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Research Article
Phenotypic and Genotypic Resistance of *Salmonella* Heidelberg Isolated From One of the Largest Poultry Production Region from Colombia

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Abstract

Background and Objective: *Salmonella enterica* is a zoonotic pathogen transmitted mainly by consumption of contaminated food from animal origin, especially poultry products. Recently, multidrug resistant *Salmonella* isolates have been reported as a public health concern, demanding active surveillance. The aim of this study was to analyze both the phenotypic and genotypic antibiotic resistance patterns of *Salmonella* isolates from healthy chickens in poultry farms of Santander, Colombia. **Materials and Methods:** *Salmonella* was isolated from cloacal swabs and characterized by microbiological methods, serotyped and molecularly confirmed by amplification of *invA* gene. Antibiotic resistance was determined by automated method and agar diffusion method as well as the presence of resistance genes was assessed by PCR. **Results:** The *Salmonella* prevalence was 2.8% (15/540) and all isolates were serotyped as *Salmonella* Heidelberg. All isolates showed phenotypic resistance to 11 out 24 antibiotics evaluated, belonging to quinolones, fluoroquinolones, cephalosporins, β-lactams, aminoglycosides and tetracyclines. Regarding genotypic resistance, all isolates showed the presence of four genes associated with antibiotic resistance, such as *strA* and *strB* genes for streptomycin, the gene bla_{CMY2} that confers resistance to ceftriaxone and the gene *sul1* associated with resistance to trimethoprim/sulfamethoxazole. **Conclusion:** These results indicate that all isolates of *Salmonella* Heidelberg from poultry farms in Santander, Colombia, are phenotypic and genotypic multiresistant, representing a potential risk to public health. The results also provide information to the resistome present in *Salmonella* strains from the broiler chicken production chain and update the serotypes present in poultry farms in Colombia.

Key words: Antibiotic resistance, egg contamination, human infection, poultry meat, *Salmonella*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Salmonella enterica subsp. *enterica* is a zoonotic pathogen which accounts with the majority of serotypes that affect human beings and domestic animals[1]. In many countries high incidence of salmonellosis in humans has been related with consumption of contaminated eggs, poultry meat and meat-products[2]. By the year 2008, 16 millions of people with typhoid fever were registered, 1.3 million with gastroenteritis and 3 million deaths throughout the world[3]. On the other hand, in poultry the transmission can be caused by the use of contaminated raw materials for the food manufacturing, poultry bedding, feed and the interaction with wild birds[4,5].

The disease is characterized by a self-limiting gastrointestinal infection with fever, diarrhea and acute abdominal pain. Nevertheless, it may progress into life-threatening disease in young children, elderly and immunocompromised patients[1,6]. On the other hand, in poultry the disease can be asymptomatic or with a clinical course characterized by diarrhea and dehydration in the affected lots, resulting in severe economic losses[5]. The treatment is based on the use of antibiotics to control the infection. However, in the last two decades multidrug resistant (MDR) *Salmonella* isolates have been increasing and become a major public health hazard[7]. The emergence of MDR strains has been associated with the inappropriate use of antibiotics and their use as growth promoters, especially in poultry and swine production[1].

The region of Santander in Colombia, participates in 25% of the poultry production of the country, producing 340 000 t of chicken meat and 2 900 million eggs annually[8]. However, in this region limited information is available on the circulating serotypes of *Salmonella* in poultry products, the serotypes responsible for human infections as well as their antibiotic resistance. In poultry farms of Santander, *Salmonella* strains (n=106) were isolated from 1-day-old chicks[9] and the overall prevalence of *Salmonella* has been reported higher than 40%[10]. The aim of the present study was to determine the phenotypic and genotypic resistance to antibiotics of the serotypes of *Salmonella* spp. from broilers located in poultry farms in the region of Santander.

MATERIALS AND METHODS

Sample collection: The study was carried out in four poultry farms located in the region of Santander with capacity for 70 000 broilers. The sample size was calculated based on the formula of Thrustfield[11] which yielded a minimum value of 256 samples. However, 540 samples were taken by cloacal swabbing from broilers of the Ross 308 genetic line on day 35 of production. All samples were deposited in test tubes with peptone water and refrigerated for further processing at Veterinary Diagnostic Laboratory of the Faculty of Veterinary Medicine and Zootechnics of the University of Tolima.

***Salmonella* isolation:** All samples were processed according to the international guidelines ISO 6579-1[12]. Briefly, samples were incubated for 24 h at 37°C in peptone-buffered water, subsequently the samples were placed in tetrathionate broth incubating at 37°C and in Rappaport Vassiliadis incubated at 42°C for selective enrichment. Then, the samples were seeded on SS agar and XLD agar. Compatible colonies were seeded in the non-selective media McConkey and Trypticase soy agar and confirmed as *Salmonella* spp. by challenge with antiserum Poly A-I and Vi (Difco, USA). The biochemical confirmation of *Salmonella* was made through the API® 20E (Bio-Mérieux, France). Furthermore, the molecular confirmation of *Salmonella* spp. was carried out with endpoint PCR by amplification of the *invA* gene (NC_003197.2) by using the forward (GTGAAATTATCG CCACGTTCGGGCAA) and reverse (TCATCGCACCG TCAAAGGAACC) primers with an amplicon size of 285 base pairs (bp)[13].

Serotyping: The isolates were serotyped following the Kauffman-White-Le Minor scheme using polyvalent antisera for groups (A-D)[14]. Serotyping was carried out at the National Veterinary Diagnostic Laboratory of the ICA-Colombia.

Antibiotic susceptibility test: The antibiotic susceptibility test was carried out through the Neg Combo 72 panel for automated system MicroScan (Beckman Coulter, USA). This process was carried out and interpreted in accordance of the guidelines described for Clinical and Laboratory Standards Institute[15] and the guidelines described by the manufacturer. Alternatively, other antibiotics of interest were assessed by Kirby-Bauer method based on the international guidelines[15] (Table 1). Isolates were considered as MDR when they showed resistance to three or more classes of antibiotics.

Genotypic resistance to antibiotics: DNA was extracted from fresh colonies using Easy-DNA kit (Invitrogen, USA) and stored

Table 1: List of antibiotics evaluated through the minimum inhibitory concentration (MIC) method using automated system Microscan (μg mL^{-1}) and Kirby-Bauer agar diffusion method (mm)

		Interpretation categories of the CIM breakpoints*		
Antibiotic	Concentration (μg)	S (≤)	I	R (≥)
Nalidixic acid (μg mL^{-1})	30	16	-	32
Ampicillin/sulbactam (μg mL^{-1})	10-10	8-4	16-8	32-16
Ampicillin (μg mL^{-1})	10	8	16	32
Cefotaxime (μg mL^{-1})	30	1	2	4
Ceftazidime (μg mL^{-1})	30	4	8	16
Ciprofloxacin (μg mL^{-1})	5	0.06	0.12	1
Ertapenem (μg mL^{-1})	10	0.5	1	2
Imipenem (μg mL^{-1})	10	1	2	4
Levofloxacin (μg mL^{-1})	5	0.12	1	2
Meropenem (μg mL^{-1})	10	1	2	4
Piperacillin/tazobactam (μg mL^{-1})	100-10	16-4	64/4	128/4
Tetracycline (μg mL^{-1})	30	4	8	16
Trimethoprim/sulfamethoxazole (μg mL^{-1})	1.25-23.75	2-38	-	4-76
Aztreonam (μg mL^{-1})	30	4	8	16
Cefazolin (μg mL^{-1})	30	2	4	8
Chloramphenicol (μg mL^{-1})	30	8	16	32
Cefepime (μg mL^{-1})	30	2	4	16
Ticarcillin/clavulanic acid (μg mL^{-1})	75/10	16-2	64-2	128-2
Ceftriaxone (mm)	30	23	20-22	19
Enrofloxacin (mm)	5	21	16-20	15
Florfenicol (mm)	30	19	15-18	14
Gentamicin (mm)	10	15	13-14	12
Streptomycin (mm)	10	15	12-14	11

*S: Susceptible, I: Intermediate, R: Resistant

at -20°C until its use. Bacterial DNA was used as a template in order to determine the presence of resistance genes for antibiotics using specific primer sets (Table 2) by endpoint PCR. For PCR, a total volume of 25 μL was prepared for each sample containing 1 μL of the DNA template, 1 μL of each primer (forward and reverse) (Invitrogen, USA), 1 μL of Taq DNA polymerase (Invitrogen, USA), 2.5 μL of DNTPs (Invitrogen, USA) and buffer, 2 μL of $MgCl_2$ and 14 μL of nuclease-free water. The PCR was run in a T100 thermal cycler (BIO-RAD, USA) with an initial denaturation step of 3 min at 95°C, followed by 35 cycles as follows: 30 sec at 95°C for denaturation, 30 sec at 55°C for annealing, 30 sec at 72°C for extension and a final extension step of 7 min at 72°C. Amplification products were revealed by horizontal electrophoresis on 2% agarose gel stained with GelGreen® (Biotium, Russia) using the PowerPac HC (BIO-RAD, USA). The gel was visualized and documented using the gel documentation system ENDURO GDS (Labnet international, USA).

RESULTS

Isolation and serotyping of *Salmonella* from broiler samples: A total of 540 samples (cloacal swabs) from broilers distributed in four poultry farms located in the region of Santander were analyzed and 15 isolates of *Salmonella* were recovered. All isolates were identified as *Salmonella* Heidelberg.

Phenotypic antibiotic resistance of *Salmonella* Heidelberg: All 15 isolates were resistant to 11 antibiotics belonging to quinolones and fluoroquinolones (nalidixic acid, ciprofloxacin and levofloxacin), cephalosporins (cefotaxime, ceftazidime, cefazolin and ceftriaxone), β-lactams (ampicillin, ampicillin/sulbactam), aminoglycosides (streptomycin) and tetracyclines (tetracycline). High resistance rates were observed for trimethoprim/sulfamethoxazole (93%), aztreonam and cefepime (46%). Lower levels of resistance were found for enrofloxacin (20%), ticarcillin/clavulanic acid (20%) and piperacillin/tazobactam (6%). All isolates were susceptible to carbapenems (ertapenem, imipenem, meropenem), phenicols (chloramphenicol, florfenicol) and aminoglycosides (gentamicin) (Table 3).

Genotypic antibiotic resistance of *Salmonella* Heidelberg: The genotypic analysis showed the presence of four genes associated with antibiotic resistance in all *Salmonella* isolates such as *strA*, *strB*, bla_{CMY2} and *sul1*. It was found low presence

Table 2: Primers used to evaluate the presence of resistance genes in *Salmonella* spp. isolates*

Antibiotic	Gene	Primer sequence	Amplicon size (bp)
Ampicillin	bla_{PSE-1}	F-GCAAGTAGGGCAGGCAATCA	422
		R-GAGCTAGATAGATGCTCACAA	
	bla_{TEM}	F-ATCAGTTGGGTGCACGAGTG	608
		R-ACGCTCACCGGCTCCAGA	
Chloramphenicol	*catA*	F-CCAGACCGTTCAGCTGGATA	454
		R-CATCAGCACCTTGTCGCCT	
	catB	F-CGGATTCAGCCTGACCACC	461
		R-ATACGCGGTCACCTTCCTG	
	cmlA	F-TGGACCGCTATCGGACCG	641
		R-CGCAAGACACTTGGGCTGC	
Gentamicin	*aadB*	F-CTAGCTGCGGCAGATGAGC	300
		R-CTCAGCCGCCTCTGGGCA	
Spectinomycin	*aadA1*	F-CTCCGCAGTGGATGGCGG	631
		R-GATCTGCGCGCGAGGCCA	
	aadA2	F-CATTGAGCGCCATCTGGAAT	500
		R-ACATTTCGCTCATCGCCGGC	
Tetracycline	*tetA*	F-GCTGTCGGATCGTTTCGG	658
		R-CATTCCGAGCATGAGTGCC	
	tetB	F-CTGTCGCGGCATCGGTCAT	615
		R-CAGGTAAAGCGATCCCACC	
Piperacillin/tazobactam	*dfrA1*	F-CAATGGCTGTTGGTTGGAC	254
		R-CCGGCTCGATGTCTATTGT	
	dfrA10	F-TCAAGGCAAATTACCTTGGC	432
		R-ATCTATTGGATCACCTACCC	
	dfrA12	F-TTCGCAGACTCACTGAGGG	330
		R-CGGTTGAGACAAGCTCGAAT	
Streptomycin	*strA*	F-TGGCAGGAGGAACAGGAGG	405
		R-AGGTCGATCAGACCCGTGC	
	strB	F-GCGGACACTTTTCCAGCCT	621
		R-TCCGCCATCTGTGCAATGCG	
Ceftriaxone	bla_{CMY2}	F-AAATCGTTATGCTGCGCTCT	224
		R-CCGATCCTAGCTCAAACAGC	
	bla_{CTX-M}	F-TTCGCTAAATACCGCCATTC	236
		R-TATCGTTGGTTGTGCCGTAA	
Trimethoprim/sulfamethoxazole	*sul1*	F-CGGACGCGAGGCCTGTATC	591
		R-GGGTGCGGACGTAGTCAGC	
	sul2	F-GCGCAGGCGCGTAAGCTGAT	514
		R-CGAAGCGCAGCCGCAATTC	
	sul3	F-GGGAGCCGCTTCCAGTAAT	500
		R-TCCGTGACACTGCAATCATTA	
quinolones and fluoroquinolones	*oqxA*	F-GGTGAAGTCGATCAGTCAGT	154
		R-ATCTATCGTGAACAGCACCT	
Nalidixic acid	*qnrA*	F-CCGCTTTTATCAGTGTGACT	188
		R-ACTCTATGCCAAAGCAGTTG	

*Based in Chuanchuen and Padungtod[7]

of the *aadA1* gene (20%) and high presence of *sul2* gene (86%). None of the isolates were positive for the genes bla_{PSE-1}, bla_{TEM}, *catA*, *catB*, *cmlA*, *tetA*, *tetB*, *dfrA1*, *dfrA10*, *dfrA12*, *sul3*, *oqxA*, *qnrA*, *aadA2* and *aadB* (Table 2).

DISCUSSION

Prevalence and serotyped: In the present study, all isolates were identified by biochemical, serological and molecular methods. The *Salmonella* prevalence was 2.8%, which is lower than those reported in other regions of the world and in Colombia. In United States the prevalence of *Salmonella* in poultry farms was 7.7%[16], 10.9% in Egypt [2] and in Ethiopia was 4.7%[17]. In case of Latin America, the prevalence of isolates from poultry farms in Ecuador was 20.1%[18], in Brazil was 5.3%[19] and in backyard poultry in Argentina was 0.6%[20]. In Colombian regions the prevalence in commercial broiler farms was 40%[10], in commercial egg-laying hen farms was 33.3%[5] and in raw chicken was 17.41%[13].

Our study found that all *Salmonella* isolates belong to serotyped Heidelberg. In agreement with World Health Organization[21], *Salmonella* Heidelberg is one of the most common serotyped isolated from poultry and egg-containing products in North America. However, recent studies in several

Table 3: Phenotypic and genotypic profiles of resistance in *Salmonella* Heidelberg isolated from poultry farms in Santander, Colombia

Sample No.	Phenotypic resistance to antibiotics (a)	Genotypic resistance to antibiotics (b)
1	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, LVX, S, TE, T/S	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
2	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, LVX, S, TE, T/S	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
3	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, FOS, LVX, S, TE, T/S	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
4	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, LVX, S, TE, T/S, TIM	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
5	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, LVX, S, TE, T/S	bla_{CMY2}, *strA*, *strB*, *sul1*
6	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, FOS, LVX, S, TE, T/S	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
7	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, FOS, LVX, S, TE, T/S	bla_{CMY2}, *strA*, *strB*, *sul1*
8	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, FOS, LVX, S, TE, T/S	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
9	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, FOS, LVX, S, TE, T/S, AZT, CPE, TIM	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
10	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, FOS, LVX, S, TE, T/S, AZT, CPE	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
11	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, ENR, LVX, S, TE, AZT, CPE, TIM	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
12	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, FOS, LVX, S, P/T, TE, T/S, AZT, CPE	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
13	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, ENR, LVX, S, TE, T/S, AZT, CPE	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
14	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, FOS, LVX, S, TE, T/S, AZT, CPE, TIM	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*
15	NA, A/S, AM, CFT, CFZ, CAZ, CP, CRO, ENR, LVX, S, TE, T/S, AZT, CPE, TIM	bla_{CMY2}, *strA*, *strB*, *sul1*, *sul2*

(a) Profiles of phenotypic resistance to antibiotics determined by MicroScan and Kirby-Bauer method. NA: Nalidixic acid, A/S: Ampicillin/sulbactam, AM: Ampicillin, CFT: Cefotaxime, CFZ: Cefazolin, CAZ: Ceftazidime, CP: Ciprofloxacin, CRO: Ceftriaxone, ENR: Enrofloxacin, FOS: Fosfomycin, LVX: levofloxacin, S: Streptomycin, TE: Tetracycline, T/S: Trimethoprim/sulfamethoxazole, AZT: Aztreonam, CPE: Cefepime, P/T: Piperacillin/tazobactam, TIM: Ticarcillin/clavulanic acid. (b) Profiles of genotypic resistance to antibiotics determined by PCR bla_{CMY2} ceftriaxone and ceftiofur, *strA*, *strB* streptomycin, *sul1, sul2* sulfamethoxazole

regions of Colombia indicates that *Salmonella* Heidelberg was the second most prevalent serotyped isolated from broiler farms (22.7%) and chicken meat (19%)[10,22], the third most prevalent serotyped isolated from broiler farms in 3 Brazilian states (7.31%)[19] and the second most prevalent serotype from poultry at slaughterhouses in Venezuela (31%)[23]. This shows that *S.* Heidelberg has emerged as a predominant serotype in different parts of poultry production chain in South America.

Noteworthy is that this serotyped has been associated with invasive human infections through poultry products. *Salmonella* Heidelberg was recovered from 110 samples of chicken breast collected during 2 002-2 006 in U.S.[24] and in 7 samples of raw poultry in retail markets in Guatemala[25] and recently, *Salmonella* Heidelberg has been isolated from 643[26] and 263[27] patients infected by consumption of poultry products.

Phenotypic resistance: All isolates of *Salmonella* Heidelberg were classified as MDR which has particular concern since MDR strains have been implicated in human outbreaks[27–29]. However, in South America there are few studies about MDR *S.* Heidelberg in poultry and by-products. In Brazil MDR *Salmonella* Heidelberg was reported in 54 and 6 isolates from poultry farms[30] and slaughterhouses[31], respectively. In Colombia, 93% of *Salmonella* Heidelberg isolated from poultry farms were MDR[10]. The high resistance to antibiotics of *Salmonella* Heidelberg in poultry production may be the result of the appearance of emerging serotypes which displaced the common isolated serotypes[10,30,32]. In other studies, in *Salmonella* Heidelberg in U.S. was reported that only the 9.3% of the isolates from poultry were MDR[33]. In human patients from U.S. *Salmonella* Heidelberg showed MDR in 34.7% of the isolates from an outbreak linked to a poultry company[26] and in 14.8% of the isolates from North Carolina state[32].

In our study, *Salmonella* isolates showed resistance to at least four families of antibiotics (β-lactams, quinolones and fluoroquinolones, cephalosporins and tetracyclines). Regarding β-lactams family, ampicillin resistance (100%) was similar to the findings in *Salmonella* isolated in the poultry industry in Ecuador (91.7%)[18] and higher than reported in poultry at slaughterhouses in Venezuela (10%)[23]. Resistance to piperacillin/tazobactam (6%) was higher than reported in Colombia in commercial egg-laying hen farms (0%)[5] and in chicken carcasses (0%)[6]. Regarding quinolones and fluoroquinolones family, the resistance to nalidixic acid (100%) was similar as reported in *Salmonella* Heidelberg isolated from poultry origin samples in Brazil (100%)[30] and higher than poultry farms in Colombia (80.3%)[10]. Resistance to ciprofloxacin (100%) was higher than the reported in *Salmonella* Heidelberg isolated from poultry at slaughterhouses in Venezuela (30%)[23] and in *Salmonella* isolated from Cundinamarca (56.8%) and Santander (40.9%) in Colombia[10]. In the case of levofloxacin (100%) the resistance was higher than reported in Colombia in isolates from poultry farms Cundinamarca (2.3%)[10] and in isolates from poultry and humans with gastroenteritis (0%)[29].

Regarding to cephalosporin family, ceftriaxone resistance (100%) was also high compared with findings in *Salmonella* Heidelberg isolated from poultry farms in Brazil (9.3%)[30] and in isolates from poultry and humans with gastroenteritis (0%) in Colombia[29]. This result is particularly critical due to the importance of this antibiotic for the treatment of *Salmonella* infections especially in children and pregnant women[34].

Cefotaxime resistance (100%) was higher than reports in poultry farms (59%), slaughterhouses (59%) and chicken meat (33%) in Colombia[28] and similar as reported in poultry farms of Ecuador (91.7%)[18]. All isolates showed resistance to ceftazidime, which is higher than reported in *Salmonella* isolated from poultry farms in Colombia (18.2%)[10] and from poultry at slaughter in Venezuela (0%)[23]. Cefepime resistance (46%) was higher than reported in Colombian isolates from poultry farms and humans with gastroenteritis (0%)[29] and in poultry farms from two different regions (0%)[10]. Cefazolin resistance was present in all isolates and was higher than the finding in isolates from Cundinamarca (18.6%) and Santander (69.7%) (Colombia)[10]. Conversely, was reported that none of the isolates from chicken carcasses showed resistance[6].

Tetracycline resistance (100%) was similar to the findings in *S.* Heidelberg from poultry at slaughterhouses in Venezuela (100%)[23] and higher than reported in Brazilian poultry farms (64.8%)[30]. In case of carbapenem family, all isolates were susceptible to ertapenem, imipenem and meropenem, similar to the results obtained in Colombian poultry farms from Santander and Tolima regions[10,29].

None of the isolates showed resistance to enrofloxacin, similar to the findings in *Salmonella* from poultry farms in Brazil[19] and from backyard chickens in Argentina[20]. In the same way, none of the isolates was resistant to phenicols. However, reports of isolates from chicken markets in Colombia exhibited resistance to chloramphenicol at a frequency of 6.38%[6] and reports in *Salmonella* Heidelberg from poultry at slaughterhouses showed resistance to chloramphenicol at a frequency of 100%. The lack of use of these antibiotic in most of the animal productions may explain the absence of resistance in the isolates in our study[23].

In case of aminoglycosides family, all the isolates were resistant to streptomycin, higher than reports in *Salmonella* from broiler farms in Brazil (24.39%)[19], as well as from raw chicken meat in Colombia (66.8%)[22]. Conversely, in our study all the isolates were susceptible to gentamicin. In contrast, in egg-laying hen farms from Ibagué was reported that all isolates were resistant[5].

Regarding monobactams family, aztreonam resistance (46%) was higher than reported in Colombian isolates from poultry farms in Cundinamarca (0%) and Santander (13.6%)[10] and in chicken carcasses in Tolima (0%)[6]. The resistance to trimethoprim/sulfamethoxazole (93%) was higher than reported in isolates from poultry farms in Brazil (17.07%)[19] and from poultry farms in Colombia (71.2%)[10]. Ticarcillin/clavulanic acid resistance (20%) was higher than reported in Colombian isolates from egg laying hen farms[5] and from chicken carcasses (0%)[6].

Genotypic resistance: The results of molecular analysis showed that none of the isolates carried the genes bla_{PSE-1} and bla_{TEM} that confer resistance to ampicillin; however, phenotypically all isolates were resistant to ampicillin. This was similar in the genes *qnrA* associated with resistance to nalidixic acid and *tetA* and *tetB* associated with resistance to tetracycline in which all isolates showed resistance, suggesting that phenotypical resistance may be mediated by others mechanisms different that the proteins coded by genes assessed in this study (Table 4). The gene bla_{CMY2} was present

Table 4: Phenotypic and genotypic percentage of resistance in *Salmonella* Heidelberg isolated from broilers in poultry farms of Santander, Colombia

Antibiotic	Phenotypic antibiotic resistance (%)	Resistance gene	Genotypic antibiotic resistance (%)
Nalidixic acid	100	*qnrA*	0
Ampicillin	100	bla_{PSE-1}	0
		bla_{TEM}	0
Ceftriaxone	100	bla_{CMY2}	100
		bla_{CTX-M}	0
Chloramphenicol	0	*catA*	0
		catB	0
		cmlA	0
Streptomycin	100	*strA*	100
		strB	100
		aadA1	0
		aadA2	0
Gentamicin	0	*aadB*	0
Trimethoprim/sulfamethoxazole	93	*sul1*	100
		sul2	86
		sul3	0
		dfrA1	0
		dfrA10	0
		dfrA12	0
Tetracycline	100	*tetA*	0
		tetB	0

in all the isolates and the gene bla_{CTX-M} was absent. In contrast, a high number of isolates from poultry farms in Brazil carry the bla_{CMY} gene, also presenting the phenotypic resistance[35]. On the other hand, in isolates from different poultry production levels in Colombia was reported a higher presence of bla_{CMY2} (n = 168) than bla_{CTX-M} (n = 52). This result is pivotal due the appearance of extended spectrum β-lactamase genes is of particular concern in poultry and public health around the world since these antibiotics are on the list of essential medicines of WHO[36].

Among chloramphenicol susceptible strains, none harbored the genes *catA*, *catB* and *cmlA*, which agrees with susceptibility in all the isolates. The *aadB* gene was not detected in the strains susceptible to gentamicin, which differs with reports of isolates from chicken carcasses in Colombia[6]. Regarding trimethoprim/sulfamethoxazole-resistant isolates, none harbored the genes *dfrA1*, *dfrA10* and *dfrA12* suggesting that resistance totrimethoprim / sulfamethoxazole is probably mediated only by *sul1* and *sul2* genes. Currently, *sul1* has had great relevance due to class I integrons are always associated with these genes facilitating its horizontal transfer to other bacteria. Most of streptomycin-resistant isolates contained *strA* and *strB* genes but none carry the genes *aadA1* and *aadA2*, which in agreement with *Salmonella* isolates from leafy vegetables and chicken carcasses in Malaysia[37].

CONCLUSION

In conclusion, this study found *Salmonella* Heidelberg isolates from poultry farms in Santander were resistant to multiple antibiotics by both phenotypic and genotypic tests. The results also provide information to the resistome present in *Salmonella* strains from the broiler chicken production chain and update the serotypes present in poultry farms in Colombia.

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