First report of multidrug-resistant <i>Salmonella</i> Infantis in broiler litter in Tolima, Colombia

Mayra A. Bonilla-Caballero, María P. Lozano-Puentes, María A. Ospina and Maryeimy Varón-López

Department of Biology, Research Group on Plant and Microbial Biotechnology - GEBIUT, Faculty of Sciences, University of Tolima, PO Box 73006299, Ibagué, Colombia.

Corresponding author: Mayra A. Bonilla-Caballero, e-mail: mabonillac@ut.edu.co
Co-authors: MPL: mplozanop@ut.edu.co, MAO: maospina@ut.edu.co, MV: mvaronl@ut.edu.co

Received: 11-12-2021, Accepted: 12-05-2022, Published online: 28-06-2022

doi: www.doi.org/10.14202/vetworld.2022.1557-1565
How to cite this article: Bonilla-Caballero MA, Lozano-Puentes MP, Ospina MA, Varón-López M (2022) First report of multidrug-resistant <i>Salmonella</i> Infantis in broiler litter in Tolima, Colombia, Veterinary World, 15(6): 1557–1565.

Abstract

Background and Aim: <i>Salmonella</i> has been identified as one of the most widely distributed zoonotic pathogens in broiler litter. Multidrug-resistant strains have been isolated from salmonellosis outbreaks, compromising the success of their treatment. This study aimed to isolate and identify <i>Salmonella</i> spp. serovars in healthy broiler litter in Tolima (Colombia), determine their resistance to different antimicrobials, and detect genes associated with β-lactam resistance that could be useful to control <i>Salmonella</i> spp. in poultry.

Materials and Methods: In total, 45 broiler litter samples were collected. <i>Salmonella</i> spp. was isolated and identified using selective and differential culture media and biochemical tests. Molecular confirmation of the pathogen was performed with the invA gene and serotyping by Kauffman–White scheme. Antimicrobial susceptibility to 15 antibiotics was determined by Kirby–Bauer method. In cefotaxime-resistant strains, <i>blaCTX-M</i>, <i>blaCTX-M-1</i>, <i>blaCMY</i>, and <i>blaTEM</i> genes were evaluated by polymerase chain reaction (PCR).

Results: In total, 817 presumptive strains were obtained from xylose lysine deoxycholate and <i>Salmonella</i> <i>Shigella</i> agars and subcultured on xylose-lysine-tergitol 4 and MacConkey agars, from which 150 strains were isolated; 29 of these strains were presumptive for <i>Salmonella</i> spp. after performing biochemical tests and 16 were confirmed by PCR as <i>Salmonella</i> Infantis (15) and Gallinarum (1). All strains were found to be multiresistant to antibiotics, showing three different profiles and isolates resistant to cefotaxime, and the <i>blaCTX-M</i> gene was detected.

Conclusion: This is the first study to isolate <i>S.</i> Infantis from broiler litter in Colombia. All isolates exhibited resistance to the evaluated antimicrobials, suggesting the misuse of antimicrobials in small- and medium-sized poultry farms. The presence of <i>Salmonella enterica</i> serovar Infantis is a public health problem. Thus, regular monitoring of poultry litter is recommended, as these bacteria can be transmitted to humans through animal products or contaminated environments.

Keywords: antibiotics, cefotaxime, poultry, <i>Salmonella</i>.

Introduction

<i>Salmonella</i> is a zoonotic pathogen with a wide range of animal and human hosts; it has been identified as a genus of global public health importance and the leading cause of foodborne illnesses responsible for thousands of deaths worldwide [1, 2]. This microorganism is one of the most prevalent pathogens in broiler litter (substrate that covers the floor of the shed with a high microbiological load) and can be transmitted from one production cycle to another. This can happen when litter is used in several consecutive batches and without adequate treatments to colonize the digestive tract of broilers, as broilers typically are in direct contact with litter and consume it [3–5]. In addition, residues of antibiotics fed to broilers can be found in the litter. When incompletely metabolized, 60–90% of antibiotics can be excreted in the broiler’s excreta [6, 7]. Different studies have shown that <i>Salmonella</i>, especially serotypes Infantis, Gallinarum, Kentucky, and Saintpaul [8–10] isolated in poultry environments, has high resistance to β-lactams, associated with the presence of <i>blaCTX-M</i>, <i>blaCTX-M-1</i>, <i>blaCMY</i>, and <i>blaTEM</i> genes [11–14].

Identifying propagation sources of pathogens on farms, such as poultry litter has been deemed vital for controlling microorganisms such as <i>Salmonella</i>. Monitoring poultry flocks are the first step to reduce the transmission of pathogens to humans and the antimicrobial resistance of these pathogens and indirectly increases disease treatment options [15].

Therefore, this study aimed to isolate and identify <i>Salmonella</i> spp. serovars in litter from healthy broilers in the department of Tolima (Colombia), estimate resistance to different antimicrobials, and detect genes associated with β-lactam resistance.

Materials and Methods

Ethical approval
The experiment was approved by the Animal Ethics Committee of University of Tolima, Colombia.
Farms were selected after consideration of the willingness of the farmers. During the collection of samples, written consent was taken from each of the farm owners or farm managers.

**Study period and location**

The study was conducted from June to December 2020. The experiment was carried out on three broiler farms located in the department of Tolima, Colombia.

**Sample collection and preparation**

In total, 45 rice husk litter samples (each sample comprised of 10 subsamples) were collected from broiler farms (1200–22,000 broilers per house). Fifteen samplings were performed on 0 (before the broilers arrived), 3, 15, 28, and 43 days of the production cycle. Samples were collected from the entire depth of the litter without scratching the original soil surface and placed in sterile Ziploc bags. After collection, 10 g of a combination of litter samples was transferred to the laboratory for immediate analysis and placed into sterilized stomacher bags containing 90 mL of buffered peptone water (BPW) (Acumedia, Lansing, Michigan).

The sample size was calculated using the following formula [16]:

\[
n = \frac{1.96^2 \cdot P_{\text{exp}} \cdot (1 - P_{\text{exp}})}{d^2}
\]

This formula was selected as the best fit for this study, as no studies of *Salmonella* spp. in poultry litter in Tolima were carried out before; therefore, no other formulas were available for this specific case. \(n\) is the required sample size, \(P_{\text{exp}}\) is the expected prevalence, and \(d\) is the desired absolute precision. The confidence level was 95%, the precision level was 5%, and the expected prevalence was 3. Applying this formula, the minimum number of samples was 44.71. Therefore, in total, 45 samples were collected. Sample selection was carried out through the non-probabilistic method, as it depended on the willingness of the farm owners to participate in this study. Before sampling, a survey was conducted; variables, such as the number of birds; biosecurity measures; material of the litter; reuse and sanitation of the litter; management of physicochemical factors; control of rodents, wild birds, and insects; and management and sanitary status of the animals, were included in the survey.

**Isolation of Salmonella**

All samples were pre-enriched in 9:1 BPW (Acumedia) for 18–24 h before transport to the Laboratory of Microbiology and Mucor rhizae for further analysis. All samples were subjected to selective culture and biochemical testing for the presence of *Salmonella enterica*. The pre-enriched samples were inoculated in Rappaport-Vassiliadis broth (Oxoid; Basingstoke, Hampshire, England) at 41°C for 24 h. The samples were subsequently cultured in xylose lysine deoxycholate (XLD) (Oxoid) and *Salmonella-Shigella* (SS) (Oxoid) agars at 37°C. Subsequently, 817 colonies were taken for subculture in xylose-lysine-tergitol 4 (XLT4) (Conadal; Madrid, Spain) and MacConkey agars (MCA; Acumedia, Michigan, US) at 37°C overnight for 24 h.

**Identification of Salmonella spp.**

Biochemical identification was made using Gram staining and oxidase test. All isolates that were Gram-positive and/or oxidase-positive were discarded. The other isolates were biochemically tested using triple sugar iron agar (Oxoid), Simmons citrate (Oxoid), lysine iron agar (Acumedia), and sulfide indole motility agar (Merck, Darmstadt, Germany), and urease.

**DNA extraction**

DNA extraction of presumptive *Salmonella* spp. strains was done by boiling method [17]. The quantity and purity were assessed using NanoDrop and Qubit, and DNA quality was checked on a 1.5% (w/v) agarose gel and visualized using an ultraviolet (UV)–visible spectrophotometer (Cole-Parmer UV Transilluminator, Vernon Hills, USA). DNA was stored at −20°C until use.

**Polymerase chain reaction (PCR)**

All isolates were confirmed by PCR by the amplification of the *invA* gene. *S. enterica* subsp. *enterica* serovar Enteritidis ATCC® 13076 strain (Thermo Scientific, United Kingdom) was used as a positive control. Amplification was performed in a T-100™ thermocycler (Bio-Rad, USA). All PCRs were performed in a total volume of 12.5 μL consisting of 6.25 μL of 2× PCR MasterMix (Corpogen, Bogota, Colombia), 2.5 μL DNA template, and 0.65 μL of each primer brought to 2.5 μL using DNA/RNA-free water. PCR and thermal conditions were performed according to the referenced authors (Supplementary data not shown). Amplicons were then visualized on a 1% agarose gel by electrophoresis (PowerPac™ HC, Bio-Rad) using 1 kb DNA ladder Load Ready™ (Amplexus, USA). The gel was stained with HydraGreen™ (ACTGene, USA) and visualized under the E-GeITM Imager System with UV Light Base (Thermo Fisher Scientific, Waltham, MA).

**Serotyping**

Serotyping of *Salmonella* spp. strains was carried out at the Colombian Agricultural Institute (ICA), following the Kauffmann–White scheme, to determine the O (somatic), Vi (capsular), and H (flagellar) antigens [18].

**Antibiotic sensitivity assay**

Isolated *Salmonella* spp. were subjected to an antimicrobial sensitivity test by the Kirby–Bauer disk diffusion method [19]. Overnight grown bacterial inocula were adjusted to the 0.5 McFarland standard, swabbed on pre-incubated Mueller-Hinton agar.
Antibiotics were recorded for each farm. Biosecurity characteristics, cleaning practices, and disinfection were identified as Gram-negative bacilli through Gram staining and identified as presumptive strains of Salmonella spp. through biochemical tests. The latter two were the most abundant. The biosecurity characteristics, cleaning practices, disinfection, reuse of the litter, and sanitary state of the animals were recorded for each farm.

**Results**

**Isolation of Salmonella spp.**

In total, 817 strains were obtained from XLD and SS agars, of which, after culture in MacConkey and XLT4 agars, 150 presumptive strains for Salmonella were determined. After identification by biochemical tests, 29 presumptive isolates were acquired, and all came from the same litter sample. The isolates were molecularly confirmed with the invA gene, which was present in 16 isolates, to which the serotyping test was performed at the ICA. Fifteen strains corresponded to Salmonella Infantis and one corresponded to Salmonella Gallinarum (Table-1).

In XLD and SS agars, the growth of other microorganisms could be observed. These microorganisms were identified as Gram-negative bacilli through Gram staining and identified as presumptive strains of Pseudomonas spp., Citrobacter spp., Klebsiella spp., Escherichia coli, and Proteus spp. through biochemical tests. The latter two were the most abundant. The biosecurity characteristics, cleaning practices, disinfection, reuse of the litter, and sanitary state of the animals were recorded for each farm.

**Salmonella phenotypic resistance**

All strains exhibited multidrug resistance (Table-1). The 16 isolates confirmed as Salmonella from the same farm were resistant to cefotaxime, gentamicin, penicillin, erythromycin, nalidixic acid, and ceftiofur. Of the isolates, 81.25% (13 of 16) were tetracycline resistant and the other (18.75% [3 of 16]) isolates had intermediate resistance; 93.75% (15 of 16) and 62.5% (10 of 16) had intermediate resistance to ciprofloxacin and doxycycline, respectively, and the remaining isolates were sensitive to both antibiotics. All isolates were sensitive to amoxicillin, ampicillin-sulbactam, enrofloxacin, streptomycin, colistin, and trimethoprim-sulfamethoxazole (Table-2).

**Identification of ESBL resistance genes**

In Salmonella spp. isolates resistant to cefotaxime, genes encoding ESBL were blaCTX-M-F, blaCTX-M-1, blaCMY, and blaTEM. All the isolates were positive for blaCTX-M-F (592 bp), but were negative for blaCTX-M-1, blaCMY, and blaTEM genes.

**Discussion**

*S. enterica* has been determined to be responsible for salmonellosis disease in several species of production animals, including poultry. Poultry is also referred to as a vehicle for transmitting salmonellosis to humans through the consumption of broiler meat or eggs contaminated with this pathogen, thus generating economic losses and impact on public and animal health [15, 21]. *Salmonella* spp. can survive for long periods on abiotic surfaces in broiler farms [22]. One such surface is broiler litter. Litter is, therefore, considered as a source for spreading pathogens between batches of broilers [22–25]. *Salmonella* Typhimurium, *Salmonella* Enteritidis, *S. Kentucky, and S. Newport are the serotypes most isolated from poultry litter [21, 22]. *S. Infantis* has been described as an emerging serotype in the poultry industry [26], as it generally does not produce symptoms in poultry. This specific serotype is, therefore, difficult to identify [27]. In Colombia, this serotype has been isolated in commercial laying hen farms in the department of Antioquia and samples of litter, feed, and drinking water, cloacal swabs, cecal content, and ovaries [28]. *S. Infantis* has also been isolated in broiler carcasses and feaces samples in three processing plants in the departments of Antioquia, Meta, and Cundinamarca [29]. *S. Infantis* in broiler litter has also been reported in tropical countries, such as in Ecuador and Brazil [30–32]. However, until now, the presence of *S. Infantis* has not been reported in Colombian broiler litter. Therefore, this study is the first to find *S. Infantis* in broiler litter in Colombia.

*S. Gallinarum*, the second serotype isolated in this study, generally has a low production yield and high mortality in broilers depending on the management conditions and the state of the immune system of the flock [25, 33]. In other studies, *S. Gallinarum* has been isolated from litter and cloacal swabs in Brazil.
and India [34, 35] and broiler organ samples in India and China [36, 37]. In Colombia, S. Gallinarum has been recovered mainly from carcasses [38]. Although the spread of Salmonella in broiler litter and farms may be due to various factors, the number of broilers on the farm is considered one of the most important determining factors [39]. Farms with small bird populations are less prone to infections than those with large populations, as higher animal density can lead to broilers experiencing more stress and having more contact with other broilers, feces, and litter, which all increase the risk of pathogen transmission, even when greater biosecurity measures are taken [4].

In addition to the threat posed by the presence of Salmonella in broiler litter as a source of indirect transmission to humans, a second threat is an increase in antibiotic-resistant strains used in poultry farming, as these could recirculate on farms for several production cycles and could be transferred to humans through consumption of contaminated poultry products. This could eventually lead to a failure in the treatment of salmonellosis in humans [40, 41]. In recent years, S.

### Table 1: Results of the microbiological and molecular tests carried out on the isolated strains of Salmonella spp.

| Strain | Biochemical confirmation | Molecular confirmation (InvA) | Serotyping | Phenotypic resistance | Genotypic resistance |
|--------|--------------------------|-------------------------------|------------|-----------------------|---------------------|
|        | Simmons citrate | Motility | Indole | TSI | LIA | SIM | Urea | SH |                | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS1*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS2*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS3*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS4*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS5*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS6*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS7*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS8*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS9*  | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS10* | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS11* | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS12* | + | K/A | ± | + | - | - | + | + | S. Gallinarum | CIP, GM, CTX, blaCTXM-F |
| FCS13* | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS14* | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS17* | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |
| FCS18* | + | K/A | ± | + | - | - | + | + | S. Infantis | CIP, GM, CTX, blaCTXM-F |

*All isolates obtained in this study were from the same farm. FC=Farm C, S=Strain number, BR=Before receiving the birds, FI=Finisher, CIP=Ciprofloxacin, GM=Gentamicin, CTX=Cefotaxime, NA=Nalidixic acid, TE=Tetracycline, E=Erythromycin, P=Penicillin, CTF=Ceftiofur, S=Streptomycin, DO=Doxycycline*
Infantis has become a public health concern in various regions worldwide due to its high resistance levels to multiple antimicrobials [42]. In fact, in this study, all S. Infantis and S. Gallinarum strains were found to be resistant to the antibiotics evaluated in the aminoglycoside group (gentamicin and streptomycin). These results were similar to those reported for S. Gallinarum in Nigeria and Mali in cloacal swabs and poultry organs [43, 44] and S. Infantis in Ecuador, England, and Japan in environmental samples from farms and poultry meat [12, 14, 45].

Likewise, in the β-lactam group, 100% resistance to cefotaxime was found, similar to the percentages reported in S. Infantis strains in Ecuador (87.5–99%) and Egypt, Korea, and Serbia (78.9–100%) [31, 46–48]. In S. Gallinarum isolates, resistance up to 85% was reported in India [49]. This resistance may be associated with cross-resistance caused by using other cephalosporins in poultry farming, such as ceftiofur [50, 51], which is an antibiotic to which all strains in this study were also resistant. Some studies have shown significant phenotypic resistance in S. Infantis strains to this antimicrobial in Chile (60.92–92%) [52, 53].

In addition, all Salmonella isolates were determined to be resistant to penicillin. These results agreed with the high resistance levels reported among other Salmonella serotypes, such as S. Enteritidis and S. Typhimurium, in poultry meat samples in Egypt [54]. Furthermore, all S. Infantis strains were sensitive to amoxicillin and ampicillin-sulbactam, in agreement with those reported in India [46] and Egypt [54]. However, results contrasted with different studies worldwide that have shown moderate resistance in S. Infantis and high in S. Gallinarum toward both antibiotics [42, 46, 55–57].

Regarding the group of quinolones, the high resistance to nalidixic acid found in this study (100%) was similar to that found in Romania and Serbia in S. Infantis strains from broiler meat and eggshells and that in Romania and Bangladesh in S. Gallinarum from healthy and sick broilers [58, 59]. Ciprofloxacin only managed to inhibit the growth of S. Gallinarum strain, similar to that obtained in isolates of this serotype in broiler meat in Egypt [60] and S. Infantis isolates from various poultry sources in Brazil [61]. The remaining 93.75% of the isolates had intermediate sensitivity to ciprofloxacin, which is similar to the sensitivity levels reported in Switzerland for S. Infantis from broiler meat and India for S. Gallinarum from internal organs of poultry [35, 62].

All isolates in this study were sensitive to enrofloxacin (100%). This sensitivity level was also described in S. Infantis in samples of cloacal swabs and broiler meat in Chile and Iran [52, 63]. Likewise, 100% of the strains were sensitive to colistin and trimethoprim-sulfamethoxazole. This result was in line with results obtained for S. Infantis in South Africa and S. Gallinarum in broiler embryos in China and broiler viscera in Brazil [64–66]. However, another study carried out in Argentina in broiler liver samples reported 100% sensitivity for other serovars [67].

All Salmonella spp. isolates were resistant to erythromycin, in line with results from South Africa in broiler samples from informal markets and results from Egypt with 83.33% of Salmonella strains isolated from broiler meat sourced from different supermarkets showing resistance to this antibiotic [47, 64]. Finally, the resistance levels obtained in this study for the tetracycline group differed from other studies. For example, 37.5% of the isolated Salmonella strains were sensitive to doxycycline. In comparison, 62.5% had intermediate sensitivity to this antibiotic, different from those reported for S. Infantis in South Africa for samples of broiler meat (50% resistance) and those reported in Indian farms for environmental samples.

### Table-2: Results of the phenotypic resistance of the isolates of S. Infantis and S. Gallinarum obtained from the litter in poultry farms.

| Antimicrobial               | Conc. (µg) | Diameter of the zone of inhibition (mm) | Number and percentage of microorganisms S. Infantis (15) | S. Gallinarum (1) |
|-----------------------------|------------|----------------------------------------|--------------------------------------------------------|-------------------|
|                             |            | S I R                                  | S I R                                                   | S I R             |
| Ampicillin-sulbactam        | 10         | ≥ 15 12–14 ≤ 11                        | 15 (93.75)                                              | -                 |
| Amoxicillin                 | 10         | ≥ 18 14–17 ≤ 13                        | 15 (93.75)                                              | -                 |
| Cefotaxime                  | 30         | ≥ 8 16–32 ≤ 64                         | -                                                       | 15 (93.75)        |
| Ciprofloxacin               | 10         | ≥ 31 21–30 ≤ 20                        | -                                                       | 15 (93.75)        |
| Gentamicin                  | 10         | ≥ 4 8 ≤ 16                             | -                                                       | 15 (93.75)        |
| Erythromycin                | 15         | ≥ 23 14–22 ≤ 13                        | -                                                       | 15 (93.75)        |
| Nalidixic Acid              | 30         | > 19 14–18 ≤ 13                        | 15 (93.75)                                              | -                 |
| Penicillin                  | 10         | ≥ 22 12–21 ≤ 11                        | 15 (93.75)                                              | -                 |
| Trimetoprim-sulfamethoxazole| 25         | > 16 11–15 ≤ 10                        | 15 (93.75)                                              | 1 (6.25)          |
| Tetracycline                | 30         | > 15 12–14 ≤ 11                        | 3 (18.75)                                               | 12 (75)           |
| Ceftiofur                   | 30         | ≥ 21 18–20 ≤ 17                        | 15 (93.75)                                              | 1 (6.25)          |
| Enrofloxacin                | 5          | ≥ 21 17–20 ≤ 16                        | 15 (93.75)                                              | 1 (6.25)          |
| Colistin                    | 10         | ≥ 14 ≤ 10                              | 15 (93.75)                                              | 1 (6.25)          |
| Doxycycline                 | 30         | ≥ 14 11–13 ≤ 10                        | 5 (31.25)                                               | 10 (62.5)         |
| Streptomycin                | 10         | > 15 12–14 ≤ 11                        | -                                                       | 15 (93.75)        |

R=Resistant, I=Intermediate, S=Sensitive
(100% resistance). In contrast, resistance levels to tetracycline were similar to those found in Brazil and Japan in meat samples, which were as high as 84.8% and 96.5%, respectively [67, 68].

Antibiotic resistance in Salmonella spp. generally depends on the acquisition of resistance genes from their environment [69]. For β-lactams, blaCTX-M genes are commonly related to resistance to cephalosporins [70]. This gene has numerous variants, such as blaCTX-M-F, detected in all isolates with phenotypes resistant to cefotaxime. These results were in line with the data reported in Brazil and Ecuador in S. Infantis isolates from broiler ceca [8, 14]. Other genes associated with the resistance of Salmonella spp. β-lactams are blaTEM and blaCMY [71], which are genes also analyzed in this study but were not detected in any isolated Salmonella samples. However, blaTEM has been reported in various serotypes from poultry environments in India (S. Typhimurium), Colombia (S. Newport, S. Paratyphi B, and S. Manhattan), and China (S. Gallinarum) [9, 46, 72, 73]. The blaCMY gene was present with the blaCMY-2 variation in S. Infantis in Japan and Egypt in broiler meat samples [11, 69]. These resistance genes in Salmonella spp. from poultry samples represent a significant risk to public health, as they can be transferred horizontally through mobile elements, such as plasmids [12]. Through plasmids, genes could not only reach hosts through poultry products contaminated with resistant bacteria but also through mechanical and biological vectors, such as flies and Alphitobius diaperinus, found in high densities in the litter analyzed in this study [70, 74].

Conclusion

Serotypes S. Infantis and S. Gallinarum were detected in the litter of broilers in Tolima. These serotypes increase the risk of the spread of Salmonella on farms and are a possible source of transfer of the pathogen and its genes to humans. All strains resistant to antibiotics are ranked by the World Health Organization as drugs of highest priority for treating diseases in humans and identified by the World Organization for Animal Health as critically important veterinary antimicrobial agents. Furthermore, all isolates carried the blaCTX-M-F gene associated with resistance to cefotaxime. These results showed that Salmonella serotypes are changing in poultry litter in Colombia.

Future studies are expected to intensify the collaboration with and participation of the farms in the Tolima region to increase the number of samples. Furthermore, this study was expected to serve as a base to characterize the state of resistance of pathogenic microorganisms and identify the Salmonella serotypes present in litter in different communities dedicated to raising broilers in surrounding areas. This would allow for developing prevention and control plans for these microorganisms. The first avenue of future research would be to study if results were similar in other regions in Colombia among small producers. The second avenue would be to distinguish between different production systems (small scale and industrial) in the study on Salmonella in broiler litter.

Data availability

Supplementary data can be available from the corresponding author on a reasonable request.

Authors’ Contributions

MV and MAO: Designed the study and revised the manuscript. MAB and MPL: Collected data, performed the experimental work, and drafted the manuscript. All authors have read and approved the final manuscript.

Acknowledgments

This study was funded by the Central Research Office of the University of Tolima, Colombia (Project/Ta360130517) and the convocation 755 of MINCIENCIAS (Ministry of Science, Technology, and Innovation of Colombia).

Competing Interests

The authors declare that they have no competing interests.

Publisher’s Note

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

References

1. Odoch, T., Wasteson Y., L’Abée-Lund, T., Muwonge, A., Kankya, C., Nyakarahuka L., Jegule, S. and Skjerve, E. (2017) Prevalence, antimicrobial susceptibility and risk factors associated with non-typhoidal Salmonella on Ugandan layer hen farms. BMC Vet. Res., 13(1): 365.
2. Christidis, T., Hurst, M., Rudnick, W., Pintar, K.D.M. and Pollari, F. (2020) A comparative exposure assessment of foodborne, animal contact and waterborne transmission routes of Salmonella in Canada. Food Control, 109: 106899.
3. Dunlop, M.W., Blackall, P.J. and Stuetz, R.M. (2016) Odour emissions from poultry litter-a review litter properties, odour formation and odorant emissions from porous materials. J. Environ. Manage., 177: 306–309.
4. Wang, L., Lilburn, M. and Yu, Z. (2016) Intestinal microbiota of broiler chickens as affected by litter management regimes. Front. Microbiol., 7: 593.
5. Stojic, M.D., Bajdev, S., Zikic, D., Peric, L. and Milosevic, N. (2016) Effect of straw size and microbial amendment of litter on certain litter quality parameters, ammonia emission, and footpad dermatitis in broilers. Arch. Anim. Breed., 59(1): 131–137.
6. Abdukhalilova, G., Kaftyveva, L., Wagenaar, J.A., Tanygarikov, B. and Bektimirov, A. (2016) Occurrence and antimicrobial resistance of Salmonella and Campylobacter in humans and broiler chicken in Uzbekistan. Public Health Panor., 2(3): 340–3347.
7. Xiong, W., Wang, M., Dai, J., Sun, Y. and Zeng, Z. (2018) Application of manure containing tetracyclines slowed down the dissipation of tet resistance genes and caused changes in the composition of soil bacteria. Ecotoxicol. Environ. Saf., 147: 455–460.
8. Mendonça, E.P., Melo, R.T., Oliveira, M.R.M.,
Diseases of Poultry. Wiley, United States. p719–730.

26. Pulido, M. (2019) Food safety-Salmonella update in broilers. *Anim. Feed Sci. Technol.*, 250(9): 53–58.

27. Vellmer, M., Milanov, D. and Kozodarcovic, G. (2018) *Salmonella* spp. in poultry: Constant challenge and new insights. *J. Hell. Vet. Med. Soc.*, 69(2): 899–910.

28. Ruiz, J., Suárez, M. and Uribe, C. (2006) Susceptibility antimicrobiana de cepas de *Salmonella* spp. en granjas de ponederas comerciales del departamento de Antioquia. *Rev. Colom. Cienc. Pecua.*, 19(17): 297–305.

29. Ramírez-Hernandez, A., Carrascal-Camacho, A.K., Varón-García, A., Brashears, M.M. and Sanchez-Plata, M.X. (2021). Genotypic characterization of antimicrobial-resistant *Salmonella* spp. strains from three poultry processing plants in Colombia. *Food*, 10(3): 491.

30. Scur, M.C., da Silva, F.G., Mira, E.A., Weber, D.L., Angeli, L.F. and Carvalho, A. (2014) Occurrence and antimicrobial resistance of *Salmonella* serotypes isolates recovered from poultry of Western Paran, Brazil. *Afr. J. Agric. Res.*, 9(9): 823–830.

31. Villagómez Estrada, S., Logacho Pilatani, M. and Vinueza Burgos, C. (2017) Presencia y resistencia a los antimicrobianos de serovariedades de *Salmonella enterica* aisladas en una empresa avícola integrada del Ecuador. *REMCA*, 38(1): 11–24.

32. Vinueza-Burgos, C., Baquero, M., Medina, J. and De Zutter, L. (2019) Occurrence, genotypes and antimicrobial susceptibility of *Salmonella* collected from the broiler production chain within an integrated poultry company. *Int. J. Food Microbiol.*, 299: 1–7.

33. Shoaib, M., Dasti, J.I., Shah, M.A.A., Zafar, M.A., Hasan, M.U., Riaz, A., Rehman, S.U. and Khan, M.A. (2017) *Salmonellosis* in poultry, new prospects of an old disease: A review. *J. Anim. Poult. Sci.*, 69(4): 361–368.

34. Abuna, F., Bedasa, M., Beyene, T., Ayana, D., Mamo, B. and Duguma, R. (2016) *Salmonella*: Isolation and antimicrobial susceptibility tests on isolates collected from poultry farms in and around Modjo, Central Oromia, and Ethiopia. *J. Anim. Poult. Sci.*, 5(2): 21–35.

35. Penha Filho, R.A.C., Ferreira, J.C., Kanashiro, A.M.I., da Costa Darnin, A.L. and Schierack, P., Sarwar, Y. and Ali, A. (2018) Multiple drug resistance and virulence characterization of non-typhoidal *Salmonella* isolated from retail chicken meat shops in Luxor city, Egypt. *Int. J. Vet. Sci.*, 2(1): 20–35.

36. Shang, K., Wei, B. and Kang, M. (2018) Distribution and dissemination of antimicrobial-resistant *Salmonella* spp. in chickens in China. *Int. J. Food Microbiol.*, 262: 23–30.

37. Castañeda Salazar, R., Pereira Bazurdo, A.N., Pulido Villamarín, A.D.P. and Mendoza Gómez, M.F. (2018) Estimación de la prevalencia de *Salmonella* spp. en pechugas de pollo para consumo humano provenientes de cuatro localidades de Bogotá-Colombia. *Infectio*, 23(1): 27.

38. Wang, Y., Zhang, A., Yang, Y., Lei, C., Jiang, W., Liu, B. and Wang, H. (2017) Emergence of *Salmonella enterica* serovar Indiana and California isolates with concurrent resistance to cefotaxime, amikacin and ciprofloxacin from chickens in China. *Int. J. Food Microbiol.*, 262: 1–8.

39. Pullido, M. (2019) Food safety-Salmonella update in broilers. *Anim. Feed Sci. Technol.*, 250(9): 53–58.

40. Pulido, M. (2019) Food safety-Salmonella update in broilers. *Anim. Feed Sci. Technol.*, 250(9): 53–58.

41. Arido, S., Toso, R., Toribio, M., Álvarez, H., Mariani, E., Cachau, P., Mancilla, M. and Oriani, D. (2017) Antimicrobianos como promotores de crecimiento (AGP) en alimentos balanceados para aves: Uso, resistencia bacteriana, nuevas alternativas y opciones de reemplazo. *Cienc. Vet.*, 19(1): 50–66.

42. Shang, K., Wei, B. and Kang, M. (2018) Distribution and dissemination of antimicrobial-resistant *Salmonella* in broiler farms with or without enrofloxacin use. *BMC Vet. Res.*, 14(1): 1–14.

43. Franco, A., Leekitcharoephon, P., Feltrin, F., Alba, P.,
Cordaro, G., Iurescia, M., Tolli, R., D’Incau, M., Staffolani, M., Di Giannatale, E., Hendriksen, R.S. and Battisti, A. (2015) Emergence of a clonal lineage of multidrug-resistant ESBL-producing *Salmonella* Infantis transmitted from broilers and broiler meat to humans in Italy between 2011 and 2014. *PLoS One.*, 10(12): 1–15.

43. Nwiyi, P., Chah, K. and Shoynyka, S. (2018) Detection of some resistance genes in *Salmonella* isolated from Poultry farms in Abia and Imo States, Southeastern Nigeria. *Niger Vet. J.*, 39(2): 124–132.

44. Sibidé, S., Traoré, A.B., Koné, Y.S., Fané, A., Coulibaly, K.W., Doumbia, A.B. and Traoré, O. (2019) Antibiotérésistance des souches de *Salmonella gallinarum* isolées en aviculture moderne en zones périurbaines au Mali. *Rev. Élev. Méd. Vét. Pays Trop.*, 72(4): 167–171.

45. Mori, T., Okamura, N., Kishino, K., Wada, S., Zou, B., Nanba, T. and Ito, T. (2017) Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from poultry meat in Japan. *Food Saf.*, 6(3): 126–129.

46. Choi, D., Chon, J.W., Kim, H.S., Kim, D.H., Lim, J.S., Yim, J.H. and Seo, K.H. (2015) Incidence, antimicrobial resistance, and molecular characteristics of non-typhoidal *Salmonella* including extended-spectrum β-lactamase producers in retail chicken meat. *J. Food Prot.*, 78(11): 1932–1937.

47. Hassan, A.H.A., Salam, H.S.H. and Abdel Latef, G.K. (2019) Identification of virulence genes, β-lactams and quinolones resistance-associated genes and integrons in *Salmonella* isolated from retail chicken meat and giblets in Egypt. *J. Microbiol. Biotechnol. Food Sci.*, 8(6): 1320–1325.

48. Nikoloi, A., Balti, T., Velebit, B., Babić, M., Milojčević, L. and Dordević, V. (2017) Antimicrobial resistance among *Salmonella* enterica serovar Infantis from broiler carcasses in Serbia. *IOP Conf. Ser. Earth. Environ. Sci.*, 85(1): 012077.

49. Arora, D., Kumar, S., Jindal, N., Narang, G., Kapoor, P.K. and Mahajan, N.K. (2015) Prevalence and epidemiology of *Salmonella enterica* serovar Gallinarum from poultry in some parts of Haryana, India. *Vet. World*, 8(11): 1300.

50. Organizacion Mundial de Sanidad Animal, OIE. (2015) Lista de Los Agentes Antimicrobianos Importantes Para la Medicina Veterinaria. OIE, Paris, France.

51. Voss-Rech, D., Vaz, C.S.L., Alves, L., Coldebella, A., Leao, J.A., Rodrigues, D.P. and Back, A. (2015) A temporal analysis of *Salmonella enterica* serotypes isolated from chicken hatcheries in Henan, China. *IOP Confer. Ser. Earth. Environ. Sci.*, 21(1): 827.

52. Lapierre, L., Cornejo, J., Zavala, S., Galarce, N., Sánchez, F., Benavides, M.B. and Sáenz, L. (2020) Phenotypic and genotypic characterization of virulence factors and susceptibility to antibiotics in *Salmonella enterica* Infantis strains isolated from chicken meat: First findings in Chile. *Animals*, 10(6): 1049.

53. Paredes-Osses, E.A., Ricci, A.F., Boke, S.D., Aguilar, K.E., Orellana, A.V., Bello, M.U. and Hernández, M.C.M. (2020) Epidemiological investigation and antimicrobial resistance profiles of *Salmonella* enterica isolated from breeder chicken hatcheries in Guelph, Ontario, Canada. *J. Worlds Poult. Res.*, 75(1): 23–30.

54. Procura, F., Bueno, D.J., Bruno, S.B. and Rogé, A.D. (2019) Prevalence, antimicrobial resistance profile and comparison of methods for the isolation of *Salmonella* in chicken liver from Argentina. *Food Res. Int.*, 119: 541–546.

55. Shigemura, H., Matsui, M., Sekizuka, T., Noda, T., Yamashita, A. and Murakami, K. (2018) Decrease in the prevalence of extended-spectrum cephalosporin-resistant *Salmonella* following cessation of cefotiofur use by the Japanese poultry industry. *Int J Microbiol.*, 2019: 5129–5137.

56. Solá-Gálvez, M., González-López, J.J., Cameron-Vees, K., Piedra-Carrasco, N., Cerdá-Cuéllar, M. and Miguera-García, L. (2015) Houseflies (*Musca domestica*) as vectors of *Salmonella* serovars and antimicrobial resistance profiles in commercial layer flocks. *Isr. J. Vet. Med.*, 75(1): 23–30.

57. Pereira, E., Torres, R., Nalevaiko, P.C., Monteiro, G.P., Fonseca, B.B., Galvão, N.N., Giombelli, A. and Rossi, D.A. (2019) Spread of the serotypes and antimicrobial resistance in strains of *Salmonella* spp. isolated from broiler. *Brazil J. Microbiol.*, 50(2): 515–522.

58. Tirzu, E., Bărbălean, G., Morar, A., Herman, V., Cristina, R.T. and Imre, K. (2020) Occurrence and antimicrobial susceptibility profile of *Salmonella* spp. in raw and ready-to-eat foods and *Campylobacter* spp. in retail raw chicken meat in Transylvania, Romania. *Foodborne Pathog. Dis.*, 17(8): 479–484.

59. Sarker, B.R., Ghosh, S., Chowdhury, S., Dutta, A., Chandra Deb, L., Krishna Sarker, B. and Mozaffir Hossain, K.M. (2021) Prevalence and antimicrobial susceptibility profiles of non-typhoidal *Salmonella* isolated from chickens in Rajshahi, Bangladesh. *Vet. Med. Sci.*, 7(3): 820–830.

60. Xie, Z., Liu, D., Liu, L., Sun, H. and Li, J. (2019) Prevalence of multidrug resistance non-typhoidal *Salmonella* isolated from layer farms and humans in Egypt. *J. World Poult. Res.*, 9(4): 280–288.

61. Borges, K.A., Furian, T.F., Souza, S.N., Salle, C.T.P., Moraes, H.L.S. and Nascimento, V.P. (2019) Antimicrobial resistance and molecular characterization of *Salmonella enterica* serotypes isolated from poultry sources in Brazil. *Rev. Bras. Cienc. Avic.*, 21(4): 1–7.

62. Hindermann, D., Gopinath, G., Chase, H., Negrete, F., Althaus, D., Zurfluh, K., Tall, B.D., Stephan, R. and Nièsch Inderbinnen, M. (2017) *Salmonella enterica* serovar Infantis from food and human infections, Switzerland, 2010–2015: Poultry-related multidrug-resistant clones and an emerging ESBL producing clonal lineage. *Front. Microbiol.*, 8: 1322.

63. Azizpour, A. (2021) Prevalence and antibiotic resistance of *Salmonella enterica* serotypes in chicken meat of Ardabil, Northwestern Iran. *Iran. J. Microbiol.*, 15(2): 232–246.

64. Mokgoph, T.M., Geebe, N., Fasina, F. and Adesiyun, A.A. (2021) Antimicrobial resistance profiles of *Salmonella* isolates on chickens processed and retailed at outlets of the informal market in Gauteng Province, South Africa. *Pathogens*, 10(3): 273.

65. Xu, Y., Zhou, X., Jiang, Z., Qi, Y., Ed-Dra, A. and Yue, M. (2020) Epidemiological investigation and antimicrobial resistance profiles of *Salmonella* enterica isolated from chicken carcasses in the stade of Mato Grosso, Brazil: Antibiotic resistance profile, serotyping, and characterization by repetitive sequence-based PCR system. *Poult. Sci.*, 97(4): 1373–1383.

66. de Cunha-Neto, A., Carvalho, L.A., Carvalho, R.C.T., dos Prazeres Rodrigues, D., Mano, S.B., de Souza Figueiredo, E.E. and Conte-Junior, C.A. (2018) *Salmonella* isolated from chicken carcasses from a slaughterhouse in the state of Mato Grosso, Brazil: Antibiotic resistance profile, serotyping, and characterization by repetitive sequence-based PCR system. *Poult. Sci.*, 97(4): 1373–1383.

67. Procura, F., Bueno, D.J., Bruno, S.B. and Rogé, A.D. (2019) Prevalence, antimicrobial resistance profile and comparison of methods for the isolation of *Salmonella* in chicken liver from Argentina. *Food Res. Int.*, 119: 541–546.

68. Furukawa, I., Ishihara, T., Teranishi, H., Saito, S., Yatsuyanagi, J., Wada, E. and Kuroki, T. (2016) Prevalence and characteristics of *Salmonella enterica* and *Campylobacter* in retail poultry meat in Japan. *Jpn. J. Infect. Dis.*, 70(3): 239–247.

69. Shimamura, H., Matsui, M., Sekizuka, T., Onozuka, D., Yoshiba, A. and Murakami, K. (2018) Decrease in the prevalence of extended-spectrum cephalosporin-resistant *Salmonella* following cessation of ceftiofur use by the Japanese poultry industry. *Int J Microbiol.*, 274: 45–51.

70. Solá-Gálvez, M., González-López, J.J., Cameron-Vees, K., Piedra-Carrasco, N., Cerdá-Cuéllar, M. and Miguera-García, L. (2015) Houseflies (*Musca domestica*) as vectors
for extended-spectrum β-lactamase-producing Escherichia coli on Spanish broiler farms. Appl. Environ. Microbiol., 81(11): 3604–3611.

71. Wong, M.H.Y., Yan, M., Chan, E.W.C., Biao, K. and Chen, S. (2014) Emergence of clinical Salmonella enterica serovar Typhimurium isolates with concurrent resistance to ciprofloxacin, ceftriaxone, and azithromycin. Antimicrob. Agents Chemother., 58(7): 3752–3756.

72. Rada, A., Hernández, C., Restrepo, E. and Villegas, M. (2019) Distribución y caracterización molecular de betalactamasas en bacterias Gram negativas en Colombia, 2001–2016. Biomédica, 39(1): 199–220.

73. Cortes Vélez, D., Rodríguez, V. and Verjan García, N. (2017) Phenotypic and genotypic antibiotic resistance of Salmonella from chicken carcasses marketed at Ibague, Colombia. Rev. Bras. Cienc. Avic., 19(2): 347–354.

74. Donoso, A., Paredes, N. and Retamal, P. (2020) Detection of antimicrobial-resistant Salmonella enterica strains in larval and adult forms of lesser mealworm (Alphitobius diaperinus) from industrial poultry farms. Front. Vet. Sci., 7: 736.