RESEARCH ARTICLE

Prevalence and Diversity of *Salmonella* Serotypes in Ecuadorian Broilers at Slaughter Age

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Abstract

*Salmonella* is frequently found in poultry and represent an important source for human gastrointestinal infections worldwide. The aim of this study was to investigate the prevalence, genotypes and antimicrobial resistance of *Salmonella* serotypes in broilers from Ecuador. Caeca content from 388 at random selected broiler batches were collected in 6 slaughter-houses during 1 year and analyzed by the ISO 6579/Amd1 protocol for the isolation for *Salmonella*. Isolates were serotyped and genotypic variation was acceded by pulsed field gel electrophoresis. MIC values for sulfamethoxazole, gentamicin, ciprofloxacin, ampicillin, cefotaxime, ceftazidime, tetracycline, streptomycin, trimethoprim, chloramphenicol, colistin, florfenicol, kanamycin and nalidixic acid were obtained. Presence of *bla*CTX-M, *bla*TEM, *bla*SHV and *bla*CMY; and *mcr-1* plasmid genes was investigated in resistant strains to cefotaxime and colistin respectively. Prevalence at batch level was 16.0%. The most common serotype was *S. Infantis* (83.9%) followed by *S. Enteritidis* (14.5%) and *S. Corvallis* (1.6%). The pulsed field gel electrophoresis analysis showed that *S. Corvallis*, *S. Enteritidis* and *S. Infantis* isolates belonged to 1, 2 and 12 genotypes respectively. *S. Infantis* isolates showed high resistance rates to 12 antibiotics ranging from 57.7% (kanamycin) up to 98.1% (nalidixic acid and sulfamethoxazole). All *S. Enteritidis* isolates showed resistance to colistin. High multiresistant patterns were found for all the serotypes. The *bla*CTX-M gene was present in 33 *S. Infantis* isolates while *mcr-1* was negative in 10 colistin resistant isolates. This study provides the first set of scientific data on prevalence and multidrug-resistant *Salmonella* coming from commercial poultry in Ecuador.

Introduction

Foodborne infections in humans caused by *Salmonella* are of primary importance around the world. Majowicz et al. [1] estimated that non-typhoidal *Salmonella* was the cause of 93.8...
Salmonella spp. in Ecuadorian Broilers

Materials and Methods

Study design and sampling

Pichincha, the province where Quito the capital city of Ecuador is located, was selected as the area to collect samples since it is an important region within Ecuador for the production of broiler meat. Big slaughterhouses were contacted and asked for their willingness to cooperate in the study. Based on these results sampling was performed in 6 slaughterhouses. From June 2013 to July 2014, a total of 388 batches (birds coming from one broiler house and slaughtered on the same day) were sampled. Each batch originated from a different epidemiological unit. All sampled batches were commercially reared and slaughtered at the age of 6 to 7 weeks.

From each batch one caecum from 25 randomly selected chickens were collected, and transported in an ice box within 1 hour to the laboratory for bacteriological analysis.

Isolation and Identification of Salmonella

From each of the 25 caeca content was aseptically pooled. Therefore, all caeca were immersed in ethanol, and after evaporation of the ethanol approximately 1 g content/cecum was collected in a sterile plastic bag. All samples were homogenized by hand during 1 min. after the addition of 225 ml Buffered Peptone Water (BPW; Difco, BD, Sparks, MD). After the incubation of the preenrichment media at 37°C for 20 hours 3 drops of each culture medium were spotted onto a
Modified Rappaport-Vassiliadis agar plate (MSRV; Oxoid, Basingstoke, UK) and incubated at 42°C for 24 hours. Plates were examined for migration and if present a loopful from the edge of the migration zone was streaked onto a Xylose Lysine Deoxycholate agar plate (XLD, Difco) and incubated at 37°C for 24 hours. Two presumptive Salmonella colonies were tested using Triple Sugar Iron agar (Difco, BD), Lysine Iron agar (BBL, BD), Urea agar (BBL, BD) and Sulfur Indole Motility medium (BBL, BD) for confirmation.

Characterization of Salmonella isolates

One Salmonella isolate per positive sample was further characterized. To limit the number of Salmonella strains to be serotyped, isolates were grouped by an enterobacterial repetitive intergenic consensus (ERIC) PCR as described by Rasschaert et al. [18]. ERIC PCR was performed on 59 strains within the same run. Based on ERIC PCR profiles 16 isolates were selected for serotyping. All these selected isolates and the 3 isolates not included in the ERIC PCR run were serotyped according to the Kauffmann-White scheme.

To characterize the Salmonella strains within each serotype, all isolates were genotyped by pulse field gel electrophoresis (PFGE) after digestion with XbaI enzyme [19]. The relatedness among the PFGE profiles was analyzed with GelCompar II software v. 6.6 (Applied Maths, Sint-Martens-Latem, Belgium). Bands representing fragments between 35 kb and 1140 kb in size were included in the analysis. A similarity dendrogram was constructed by the unweighted pair group method using arithmetic averages algorithm (UPGMA). DICE similarity coefficient with a position tolerance of 1.4 was calculated. A PFGE genotype was assigned on the basis of the difference in the presence of at least one band in the XbaI fingerprint [20]. Genotypes were identified by numerical suffixes after a capital indicating the serotype (e.g. I-1 refers to serotype Infantis).

Antimicrobial Resistance

Antimicrobial resistance was evaluated by determining the minimum inhibitory concentration (MIC) using the EUMVS2 plates (Thermo Scientific, West Palm Beach, USA). The tests were performed according to the manufacturer instructions. The following antibiotics were evaluated: sulfamethoxazole, gentamicin, ciprofloxacin, nalidixic acid, ampicillin, cefotaxime, ceftazidime, tetracycline, streptomycin, trimethoprim, chloramphenicol, colistin, florfenicol and kanamycin. Escherichia coli ATCC 25922 was used as the quality control strain. Clinial breakpoints values from the Clinical and Laboratory Standards Institute [21] were considered to determine bacterial antibiotic resistance for kanamycin and sulfamethoxazole. For all other antibiotics epidemiological breakpoint values from the European Committee on Antimicrobial Susceptibility Testing were considered [22]. Salmonella isolates resistant to cefotaxime where further examined for the presence of ESBL or AmpC phenotypes by disk diffusion tests [23,24]. According to the disk diffusion results PCR tests were performed to identify \( \text{bla}_{\text{CTX-M}} \), \( \text{bla}_{\text{TEM}} \) and \( \text{bla}_{\text{SHV}} \) genes in ESBL isolates and \( \text{bla}_{\text{CMY}} \) in AmpC isolates. PCR conditions and primers were the ones described by Hasman et al. [25] for \( \text{bla}_{\text{CTX-M}} \), Olesen et al. [26] for \( \text{bla}_{\text{TEM}} \), Arlet et al. [27] for \( \text{bla}_{\text{SHV}} \) and; Hasman et al. [25] and Kruger et al. [28] for \( \text{bla}_{\text{CMY}} \).

Isolates with phenotypic resistance to colistin were tested for the presence of the new described mcr-1 plasmid gene by primers described by Liu et al. [29]. For the PCR reaction mixture the Maxima Hot Start Green PCR Master Mix (Promega) was used. The total mixture of 25 µl contained 1X hot start PCR buffer, 400µM of each nucleotide (dNTP) 4mM MgCl₂, 0.2 µM of each primer and 1 µl of the template DNA obtained after boiling during 10 minutes of 1 colony of the bacteria in 100 µl of DNA free water. The following PCR program was used: a denaturation step at 95°C for 5 minutes, 35 cycles of 1 minute at 95°C, 0.5 minutes at 60°C, 1
minute at 72°C, and finally 10 minutes at 72°C. After the PCR, the amplification products were confirmed by gel electrophoresis using a 2% agarose gel. A PCR amplicon of 308 bp was expected. As positive control we used the Salmonella autoagglutinable strain S15FP06306, a strain isolated from poultry and confirmed to have the mcr-1 gene by sequencing of the PCR product and by performing whole genome sequencing on the strain.

**Statistical analysis**

Prevalence of Salmonella positive batches was estimated using a random-effects logistic regression model with farms and the sampling occasions per farm as random factors. The 95% confidence interval (CI95%) for the prevalence was calculated once the regression model fit the intercept. Variance components and their standard deviations and the intraclass correlation coefficient (ICC) are reported. Function *glmer* from *lme4* package [30] in R environment version 3.3.1 [31] was used to estimate the fixed and the random factors. Salmonella prevalence in farms and its CI95% were estimated under independence assumption for farms and considering a farm positive when at least one of the sampled batches was positive.

**Results**

In total 388 batches originated from 119 farms (1 to 9 flocks per farm) were sampled. From all tested batches 62 (16.0%; CI95%; 12.6–24.5) were Salmonella positive. The variance component for farms was 0.0237 (SD: 0.154) and 0.0345 (SD: 0.185) for sampling occasions per farm. Thus, the ICC estimated was 0.5928 as a measure of reproducibility in the sample results. Positive batches originated from 50 (42.0%; CI95%; 33.1–51.4) farms (Table 1). For 87 farms, more than one batch was sampled. One, two and three batches were found Salmonella positive on 41, 6 and 3 of those farms respectively.

ERIC-PCR of the 59 Salmonella isolates delivered 2 patterns. Serotyping demonstrated that pattern 1 corresponded to S. Enteritidis and pattern 2 to S. Infantis (Fig A in S1 File). Direct serotyping of the other 3 Salmonella strains resulted in 2 strains belonging to S. Infantis and 1 strain to S. Corvallis. In total 52 isolates (83.9%) were S. Infantis, 9 (14.5%) S. Enteritidis and 1 (1.6%) S. Corvallis.

The PFGE analysis (Fig B in S1 File) showed that S. Corvallis, S. Enteritidis and S. Infantis isolates belonged to 1, 2 and 12 genotypes respectively (Table 2).

**Table 1.** Salmonella positive batches in relation to the number of tested batches per farm.

| Number of batches/farm sampled | Number of farms | Number of farms with 0, 1, 2 or 3 positive batches |
|-------------------------------|----------------|-----------------------------------------------|
|                               |                | 0   | 1   | 2   | 3   |
| 1                             | 34             | 27  | 7   |     |     |
| 2                             | 18             | 12  | 6   |     |     |
| 3                             | 12             | 7   | 5   |     |     |
| 4                             | 19             | 10  | 8   | 1   |     |
| 5                             | 17             | 10  | 8   | 1   |     |
| 6                             | 15             | 6   | 4   | 4   | 1   |
| 7                             | 2              | 1   | 1   |     |     |
| 8                             | 1              | 1   |     |     |     |
| 9                             | 1              | 1   |     |     |     |
| Total                         | 119            | 70  | 41  | 6   | 3   |

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Within the S. Infantis strains the genetic similarity was minimal (87% similarity) and the different genotypes were due to the presence or absence of one band in the obtained profiles. The genotype I-1 was the dominant genotype (40.4%) within this serotype. Salmonella isolates from 9 farms with more than 1 Salmonella positive batch, belonged to different serotypes (2 farms), genotypes (5 farms) or serotypes and genotypes (1 farm) (Table 3).

Antimicrobial resistance rates within each Salmonella serotype against the 14 tested antibiotics are shown in Table 4 and the MIC distributions for the different antibiotics are shown in Table B in S1 File. S. Infantis isolates showed a resistance rate of 5.8% and 1.9% for ceftazidime and colistin respectively, whereas for the other 12 tested antibiotics the resistance rates varied from 57.7% (kanamycin) up to 98.1% (nalidixic acid and sulfamethoxazole). In contrast, all S. Enteritidis isolates showed resistance to colistin. The resistance rate for the other antibiotics ranged from 11.1% up to 33.3%.

S. Infantis isolates showed 19 resistance patterns in which resistance from 2 up to 13 antibiotics were involved (Table 5). The resistance pattern 2 (38.5%) was the most frequent one within S. Infantis isolates. S. Enteritidis isolates presented 4 antibiotic resistance patterns...

Table 2. Salmonella genotypes present in each serotype.

| Serotype       | Genotype | Nb. of strains |
|----------------|----------|----------------|
| S. Corvallis   | C-1      | 1              |
| S. Enteritidis | E-1      | 5              |
|                | E-2      | 4              |
| S. Infantis    | I-1      | 21             |
|                | I-2      | 6              |
|                | I-3      | 2              |
|                | I-4      | 6              |
|                | I-5      | 1              |
|                | I-6      | 1              |
|                | I-7      | 1              |
|                | I-8      | 10             |
|                | I-9      | 1              |
|                | I-10     | 1              |
|                | I-11     | 1              |
|                | I-12     | 1              |
| **Total**      |          | 62             |

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Table 3. Salmonella serotypes and genotypes found in farms with multiple positive batches.

| Farm | C-1 | E-1 | E-2 | I-1 | I-2 | I-8 | I-9 | I-10 | I-11 | Total |
|------|-----|-----|-----|-----|-----|-----|-----|------|------|-------|
| A    |     |     |     |     |     | 1   |     | 1    | 1    | 2     |
| B    | 1   | 1   |     |     |     |     |     | 1    |      | 2     |
| C    |     |     | 2   |     |     |     |     |      |      | 2     |
| D    | 1   | 1   |     |     |     |     |     |      |      | 2     |
| E    |     |     |     | 1   |     |     |     |      |      | 1     |
| F    |     |     | 1   | 1   |     |     |     |      |      | 2     |
| G    |     |     |     |     | 2   |     |     |      |      | 3     |
| H    | 1   | 2   |     |     |     |     |     |      |      | 3     |
| I    | 1   | 1   |     |     |     |     |     |      | 1    | 3     |

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### Table 4. Number of *Salmonella* strains resistant to each tested antibiotic.

| Antibiotic          | S. Infantis | S. Enteritidis | S. Corvallis |
|---------------------|-------------|----------------|--------------|
| Sulfamethoxazole    | 51 (98.1)   | 3 (33.3)       | 1 (100)      |
| Nalidixic acid      | 51 (98.1)   | 2 (22.2)       |              |
| Ciprofloxacin       | 49 (94.2)   | 2 (22.2)       | 1 (100)      |
| Tetracycline        | 49 (94.2)   | 1 (11.1)       |              |
| Trimethoprim        | 47 (90.4)   | 2 (22.2)       | 1 (100)      |
| Streptomycin        | 47 (90.4)   | 2 (22.2)       |              |
| Cefotaxime          | 42 (80.8)   | 2 (22.2)       |              |
| Ampicillin          | 41 (78.8)   | 1 (11.1)       |              |
| Florfenicol         | 40 (76.9)   | 2 (22.2)       |              |
| Gentamicin          | 39 (75)     | 2 (22.2)       |              |
| Chloramphenicol     | 39 (75)     | 1 (11.1)       |              |
| Kanamycin           | 30 (57.7)   | 2 (22.2)       |              |
| Colistin            | 1 (1.9)     | 9 (100)        |              |
| Ceftazidime         | 3 (5.8)     | 1 (11.1)       |              |

**Sulfamethoxazole (S), ciprofloxacin (C), nalidixic acid (L), tetracycline (T), trimethoprim (M), cefotaxime (F), ampicillin (A), florfenicol (N), gentamicin (G), chloramphenicol (H), kanamycin (K), streptomycin (R), colistin (O) and ceftazidime (Z).**

**S**: Not Applicable.

*Number of strains with ESBL or AmpC phenotype according to disk diffusion test.*

### Table 5. Antibiotic resistance patterns of *Salmonella* strains and phenotypes of cefotaxime resistant strains.

| Pattern | Resistance pattern | No. Antibiotics | S. Infantis | S. Enteritidis | S. Corvallis | Rate (%) | ESBL + strains* | bla<sub>CTX-M</sub> | AmpC + strains* |
|---------|--------------------|-----------------|-------------|----------------|--------------|----------|-----------------|----------------------|-----------------|
| 1       | SGCAFZTRMHNLK      | 13              | 2           | 2              | 3.2%        | 2        | 2               | 2                    |                 |
| 2       | SGCAFTTRMHNLK      | 12              | 20          | 32.3%         | 15          | 15       | 5               |                      |                 |
| 3       | SGCAFTTRMHNL       | 12              | 1           | 1.6%          | 1           | 1        |                 | 1                    |                 |
| 4       | SGCAFTTRMNKL       | 12              | 1           | 1.6%          | 1           | 1        |                 |                      |                 |
| 5       | SGCAFTTRMHNKL      | 12              | 1           | 1.6%          | 1           | 1        |                 |                      |                 |
| 6       | SGCAFTTRMHNL       | 11              | 6           | 9.7%          | 6           | 6        | 5               |                      |                 |
| 7       | SGCAFTTRHNL        | 11              | 2           | 3.2%          | 2           | 2        |                 |                      |                 |
| 8       | SGCAFTTRMHNL       | 10              | 1           | 1.6%          | 1           | 1        |                 |                      |                 |
| 9       | SGCAFTTRMKL        | 10              | 1           | 1.6%          | 1           | 1        |                 |                      |                 |
| 10      | SGCAFTTRHNL        | 10              | 1           | 1.6%          | 1           | 1        |                 |                      |                 |
| 11      | SGCAFMHNKL         | 10              | 1           | 1.6%          | 1           | 1        |                 |                      |                 |
| 12      | SGCTRMHNLK         | 10              | 3           | 4.8%          | NA          | NA       | NA              |                      |                 |
| 13      | SCAFTRMNKL         | 9               | 1           | 1.6%          | 1           |         |                 |                      |                 |
| 14      | GCAFTHNLK          | 9               | 1           | 1.6%          | 1           | 1        |                 |                      |                 |
| 15      | SCFTRHNL           | 8               | 1           | 1.6%          | NA          | NA       | NA              |                      |                 |
| 16      | SCAFZTRL           | 8               | 1           | 1.6%          | 1           | 1        |                 |                      |                 |
| 17      | SCAFTRL            | 7               | 3           | 4.8%          | 3           | 3        |                 |                      |                 |
| 18      | SCFTRML            | 7               | 1           | 1.6%          | 1           |         |                 |                      |                 |
| 19      | SCTRML             | 6               | 4           | 6.5%          | NA          | NA       | NA              |                      |                 |
| 20      | STRML              | 5               | 1           | 1.6%          | NA          | NA       |                 |                      |                 |
| 21      | SCM                | 3               | 1           | 1.6%          | NA          | NA       |                 |                      |                 |
| 22      | SO                 | 2               | 1           | 1.6%          | NA          | NA       |                 |                      |                 |
| 23      | SM                 | 2               | 1           | 1.6%          | NA          | NA       |                 |                      |                 |
| 24      | O                  | 1               | 6           | 9.7%          | NA          | NA       |                 |                      |                 |
| Total   |                    | 52              | 9           | 1             | 34          | 33       | 10              |                      |                 |

Sulfamethoxazole (S), ciprofloxacin (C), nalidixic acid (L), tetracycline (T), trimethoprim (M), cefotaxime (F), ampicillin (A), florfenicol (N), gentamicin (G), chloramphenicol (H), kanamycin (K), streptomycin (R), colistin (O) and ceftazidime (Z).

*Number of strains with ESBL or AmpC phenotype according to disk diffusion test.*

**S**: Not Applicable.
containing 1 (pattern 24, 6 strains), 2 (pattern 21, 1 strain) and 12 (patterns 4 and 5, both one strain) antibiotics. Two \( \text{S. Enteritidis} \) isolates were resistant to 12 antibiotics. The \( \text{S. Corvallis} \) isolate was resistant to 3 antibiotics.

From the 44 \( \text{Salmonella} \) isolates that showed resistance to cefotaxime 34 presented a ESBL phenotype and were \( \text{S. Infantis} \), while 10 presented an AmpC phenotype with 2 \( \text{S. Enteritidis} \) and 8 \( \text{S. Infantis} \). None of the ESBL isolates were positive by PCR for the \( \text{bla}_{\text{TEM}} \) or \( \text{bla}_{\text{SHV}} \) genes, while 33 of these isolates were positive for the \( \text{bla}_{\text{CTX-M}} \) gene. None of the AmpC isolates were positive for the \( \text{bla}_{\text{CMY}} \) gene. None of the 10 colistin resistant strains were positive for the \( \text{mcr-1} \) plasmid gene by PCR.

**Discussion**

To our knowledge, this is the first study about \( \text{Salmonella} \) in commercial reared broiler batches at slaughter in Ecuador. Results indicate that 15.9% of the batches slaughtered in the province of Pichincha are \( \text{Salmonella} \) positive. This result is similar to the prevalence reported in Venezuela (23%; \( n = 332 \)) [32]. In contrast prevalence in Brazil was only of 5% (\( n = 40 \)) [33] and in Colombia 65% (\( n = 315 \)) [34]. On the other hand, for the European Union member states and 3 European non-member states an overall \( \text{Salmonella} \) prevalence of 3.37% at farm level was reported with rates varying from 0.08% in Norway to 13.48% in Hungary in 2014 [35].

Only \( \text{S. Infantis} \) (83.9%), \( \text{S. Enteritidis} \) (14.5%) and \( \text{S. Corvallis} \) (1.6%) were found in positive batches. These findings contrast with data from Colombia, where a wider diversity of \( \text{Salmonella} \) serotypes were reported in broilers at slaughter age [36]. These authors found 31 serotypes among 378 examined \( \text{Salmonella} \) strains with the most common serotypes being \( \text{S. Paratyphi B dT+}, \text{S. Heidelberg}, \text{S. Enteritidis} \) and \( \text{S. Typhimurium} \). Similarly, data from Venezuela indicated that the most prevalent \( \text{Salmonella} \) serotypes at slaughterhouse level were \( \text{S. Paratyphi B} \) and \( \text{S. Heidelberg} \) [32]. On the other hand, in Brazil the most prevalent serotypes in chicken carcasses were \( \text{S. Enteritidis}, \text{S. Infantis}, \text{S. Typhimurium} \) and \( \text{S. Heidelberg} \) [37]. In the European Union the most reported serotypes at farm level were \( \text{S. Infantis} \) (43.4%) followed by \( \text{S. Mbandaka} \) (13.5%), \( \text{S. Livingstone} \) (7.3%) and \( \text{S. Enteritidis} \) (7.3%) in 2014 [35]. Accordingly, the emergence of \( \text{S. Infantis} \) in human salmonellosis has been reported [38]. The role poultry in human salmonellosis caused by \( \text{S. Infantis} \) in Ecuador needs further research.

Moreover, PFGE analysis demonstrated that the \( \text{S. Infantis} \) strains were genetically very similar. Although there were 12 identified genotypes within \( \text{S. Infantis} \), most of them varied in 1 to 2 bands with similarities above 88%, which suggest that these strains are highly related [20]. This is in accordance with other studies that showing a high similarity of \( \text{S. Infantis} \) within poultry, other animal and human isolates [39–42].

The reason why only 3 \( \text{Salmonella} \) serotypes were found and the \( \text{S. Infantis} \) strains showed a high genetic similarity in the present study is not clear and need further research for clarification. In a first step collection of samples from all over Ecuador may give a broader view of \( \text{Salmonella} \) serotypes present in broilers at national level. Moreover, such a study may also confirm the prevalence of \( \text{Salmonella} \) in broilers observed in the present study.

High antibiotic resistance rates were shown against most of the tested antibiotics within \( \text{S. Infantis} \) strains. \( \text{S. Infantis} \) strains showed also higher multiresistant patterns than \( \text{S. Enteritidis} \). Of the \( \text{S. Infantis} \) strains 44.2% showed resistance to at least 12 antibiotics, whereas 22.2% of \( \text{S. Enteritidis} \) strains presented resistant patterns to 12 antibiotics. In concordance, for Brazil 71.3% (\( n = 87 \)) of \( \text{Salmonella} \) strains isolated from poultry houses were reported to be resistant to chloramphenicol, ampicillin, ceftazidime, ciprofloxacin, nalidixic acid, tetracycline, sulfamethoxazole, and trimethoprim/sulfamethoxazole [43]. Although \( \text{S. Enteritidis} \) has been found to be susceptible to most antibiotics [44,45], antibiotic resistance has also been reported to β-
lactam antibiotics, sulfonamides, quinoxalines, fluoroquinolones and tetracyclines [46–48]. Moreover, 2 S. Enteritidis isolates presented resistance towards 12 antibiotics which is in accordance with previous findings [49]. This is of special interest since it suggests that in high antibiotic pressure environments, non-classical multidrug resistant (MDR) Salmonella serotypes can emerge.

In the present study 85.5% and 83.9% of Salmonella strains were resistant to nalidixic acid and ciprofloxacin respectively. High resistance rates to fluoroquinolones have been reported in Salmonella. For example, EFSA and ECDC reported for 2013 high to extremely high levels of resistance to these 2 antibiotics in Salmonella from broilers [45]. A study in Serbia showed that 100% of S. Infantis strains were resistant to ciprofloxacin and nalidixic acid [42] while Rahmani et al., demonstrated high fluoroquinolone resistance in both, S. Infantis and S. Enteritidis [41]. High fluoroquinolone resistance rates reported in our study may be explained by the selective pressure of resistant strains under the common use of fluoroquinolones as therapeutics in Ecuadorian broiler farms.

Low rates of colistin resistant in Salmonella has been described before [41,50,51]. However, it has been suggested that S. Enteritidis may have increased colistin MIC values [52]. This is in accordance with our results where 77.8% of S. Enteritidis and 1.9% of S. Infantis strains presented a colistin resistant phenotype. On the other hand, other studies have reported that resistance to colistin in Salmonella enterica isolated from food animals was mainly presented in S. Typhimurium but not in S. Enteritidis or S. Infantis [53,54]. Since the resistance in the phenotype positive Salmonella strains was not attributable to the mcr-1 plasmid gene, it may be assumed that mutations in the chromosomal genes were the source for the observed resistance [29]. Even though the mcr-1 plasmid gene has been mainly described in E. coli from Latin America, Europe and Asia [29,55–57] this gene has also been observed in Salmonella enterica from European countries like UK, Spain and France [58–60]. These data suggest that mcr-1 gene might be present in Salmonella enterica in Latin America, but further research is needed to confirm this assumption.

In accordance with findings from other studies carried out in Latin America, β-lactam-resistant Salmonella isolates were identified [34,61,62]. Although blaTEM and blaSHV are reported as common genes in resistant Salmonella [43,63], these resistance genes were not found in our strains. However, studies in Brazil and USA have identified the blaCTX-M genes as the most prevalent ESBL genes in Salmonella recovered from poultry [64,65] which is in accordance with our results. It should be taken into account that, even though the main families of beta-lactamases were included in this study, resistance to beta-lactams present in the negative strains could be mediated by other ESBL or AmpC genes [14,66]. The presence of these strains in Ecuadorian broilers is of public health concern since resistance to β-lactam antibiotics, listed as WHO Essential Medicines [67], may limit the options to treat human Salmonella infections.

Moreover, all antibiotics, with exception of colistin and ceftazidime, showed high rates of antimicrobial resistance indicating the necessity of a better use of antibiotics and biosecurity implementation in the primary sector to reduce the multidrug-resistant bacteria loads in broilers reared in Ecuador. It is worth to mention that there is a global trend towards an increase of antimicrobials consumption in the animal production sector [68]. This place a concern since the misuse of antibiotics in livestock production can lead to the occurrence of MDR bacteria, especially in low- and middle-income countries frequently lacking a clear legislative framework about the use of antibiotics in the animal production sector [69].

In conclusion, this study provides the first set of scientific data on prevalence and multidrug-resistant Salmonella originating from commercial poultry in Ecuador. This evidence may be useful for implementation of official policies aiming to decrease the prevalence of Salmonella in poultry farms.
Supporting Information

S1 File. Fig A, ERIC-PCR profiles of the 59 tested Salmonella isolates. Fig B, PFGE profiles of the 62 Salmonella isolates collected from the positive broiler batches. Table A, Distribution of the minimal inhibitory concentration values for the 62 Salmonella isolates collected from the positive broiler batches.

(PDF)

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Author Contributions

Conceived and designed the experiments: CVB LDZ. Performed the experiments: CVB MC SB. Analyzed the data: CVB LDZ LRG. Contributed reagents/materials/analysis tools: CVB LDZ SB MC. Wrote the paper: CVB LDZ.

References

1. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O’Brien SJ, et al. The Global Burden of Nontyphoidal Salmonella Gastroenteritis. Clin Infect Dis. 2010; 50: 882–889. doi: 10.1086/650733 PMID: 20158401

2. WHO. WHO estimates of the global burden of foodborne diseases [Internet]. 2015. Available: http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/

3. WHO. Salmonella (non-typhoidal) Fact sheet N°139 [Internet]. 2015 [cited 5 Oct 2015]. Available: http://www.who.int/mediacentre/factsheets/fs139/en/

4. Ministerio de Salud Pública del Ecuador. GACETA EPIDEMIOLÓGICA SEMANAL No. 51 [Internet]. Quito, 2014 [cited 16 Mar 2016] pp. 1–40. Available: http://instituciones.msp.gob.ec/images/Documentos/Ministerio/EPIDEMIOLOGIA/gaceta2014/GacetaN51_opt.pdf

5. FAO. Risk assessments of Salmonella in eggs and broiler chickens [Internet]. FAO/WHO, editor. Geneva: FAO/WHO; 2002. Available: http://www.fao.org/docrep/005/y4392e/y4392e00.htm

6. Herman KM, Hall a J, Gould LH. Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. Epidemiol Infect. 2015; 1–11. doi: 10.1017/S0950268815000047

7. Antunes P, Mourão J, Campos J, Peixe L. Salmonellosis: the role of poultry-meat. Clin Microbiol Infect. 2015; doi: 10.1016/j.cmi.2015.12.004

8. Fernandez J, Fica A, Ebensperger G, Caiullan H, Prat S, Fernandez A, et al. Analysis of Molecular Epidemiology of Chilean Salmonella enterica Serotype Enteritidis Isolates by Pulsed-Field Gel Electrophoresis and Bacteriophage Typing. J Clin Microbiol. 2003; 41: 1617–1622. doi: 10.1128/JCM.41.4.1617–1622.2003 PMID: 12682153

9. Pazzaglia G, Wignall FS, Batchelor R, Alexander W, Vargas Alfaro L, Zavaleta A. [Outbreak of paratyphoid fever among Peruvian naval personnel]. Bol Oficina Sanit Panam. 1992; 112: 395–405. PMID: 1610504

10. Mercado M, Avila J, Rey M, Montoya M, Gamboa A, Carrascal AK, et al. Brotes por Salmonella spp., Staphylococcus aureus y Listeria monocytogenes asociados al consumo de pollo. Revisión sistemática de la literatura. Biomédica. 2012; 32. doi: 10.7705/biomedica.v32i2.697

11. Donado-Godoy P, Clavijo V, León M, Tafur MA, Gonzales S, Humé M, et al. Prevalence of Salmonella on retail broiler chicken meat carcasses in Colombia. J Food Prot. 2012; 75: 1134–8. doi: 10.4315/0362-028X.JFP-11-513 PMID: 22691484

12. Donado-Godoy P, Gardner I, Byrne BA, Leon M, Perez-Gutierrez E, Ovalle MV, et al. Prevalence, risk factors, and antimicrobial resistance profiles of Salmonella from commercial broiler farms in two important poultry-producing regions of Colombia. J Food Prot. 2012; 75: 874–83. doi: 10.4315/0362-028X. JFP-11-458 PMID: 22564936

13. Pulido-Landínez M, Sánchez-Ingunza R, Guard J, Nascimento VP Do. Assignment of serotype to Salmonella enterica isolates obtained from poultry and their environment in southern Brazil. Lett Appl Microbiol. 2013; 57: 288–294. doi: 10.1111/lam.12110 PMID: 23734786
14. Seiffert SN, Hilty M, Perreten V, Endimiani A. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? Drug Resist Updat. 2013; 16: 22–45. doi: 10.1016/j.drup.2012.12.001 PMID: 23395305

15. Reardon S. Antibiotic resistance sweeping developing world. Nature. 2014; 509: 141–2. doi: 10.1038/509141a PMID: 24805322

16. Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. Nat Rev Microbiol. 2014; doi: 10.1038/nrmicro3270

17. CONAVE. Estadísticas de Producción Avícola 2013 [Internet]. 2014 p. 1.

18. Rasschaert G, Houf K, Imberechts H, Grijspeerdt K, De Zutter L, Heyndrickx M. Comparison of five

19. CDC. Pathogens & Protocols. In: Standard Operating Procedure for PulseNet PFGE of Escherichia coli O157:H7, Escherichia coli non-O157 (STEC), Salmonella serotypes, Shigella sonnei and Shigella flexneri [Internet]. 2015 [cited 11 Sep 2015]. Available: http://www.cdc.gov/pulsenet/PDF/ecoli-shigella-salmonella-pfge-protocol-508c.pdf

20. Barrett TJ, Gerner-Smidt P, Swaminathan B. Interpretation of pulsed-field gel electrophoresis patterns in foodborne disease investigations and surveillance. Foodborne Pathog Dis. 2006; 3: 20–31. doi: 10.1089/ffd.2006.3.20 PMID: 16602976

21. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. Informational Supplement. Pennsylvania: Clinical and Laboratory Standards Institute; 2014.

22. EUCAST. European Committee on Antimicrobial Susceptibility Testing. Data from the EUCAST MIC distribution website [Internet]. 2015. Available: http://mic.eucast.org

23. Song W, Jeong SH, Kim JS, Kim HS, Shin DH, Roh KH, et al. Use of boronic acid disk methods to detect the combined expression of plasmid-mediated AmpC β-lactamases and extended-spectrum β-lactamases in clinical isolates of Klebsiella spp., Salmonella spp., and Proteus mirabilis. Diagn Microbiol Infect Dis. 2007; 57: 315–318. doi: 10.1016/j.diagmicrobio.2006.08.023 PMID: 17174510

24. CLSI. Performance Standards for Antimicrobial Susceptibility Testing. Informational Supplement. 2015.

25. Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. J Antimicrob Chemother. 2005; 56: 115–21. doi: 10.1093/jac/dki190 PMID: 15941775

26. Olesen I, Hasman H, Aarestrup FM. Prevalence of beta-lactamases among ampicillin-resistant Escherichia coli and Salmonella isolated from food animals in Denmark. Microb Drug Resist. 2004; 10: 334–40. doi: 10.1089/mdr.2004.10.334 PMID: 15650379

27. Arlet G, Rouveau M, Philippon A. Substitution of alanine for aspartate at position 179 in the SHV-6 extended-spectrum beta-lactamase. FEMS Microbiol Lett. 1997; 152: 163–7. Available: http://www.ncbi.nlm.nih.gov/pubmed/9228783 PMID: 9228783

28. Kruger T, Szabo D, Keddy KH, Ddeley EK, Hujer AM, et al. Infections with nontyphoidal Salmonella species producing TEM-63 or a novel TEM enzyme, TEM-131, in South Africa. Antimicrob Agents Chemother. 2004; 48: 4263–70. doi: 10.1128/AAC.48.11.4263–4270.2004 PMID: 15504851

29. Liu Y, Wang Y, Walsh TR, Yi L, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2015;3099. doi:10.1016/S1473-3099(15)00424-7

30. Bates D, Maechler M, Bolker B, Walker S, Christensen R, Singmann H, et al. Package “lme4”. Linear Mixed-Effects Models using “Eigen” and S4. [Internet]. CRAN Repository; 2016. Available: https://cran.r-project.org/web/packages/lme4/lme4.pdf

31. R Core Team. R: A Language and Environment for Statistical Computing [Internet]. [cited 21 Jun 2016]. Available: https://www.r-project.org/

32. Boscan-Duque LA, Arzaluz-Fisher AM, Ugartec C, Sanchez D, Wittum TE, Hoet AE. Reduced susceptibility to quinolones among Salmonella serotypes isolated from poultry at slaughter in Venezuela. J Food Prot. 2007; 70: 2030–2035. PMID: 17900079

33. Giombelli A, Gloria MBA. Prevalence of <i>Salmoneilla</i> and <i>Campylobacter</i> on Broiler Chickens from Farm to Slaughter and Efficiency of Methods To Remove Visible Fecal Contamination. J Food Prot. 2014; 77: 1851–1859. doi: 10.4315/0362-028X.JFP-14-200 PMID: 25364917

34. Donado-Godoy P, Gardner I, Byrne BA, Leon M, Perez-Gutiérrez E, Ovalle M V, et al. Prevalence, risk factors, and antimicrobial resistance profiles of Salmonella from commercial broiler farms in two
important poultry-producing regions of Colombia. J Food Prot. 2012; 75: 874–83. doi: 10.4315/0362-028X.JFP-11-458 PMID: 22564936

35. EFSA, ECDC. European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2013. EFSA J. 2015; 13: 1–162. doi: 10.2903/j.efsa.2015.3991

36. Donado-Godoy P, Clavijo V, León M, Arevalo A, Castellanos R, Bernal J, et al. Counts, serovars, and antimicrobial resistance phenotypes of Salmonella on raw chicken meat at retail in Colombia. J Food Prot. 2014; 77: 227–35. doi: 10.4315/0362-028X.JFP-13-276 PMID: 24490916

37. Medeiros MAN, Oliveira DCN de, Rodrigues D dos P, Freitas DRC de. Prevalence and antimicrobial resistance of Salmonella in chicken carcasses at retail in 15 Brazilian cities. Rev Panam Salud Publica. 2011; 30: 555–60. Available: http://www.ncbi.nlm.nih.gov/pubmed/22358402 PMID: 22358402

38. Hendriksen RS, Vieira AR, Carlesso M, Lo Fo Wong DMA, Jensen AB, Wegener HC, et al. Global monitoring of Salmonella serovar distribution from the World Health Organization Global Foodborne Infections Network Country Data Bank: results of quality assured laboratories from 2001 to 2007. Foodborne Pathog Dis. 2011; 8: 887–900. doi: 10.1089/fpd.2010.0787 PMID: 21492021

39. Franco A, Leekitcharoenphon P, Feltrin F, Alba P, Cordaro G, Luorescia M, et al. 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. 2015; 10: e0144802. doi: 10.1371/journal.pone.0144802

40. Hauser E, Tietze E, Helmuth R, Juncker E, Prager R, Schroeter A, et al. Clonal dissemination of Salmonella enterica serovar Infantis in Germany. Foodborne Pathog Dis. 2012; 9: 352–60. doi: 10.1089/fpd.2011.1038 PMID: 22401270

41. Rahmani M, Peighambari SM, Svendsen CA, Cavaco LM, Agera Y, Hendriksen RS. Molecular clonality and antimicrobial resistance in Salmonella enterica serovars Enteritidis and Infantis from broilers in three Northern regions of Iran. BMC Vet Res. 2013; 9: 66. doi: 10.1186/1746-6148-9-66 PMID: 23561048

42. Velthner M, Kozoderovi?? G, Grego E, Galí?? N, Stojanov I, Jelesi?? Z, et al. Clonal spread of salmonella enterica serovar infantis in Serbia: Acquisition of mutations in the topoisomerase genes gyrA and parC leads to increased resistance to fluoroquinolones. Zoonoses Public Health. 2014; 61: 364–370. doi: 10.1111/zph.12081 PMID: 24119387

43. Mattiello SP, Drescher G, Barth VC, Ferreira C a, Oliveira SD. Characterization of antimicrobial resistance in Salmonella enterica strains isolated from Brazilian poultry production. Antonie Van Leeuwenhoek. 2015; doi: 10.1007/s10482-015-0577-1

44. Hur J, Jawale C, Lee JH. Antimicrobial resistance of Salmonella isolated from food animals: A review. Food Res Int. 2012; 45: 819–830. doi: 10.1016/j.foodres.2011.05.014

45. EFSA. SCIENTIFIC REPORT OF EFSA AND ECDC. EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2013. EFSA J. 2015; 13: 1–178. doi: 10.2903/j.efsa.2015.4036

46. Kuang X, Hao H, Bai M, Wang Y, Ahmad I, Liu Z, et al. Serotypes and antimicrobial susceptibility of Salmonella spp. isolated from farm animals in China. Front Microbiol. 2015; 6: 602. doi: 10.3389/fmicb.2015.00602 PMID: 26157426

47. Diarra MS, Delaquais P, Rempel H, Bach S, Hartlon C, Aslam M, et al. Antibiotic resistance and diversity of Salmonella enterica serovars associated with broiler chickens. J Food Prot. 2014; 77: 40–49. doi: 10.4315/0362-028X.JFP-13-251 PMID: 24405997

48. Turki Y, Mehr I, Ouzari H, Khessairi A, Hassen A. Molecular typing, antibiotic resistance, virulence gene and biofilm formation of different Salmonella enterica serotypes. J Gen Appl Microbiol. 2014; 60: 123–130. doi: 10.2323/jgam.2014.60.123 PMID: 25273985

49. Hur J, Kim JK, Park JH, Lee YJ, Lee JH. Molecular and virulence characteristics of multi-drug resistant Salmonella Enteritidis strains isolated from poultry. Vet J. 2011; 189: 306–311. doi: 10.1016/j.tvjl.2010.07.017 PMID: 20822940

50. Olaitan AO, Dia NM, Gautret P, Benkouiten S, Belhouchat K, Drali T, et al. Acquisition of extended-spectrum cephalosporin- and colistin-resistant Salmonella enterica subsp. enterica serotype Newport by pilgrims during Hajj. Int J Antimicrob Agents. 2015; 45: 600–4. doi: 10.1016/j.ijantimicag.2015.01.016 PMID: 25769786

51. Lu Y, Wu C-M, Wu G-J, Zhao H-Y, He T, Cao X-Y, et al. Prevalence of antimicrobial resistance among Salmonella isolates from chicken in China. Foodborne Pathog Dis. 2011; 8: 45–53. doi: 10.1089/fpd.2010.0605 PMID: 21085118

52. Agera Y, Torpdahl M, Zachariasen C, Seyfarth A, Hammerum AM, Nielsen EM. Tentative colistin epidemiological cut-off value for Salmonella spp. Foodborne Pathog Dis. 2012; 9: 367–9. doi: 10.1089/fpd.2011.1015 PMID: 22300222
53. de Jong A, Smet A, Ludwig C, Stephan B, De Graef E, Vanrobaeys M, et al. Antimicrobial susceptibility of Salmonella isolates from healthy pigs and chickens (2008–2011). Vet Microbiol. 2014; doi: 10.1016/j.vetmic.2014.01.030

54. Morales AS, Fragoso de Araújo J, de Moura Gomes VT, Reis Costa AT, Prazeres Rodrigues D dos, Porfida Ferreira TS, et al. Colistin Resistance in Escherichia coli and Salmonella enterica Strains Isolated from Swine in Brazil. Sci World J. 2012; 2012: 1–4. doi: 10.1100/2012/109795

55. Malhotra-Kumar S, Xavier BB, Lammens C, Butaye P, Goossens H. Colistin resistance gene mcr-1 harboured on a multidrug resistant plasmid. Lancet Infect Dis. 2016; 3099: 7–8. doi: 10.1016/S1473-3099(16)00012-8

56. Hasman H, Hammerum A, Hansen F, Hendriksen R, Olesen B, Agersø Y, et al. Detection of mcr-1 encoding plasmid-mediated colistin-resistant Escherichia coli isolates from human bloodstream infection and imported chicken meat, Denmark 2015. Euro Surveill. 2015; 20: 30085. http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085

57. Rapoport M, Faccone D, Pasteran F, Ceriana P, Albornoz E, Petroni A, et al. mcr-1-mediated colistin resistance in human infections caused by Escherichia coli: First description in Latin America. Antimicrob Agents Chemother. 2016;563. doi: 10.1128/AAC.00573-16

58. Doumith Michel, Godbole Gauri, Ashton Philip, Lammens C, Butaye P, Hopkins Katie L and NW. Detection of the plasmid-mediated mcr-1 gene conferring colistin resistance in human and food isolates of Salmonella enterica and Escherichia coli in England and Wales. J Antimicrob Chemother. 2016;Accepted . doi: 10.1093/jac/dkw093

59. Quesada A, Ugarte-Ruiz M, Iglesias MR, Martínez R, Florez-Cuadrado D, et al. Detection of plasmid mediated colistin resistance (MCR-1) in Escherichia coli and Salmonella enterica isolated from poultry and swine in Spain. Res Vet Sci. 2016; 105: 134–135. doi:10.1016/j.rvsc.2016.02.003

60. Van Boeckel TP, Brower C, Grenfell BT, Levin S a, Robinson TP, et al. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A. 2015; 1–6. doi:10.1073/pnas.1503141112

61. WHO. WHO Model Lists of Essential Medicines. In: Essential medicines and health products [Internet]. 2015 [cited 25 Jan 2016]. Available: http://www.who.int/medicines/publications/essentialmedicines/en/

62. Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin S a, Robinson TP, et al. Global trends in antimicrobial use in food animals. Proc Natl Acad Sci U S A. 2015; 1–6. doi:10.1073/pnas.1503141112

63. WHO. Worldwide country situation analysis: response to antimicrobial resistance [Internet]. WHO Press. 2015. ISBN: 978 92 4 156494 6