.05$) were observed between farms. Digestion results from the PCR products of the *fliC* gene with the *Sau*3AI enzyme showed a unique restriction pattern in the 80 isolates, which corresponded to that of the *S.* Typhimurium serotype (Figure 1). The *fliC* gene in *Salmonella* is approximately 1500 bp in size, depending on the serotype. In the case of *S*. Typhimurium, the reported size is 1488 bp (NLM 2026), and it has three restriction sites for the *Sau*3A1 enzyme, generating four restriction fragments, three of which are major due to their size: 700, 471, and 302 bp. In the *Salmonella* isolates recovered in this study, the observed restriction fragments match the expected size based on the restriction sites of the *S*. Typhimurium *fliC* gene, indicating that the recovered isolates belong to the *S*. Typhimurium serotype (Gallegos-Robles *et al*. 2008). Differences were observed in the number of positive samples for *Salmonella* found in each farm, and this variation could be due to factors such as the farm size, the number of animals per house, hygiene status, management practices, animal species in the farm, among others (Denagamage *et al*. 2015). The fact of finding *Salmonella* in chicken manure, food, and water, coupled with the ubiquitous nature of *Salmonella*, facilitating a cyclical lifestyle. This cyclical lifestyle consists of moving from a host to the environment, and from the environment to a new host, and the capacity for long-term survival in a secondary environment (indicating the existence of horizontal and vertical transmission of *Salmonella* in the evaluated farms) (Heyndrickx *et al*. 2002, Winfield and Groisman 2003, Collineau *et al*. 2020). Additionally, it indicates crucial differences in the safety program's management applied in each farm and the risk that broilers contaminated with *Salmonella* are being produced.

### Salmonella distribution by type of analyzed sample

In this study, *Salmonella* was found in chicken manure (53.75%), drinking water (21.25%), and feed samples (25%). Significant differences ($X^2 = 19.56$, $p < 0.05$) were observed between type of samples. Different contamination status by *Salmonella* were observed within the different farms, as it was found from no contamination (farm A), contamination in chicken manure (farm B) and contamination in chicken manure, feed, and water (farms C, D and E).

According to Uzzau *et al*. (2000), some *Salmonella* serotypes require nutritional needs for specific amino acids and vitamins, while others are considered ubiquitous, such as *S*. Enteritidis and *S*. Typhimurium, have minimal nutrient needs. In this sense, it can be highlighted from this study that the water from the drinkers provided the minimum chemical-physical requirements necessary for the survival of *Salmonella* (Soria *et al*. 2013). Regarding the chicken manure and feed samples positive for *Salmonella*, some reports indicated its presence in this type of samples (Griffiths 2011, Muniz *et al*. 2014), with feed being considered one of the regular transmission routes to birds, which subsequently is the cause of contamination of the digestive tract of birds, feces, chicken manure, and the house, becoming a vicious circle in the farm (Park *et al*. 2011, Soria *et al*. 2011, Golden *et al*. 2021). A single chicken with *Salmonella* can infect its peers both in the incubator, in the boxes during transport, and finally on the receiving farm (Davila and Ortiz 2022). These results also indicated the potential risk of environmental contamination by *Salmonella* since chicken manure is used as a feed supplement for cattle (Lanyasunya *et al*. 2006, Bolan *et al*. 2010) and is also applied as a fertilizer to the soil by the nutrient (Griffiths 2011, Kyakuwaire *et al*. 2019). Besides, precipitation, humidity, soil properties, and pH are influential in the spread, reproduction, and survival of *Salmonella* (Robinson *et al*. 2022). Identified *S*. Gallinarum and *S*. Pullorum are the serotypes commonly found in poultry environments; however, *S*. Typhimurium and *S*. Enteritidis serotypes can also be found, which have sometimes displaced the former (Soria *et al*. 2013). In this study, the only identified serotype was the *S*. Typhimurium, one of the most common recovered serotypes in poultry, and its association with antibiotic-resistant strains represents a challenge for the poultry industry to establish control measures (Menconi *et al*. 2014). Having found the serotype *S*. Typhimurium may indicate its adaptation to these environments and high capacity to express resistance genes to antibiotics commonly used to treat infectious problems in the poultry sector (Mezali and Hamdi 2012).

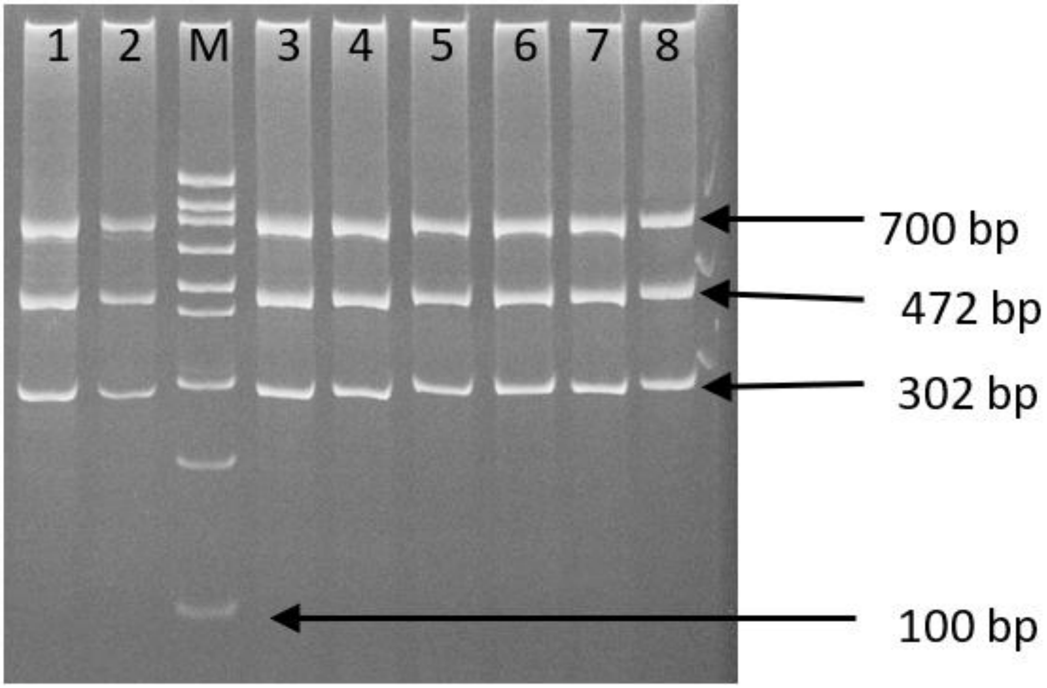

**Figure 1.** Restriction profiles of the *Salmonella fliC* gene obtained with *Sau*3AI endonuclease digestion. Lanes 1-2 and 3 through 7 restriction profile of *S*. Typhimurium; lane 8 *S*. Typhimurium (ATCC 13311). M: 100-bp molecular marker (BIOLINE).

## Molecular diagnosis of antibiotic resistance genes

Regarding the presence of ampicillin (*PSE-1* and *bla*TEM), tetracycline (*tetA* (G)), chloramphenicol (*floR*), streptomycin (*aadA2*) and sulfonamide (*sul1*) resistance genes, the results of the molecular characterization are listed in Table 2. 47.5% of the isolates (38/80) were *PSE-1* gene carriers, 83.75% (67/80) *bla*TEM gene carriers, 11.25% (9/80) *tetA* (G) gene carriers, 60.0% (48/80) *floR* gene carriers, 60.0% (48/80) *aadA2* gene carriers and 82.5% (66/80) *sul1* gene carriers. Figure 2 shows the PCR products of each of the genes used for *Salmonella* isolate molecular characterization.

**Table 2.** Results of the molecular characterization of antibiotic resistance in *S.* Typhimurium strains.

| Antibiotic resistance patterns | Gene | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | ***invA*** | ***PSE-1*** | ***bla*TEM** | ***tetA*(G)** | ***floR*** | ***aadA2*** | ***sul1*** | **†** |
| 1 (1)[€] | + | - | - | - | + | - | + | 2 |
| 2 (2) | + | - | + | - | - | - | + | 2 |
| 3 (8) | + | + | + | - | - | - | - | 2 |
| 4 (1) | + | + | - | - | - | + | - | 2 |
| 5 (1) | + | - | - | - | - | + | + | 2 |
| 6 (2) | + | + | - | - | - | - | + | 2 |
| 7 (13) | + | - | + | - | + | - | + | 3 |
| 8 (4) | + | - | + | + | - | - | + | 3 |
| 9 (1) | + | - | + | - | - | + | + | 3 |
| 10 (3) | + | + | + | - | - | + | - | 3 |
| 11 (1) | + | + | - | - | - | + | + | 3 |
| 12 (2) | + | + | + | - | + | - | - | 3 |
| 13 (7) | + | - | - | - | + | + | + | 3 |
| 14 (13) | + | - | + | - | + | + | + | 4 |
| 15 (1) | + | - | + | + | + | - | + | 4 |
| 16 (8) | + | + | + | - | - | + | + | 4 |
| 17 (8) | + | + | + | - | + | + | + | 5 |
| 18 (1) | + | + | + | + | - | + | + | 5 |
| 19 (3) | + | + | + | + | + | + | + | 6 |
| ‡ | 80 | 38 | 67 | 9 | 48 | 48 | 66 | |

[€]Number in parentheses indicates the number of isolates with that resistance pattern. † Number of antibiotic resistance genes present in each pattern. ‡ Number of isolates carrying each gene.

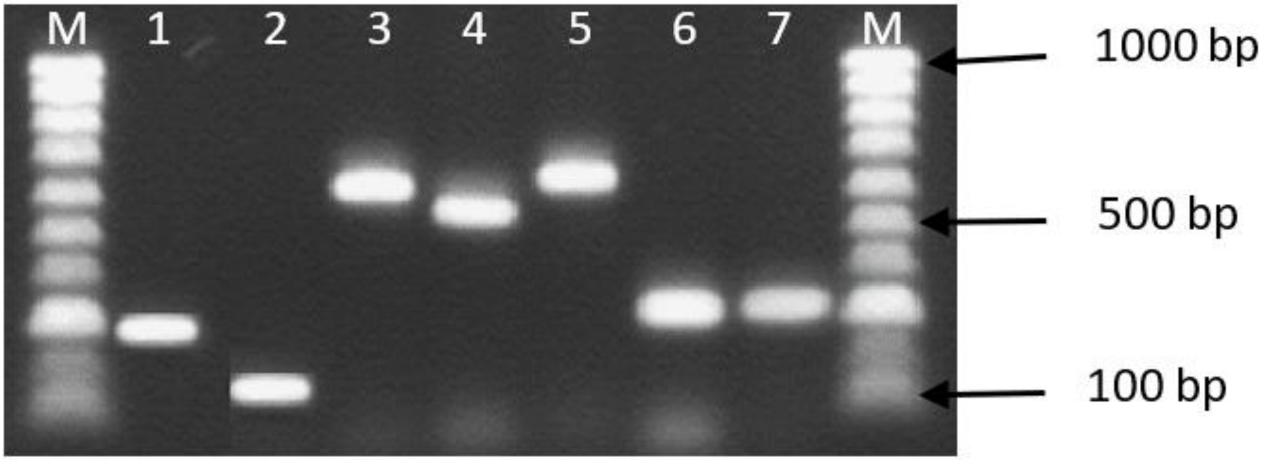

**Figure 2.** PCR products of the seven genes used to characterize *Salmonella* isolates. Lane 1: PCR product of the *invA* gene (287 bp); Lane 2: *PSE-1* gene (ampicillin, 151 bp); Lane 3: *tetA*(G) (tetracycline, 603 bp); Lane 4: *floR* (chloramphenicol, 542 bp); Lane 5: *aadA2* (streptomycin, 623 bp); Lane 6: *bla*TEM (ampicillin, 310 bp); Lane 7: *sul1* (sulfonamides, 318 bp); M: 100-bp molecular marker (BIOLINE).

All isolates turned out to be carriers of at least two of the genes that confer resistance to some of the antibiotics of interest in this study. The values for the isolate numbers and the number of antibiotic resistance genes were the following: 15 of the isolates (18.75 %) resulted in being a carrier of two of the genes; 30 of the isolates (37.5%) resulted in being a carrier of three genes; 22 of the isolates (27.5%) were carriers of four of the genes; 10 of the isolates (12.5%) were carriers of five of the genes, and three of the isolates (3.75%) were carriers of six of the genes. On average, 3.45 antibiotic resistance genes were found per isolate, which corresponds to a 57.5% average resistance in the isolates recovered in this study.

The gene that was diagnosed less frequently among the isolates was *tetA* (G) present in only nine samples, while those with the highest frequency were *bla*TEM present in 67 isolates and *sul-1* present in 66 isolates. 19 patterns of antibiotic resistance were observed. 81.25% of the isolates showed resistance genes to three or more antibiotics, and the most frequently observed resistance genetic pattern (26.25%) was the tetra-resistant ACSSu (ampicillin, chloramphenicol, streptomycin, and sulfonamides). The lowest and highest resistance gene frequencies were found in B and D farms respectively, both in manure samples. The *Salmonella* isolates carrying five and six antibiotic resistance genes of interest in this study were recovered mostly from the poultry manure samples. The gene that confers resistance to tetracycline (*tetA*(G)) is the one that was diagnosed with less frequency among the isolates recovered in this study. It may be due to the fact that the *tetA*(G) gene is inserted in some mobile genetic structure, and that this can be regulated by some chemical compound that does not allow a high presence of the gene in *Salmonella*. Ribera *et al*. (2003) publish that the *tetA* and *tetR* genes (encoding the regulatory protein of *tet*A) on mobile genetic elements (transposons) in *Acinetobacter baumannii*. Di Conza *et al*. (2013) defines that a transposon is a DNA fragment that presents extra genes that change the phenotype of the recipient genome in a predictable way, and that can also migrate from one site (donor) to another (recipient) within the genome. Liebert *et al*. (1999) argue that the *tetA* and *tetR* genes are inserted in the Tn10 transposon, and that in turn is part of the R100 transferable plasmid. Camacho *et al*. (2005) mention that the transfer of the R100 plasmid is induced by DAM methylation and repressed by the absence of leucine-sensitive regulatory protein (Lrp). Leucine is an amino acid with positive effects on muscle protein synthesis (Velázquez *et al*. 2012). Probably the diets of the farms that resulted in lower incidence of the *tetA* gene did not contain high doses of the amino acid. It differs from the results of other studies in which the gene for resistance to the antibiotic tetracycline is high (Balsalobre and Hernández-Godoy 2004, Wang *et al*. 2019). Since 88.75% of the recovered isolates do not carry the gene that confers resistance to tetracycline, this antibiotic could be used as the first choice in birds from these farms that present health problems caused by *S*. Typhimurium. This work's results also coincide with those of Balsalobre and Hernández-Godoy (2004) and Wang *et al*. (2019) concerning the high frequency of genes that confer resistance to ampicillin, chloramphenicol, streptomycin, and sulfonamides.

Three isolates recovered from the farm D carried the genes that express the penta-resistant pattern ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline). It represents a high risk to human health due to the possibility that it is the multi-resistant strain *S*. Typhimurium DT104 (Zhao *et al*. 2003), which suggests that the DT104 strain of *S*. Typhimurium could exist in the region (although with a low prevalence). A previous report in Mexico have mentioned the presence of multidrug-resistant *S*. Typhimurium in pig carcasses, from which strains of this serotype have been isolated with genes conferring resistance to the antibiotic's ampicillin, chloramphenicol, and sulfamethoxazole (Talavera *et al*. 2011). Another study in ground beef in central Mexico reports the presence of *Salmonella* serotypes (Newport, Senftenberg, Agona, 1,4,[5],12:i-, and Typhimurium) multidrug-resistant to the antibiotic's ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline, among other antibiotics evaluated. Among the multidrug-resistant isolates, almost 60% (26/45) presented both phenotypes and genotypes of penta-resistance or higher (Campos-Granados *et al*. 2023). It is essential to mention the presence in poultry environments of *Salmonella*'s multi-resistant strains to antibiotic have no previous report of in the Region Laguna.

The multi-locus variability observed in multi-resistance to antibiotics in this work may be due to the horizontal transfer of genes that promotes the simultaneous spread of resistance to several classes of unrelated antibiotics, which is highly possible since the environment of poultry farms are considered one of the hot-spot environments for genetic exchange (Peterson and Kaur 2018). The presence of *S*. Typhimurium in the three types of samples analyzed indicates hygiene problems and failures in the safety programs on the farms evaluated. The genetic variability observed in the antibiotic resistance profiles indicates horizontal genetic exchange between the strains present in these farms. The isolates that carry the six antibiotic resistance genes of interest in this study may be the multiresistant *S*. Typhimurium DT104 serotype, which for the first time, is mentioned in poultry environments in this region of Mexico. Specific management strategies for the class and antibiotic dose to be used should be applied depending on the resistance profile found in the isolates recovered from each farm. Other prevention alternatives, such as using vaccines or systems to reduce the risk of contamination against *Salmonella*, should be used in poultry farms to avoid those birds and their products constitute a risk factor for human health.

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## AUTHOR DISCLOSURE STATEMENT

The authors declare that they have no competing interests.

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