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Counts, Serovars, and Antimicrobial Resistance Phenotypes of *Salmonella* on Raw Chicken Meat at Retail in Colombia

PILAR DONADO-GODOY,[1] VIVIANA CLAVIJO,[1] MARIBEL LEÓN,[2] ALEJANDRA AREVALO,[1] RICARDO CASTELLANOS,[1] JOHAN BERNAL,[1] MC ALLISTER TAFUR,[2] MARIA VICTORIA OVALLE,[1] WALID Q. ALALI,[3]* MICHAEL HUME,[4] JUAN JOSE ROMERO-ZUÑIGA,[5] ISABEL WALLS,[6] AND MICHAEL P. DOYLE[3]

[1]CORPOICA-Corporación Colombiana de Investigación Agropecuaria–CBB–Centro de Biotecnología y Bioindustria, Km 14, Via Mosquera, Cundinamarca, Colombia; [2]Instituto Colombiano Agropecuario (ICA), Carrera 41 No. 17-81, Bogotá DC, Colombia; [3]Center for Food Safety, University of Georgia, 1109 Experiment Street, Griffin, Georgia 30223, USA; [4]Department of Agriculture, Agricultural Research Service, Food Animal Protection Research Laboratory, 2881 F&B Road, College Station, Texas 77845, USA; [5]Universidad Nacional de Costa Rica, Programa de Investigación en Medicina Poblacional Escuela de Medicina Veterinaria, P.O. Box 304-3000 Heredia, Costa Rica; and [6]United States Department of Agriculture, National Institute of Food and Agriculture, Washington, DC 20250, USA

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ABSTRACT

The objective of this study was to determine *Salmonella* counts, serovars, and antimicrobial-resistant phenotypes on retail raw chicken carcasses in Colombia. A total of 301 chicken carcasses were collected from six departments (one city per department) in Colombia. Samples were analyzed for *Salmonella* counts using the most-probable-number method as recommended by the U.S. Department of Agriculture, Food Safety Inspection Service protocol. A total of 378 isolates (268 from our previous study) were serotyped and tested for antimicrobial susceptibility. The overall *Salmonella* count (mean log most probable number per carcass $\pm$ 95% confidence interval) and prevalence were 2.1 (2.0 to 2.3) and 37%, respectively. There were significant differences ($P < 0.05$) by *Salmonella* levels (i.e., counts and prevalence) by storage temperature (i.e., frozen, chilled, or ambient), retail store type (wet markets, supermarkets, and independent markets), and poultry company (chicken produced by integrated or nonintegrated company). Frozen chicken had the lowest *Salmonella* levels compared with chicken stored at other temperatures, chickens from wet markets had higher levels than those from other retail store types, and chicken produced by integrated companies had lower levels than nonintegrated companies. Thirty-one *Salmonella* serovars were identified among 378 isolates, with *Salmonella* Paratyphi B tartrate-positive (i.e., *Salmonella* Paratyphi B dT+) the most prevalent (44.7%), followed by Heidelberg (19%), Enteritidis (17.7%), Typhimurium (5.3%), and Anatum (2.1%). Of all the *Salmonella* isolates, 35.2% were resistant to 1 to 5 antimicrobial agents, 24.6% to 6 to 10, and 33.9% to 11 to 15. Among all the serovars obtained, *Salmonella* Paratyphi B dT+ and *Salmonella* Heidelberg were the most antimicrobial resistant. *Salmonella* prevalence was determined to be high, whereas cell numbers were relatively low. These data can be used in developing risk assessment models for preventing the transmission of *Salmonella* from chicken to humans in Colombia.

Salmonella is one of the major causes of foodborne gastroenteritis in humans worldwide; in addition, salmonellosis has been associated with a huge economic burden due to the cost of medical care and labor losses *(34)*. Among foods of animal origin, chicken meat is classified as a significant source of foodborne salmonellosis *(6, 18, 21)*. The severity of salmonellosis in humans can be related to multiple factors such as *Salmonella* serovar, counts, and antimicrobial resistance characteristics of the pathogen *(23)*.

In Colombia, epidemiologic studies have been conducted mainly at preharvest broiler farms to determine risk factors associated with the prevalence of *Salmonella* spp., as well as phenotypic and genotypic characteristics of the pathogen *(9, 11)*. The authors reported that the *Salmonella* prevalence on farms was 41%, and the significant risk factors were cleaning fixed equipment and composting dead birds at the farms *(11)*. Furthermore, when comparing serovars obtained from chicken farms and retail chicken meat, similar genotypes (i.e., molecular subtypes) of *Salmonella* Paratyphi B dT+ and *Salmonella* Heidelberg were present on farms and on raw chicken meat from retail outlets, suggesting the salmonellae were disseminated along the broiler chicken production chain *(9, 11)*. However, the study conducted on retail meat was limited in geographical distribution (i.e., limited to one city [Bogota]), with a relatively small sample size, and only chicken thigh samples (25 g) were tested for *Salmonella*. Moreover, there is minimal published research for Colombia that focuses on *Salmonella* quantities (i.e., cell counts), serovars, and antimicrobial resistance phenotypes on raw chicken meat at the retail level.

Salmonella Enteritidis and Typhimurium are the most common serovars implicated in gastroenteric infections

* Author for correspondence: Tel: 770-467-6066; Fax: 770-229-3216; E-mail: walali@uga.edu.

worldwide, as well as in Colombia *(20)*. According to a study conducted by the National Institute of Health of Colombia between 1997 and 2011, 23 *Salmonella* serovars were identified from 5,641 human enteric *Salmonella* isolates *(20)*. *Salmonella* Typhimurium (32.3%) and Enteritidis (28.9%) were the most prevalent serovars.

The antimicrobial resistance phenotypes of *Salmonella* could vary, depending on the serovar, as has been revealed in previous studies *(16, 32)*. For instance, in a study conducted at chicken farms in Colombia, 89% of antimicrobial-resistant *Salmonella* Paratyphi B dT+ (tartrate-positive) isolates were resistant to more than five drugs, whereas 80.7% of antimicrobial-resistant *Salmonella* Enteritidis isolates were resistant to less than three drugs *(9, 11)*.

In our previous study conducted in 2011 to estimate the baseline *Salmonella* prevalence on raw chicken meat at the retail level in Colombia, we determined that 27% (n = 1,003) of whole chicken carcasses were *Salmonella* positive *(10)*. However, collecting quantitative data on *Salmonella* counts/cell numbers on raw chicken meat is necessary because the human health of *Salmonella* is based on a dose-response relationship, and low *Salmonella* cell numbers may not pose a major risk if the temperature abuse at retail or in the home does not occur, even though the prevalence is high. In addition, data on the frequency of common and unique *Salmonella* serovars and their antimicrobial resistance profiles on raw chicken are limited in Colombia.

As a follow-up to our study conducted in 2011 *(10)*, where we determined the baseline *Salmonella* prevalence on raw chicken at retail in 23 departments in Colombia, the objectives of this study were to (i) determine *Salmonella* cell counts on raw chicken at the retail market in six departments where *Salmonella* prevalence was the highest (based on data from our previous study) and (ii) phenotypically characterize (serovars and antimicrobial resistance) *Salmonella* isolates from both the previous and the current study to have a better geographical representation of Colombia.

MATERIALS AND METHODS

Study design and sampling procedure for quantitative analysis of *Salmonella*. A cross-sectional study was carried out between August 2011 and May 2012 to determine quantitatively the counts of *Salmonella* on retail chicken meat in Colombia. The study design and the sampling plan were based on data from our previous study *(10)*. In that, we determined the baseline *Salmonella* prevalence on raw chicken meat (whole chicken carcasses) at retail in 23 departments (i.e., provinces) in Colombia *(10)*. Based on these prevalence data *(10)*, 301 whole chicken carcasses were collected in this study from retail stores in the capital cities of six departments where *Salmonella* prevalence was the highest *(10)*. A sample size of 301 broiler carcasses was determined, using an absolute error of 5%, with a 95% level of confidence and an expected prevalence of 27% *(10)*. The sampling strategy was designed in a similar manner as in the previous study *(10)*, using a stratified sampling approach, in which the city, locality (i.e., district), and store type were the primary, secondary, and tertiary units, respectively. Sampling was conducted in the following six capital cities: Bogotá, Bucaramanga, Ibague, Barranquilla, Pasto, and Sincelejo. The population of these cities corresponded to 52% of the total population of Colombia. The chicken carcasses were collected from the retail stores that had at least one *Salmonella*-positive sample based on results of the Donado-Godoy et al. *(10)* study.

Samples were collected from three types of retail stores: supermarkets, independent stores, and wet markets. Supermarkets were nationally recognized brand chain stores that sold chickens (conventional and free range) chilled or frozen, supplied mostly by large integrated poultry companies and some small-scale poultry farmers. Independent stores were those that belonged to either integrated poultry companies or small-scale nonintegrated poultry companies. Carcasses of conventionally raised or free-range chickens were sold either chilled or frozen. Wet markets were open markets (within a shopping plaza) that included meat stores as well as fruit and vegetable stores. Carcasses of conventionally raised or free-range chickens were sold at ambient temperature, chilled or frozen. Data on the following variables were recorded when chicken samples were collected: retail store type, chicken production system (conventional or free range), storage temperature (frozen [$<-5^{\circ}$C], chilled [4 to 10°C], or ambient [no temperature control]), poultry company (integrated or nonintegrated), social economic stratum, retail store name, retail store address, locality, and chicken price.

The social economic stratum was determined using the classification from the Bogotá Planning Department based on location, income, and surrounding areas, for which stratum 1 was the lowest and stratum 6 was the highest *(14)*. The mean price per carcass was US$5.74 ($\pm$3.5).

Quantitative *Salmonella* analysis using the three-tube MPN method. Whole broiler chicken carcasses (chilled, frozen, or at ambient storage condition) were purchased from retail stores, held on ice in insulated containers, and transported to the laboratory within 24 h. Upon arrival, frozen chickens were thawed at room temperature (within 2 h). Enumeration of *Salmonella* was conducted according to the most-probable-number (MPN) method recommended by the U.S. Department of Agriculture, Food Safety and Inspection Service (USDA-FSIS) *(29)*. Each carcass was placed in a 1,000-ml Whirl-Pak bag (Nasco, Fort Atkinson, WI), 400 ml of sterile buffered peptone water (Difco, BD, Sparks, MD) was added, and the bag was hand massaged for 5 min. For the three-tube MPN method, 10-ml portions of the chicken carcass rinsate were added to three empty tubes, and then 1-ml portions were serially diluted twice (10-fold) in 9-ml buffered peptone water tubes. All nine tubes were incubated at 37°C for 24 h. After incubation, 0.5 and 0.1 ml of each preenrichment tube were added to 10 ml of tetrathionate broth (Difco) and Rappaport-Vassiliadis broth (Difco), respectively, and incubated for 24 h at 42°C. A loopful from each enrichment broth was streaked onto brilliant green sulfa agar (Difco) and on xylose lysine Tergitol 4 agar (Difco) plates and incubated for 24 h at 37°C. Up to three typical colonies of *Salmonella* were selected per plate, inoculated onto triple sugar iron agar (Difco) and lysine iron agar (Difco) slants and then incubated at 37°C for 24 h. Isolates with typical *Salmonella* biochemical reaction characteristics were confirmed by agglutination using *Salmonella* Poly-O (A and Vi; Difco) antiserum. Another confirmation test of *Salmonella* genus was performed in parallel using a BD Phoenix automated microbiological system according to the manufacturer's instructions (BD, Sparks, MD). The MPN calculations per milliliter were performed as recommended by USDA-FSIS protocol *(29)* and then multiplied by the dilution factor of 400 (i.e., 400 ml of buffered peptone water rinsate) to obtain MPN per carcass. Colonies confirmed as *Salmonella* were preserved in skim milk at -70°C for serotyping

TABLE 1. Salmonella *prevalence and counts on chicken carcasses from six cities in Colombia*

		Donado-Godoy et al. *(10)*:		This study:		
Department	City	No. of samples	No. (%) of *Salmonella*-positive samples[a]	No. of samples	No. (%) of *Salmonella*-positive samples	Mean log MPN/carcass (95% CI)[b]
Sucre	Sincelejo	11	6 (54.5)	23	11 (47.8)	3.0 (2.4–3.6)
Santander	Bucaramanga	30	11 (36.7)	30	16 (53.3)	2.5 (2.1–2.8)
Tolima	Ibagué	27	8 (29.6)	27	9 (33.3)	2.0 (1.3–2.7)
Cundinamarca	Bogotá	368	159 (43.2)	146	50 (34.2)	2.0 (1.8–2.1)
Nariño	Pasto	20	5 (25.0)	20	8 (40)	2.0 (1.6–2.3)
Atlántico	Barranquilla	54	18 (33.3)	55	16 (29.1)	1.8 (1.5–2.0)
Total		510	268 (26.7)	301	110 (36.5)	2.1 (2.0–2.3)

[a] Number (percentage) of *Salmonella*-positive samples reported by Donado-Godoy et al. *(10)* ($n = 1{,}003$ samples).
[b] Mean log most probable number (MPN) and 95% confidence interval (CI) of *Salmonella* on chicken carcasses.

and antimicrobial susceptibility testing. The limit of detection was estimated at 12 MPN salmonellae per carcass.

Serotyping of *Salmonella*. All *Salmonella* isolates (a total of 378) were serotyped according to the Kauffmann-White scheme *(27)* and manufacturer's instructions (Difco). Among the 378 isolates serotyped, 268 *Salmonella* were from our previous project *(10)* and 110 from this study. One isolate per sample was used for serotyping. *Salmonella* Paratyphi B serovar was differentiated by the fermentation of d-tartrate based on the lead acetate test according to the protocol described by Malorny et al. *(22)*.

Antimicrobial susceptibility testing. The MIC of all serovar *Salmonella* isolates ($n = 378$) to a panel of antimicrobial agents was evaluated using a Phoenix automated microbiological system (BD) *(5)*. The Phoenix NMIC/ID-121 panel was selected because it involved antimicrobial agents used in animal and human health. This panel included the following 17 agents: amoxicillin–clavulanic acid, amikacin, ampicillin, cefazolin, cefepime, cefotaxime, cefoxitin, ceftriazone, ertapenem, gentamicin, imipenem, levofloxacin, nitrofurantoin, piperacillin/tazobactam, tetracycline, tobramycin, and trimethoprim-sulfamethoxazole. Specifications for the concentration range of each antimicrobial agent in the panel are listed elsewhere *(11)*. The MICs for the *Salmonella* isolates tested were interpreted into resistant or susceptible based on the Clinical and Laboratory Standards Institute breakpoints *(7)*. Intermediate MIC results were reclassified as resistant. Moreover, antimicrobial susceptibility of the isolates to five additional antimicrobials, ceftiofur, enrofloxacin, streptomycin, nalidixic acid, and ciprofloxacin, were evaluated according to an agar disk diffusion method on Mueller-Hinton agar (Difco), and results for veterinarian antimicrobial agents (enrofloxacin and ceftiofur) were interpreted based on animal health criteria published by the Clinical and Laboratory Standards Institute, while the rest of the antimicrobial agents were interpreted according to the standard Clinical and Laboratory Standards Institute human health criteria *(7)*. The cutoff criteria for these antimicrobial agents are listed elsewhere *(10)*. *Escherichia coli* ATCC 25922 was used as a quality control bacterium in both methodologies for each assay. However, the ATCC 25922 organism panel did not cover all the ranges of the antimicrobial agent concentrations used in the study, which is a limitation associated with using customized premade quality control panels that are used in automated testing equipment. That being said, based on our previous studies, the consistency of the antimicrobial susceptibility quality control testing has been very high.

Statistical analysis. Sample size was determined by WinEpiscope 2.0 *(2)*. Prior to statistical analysis, variables were codified by numbers. The statistical software (SAS) version 9.2 (Cary, NC) was used for the univariate and multivariate analysis. The likelihood chi-square or Fisher's exact test was used to evaluate associations between categorical variables (type of retail store, storage temperature, socioeconomic stratum, city, chicken production system, and poultry company) and *Salmonella* prevalence and serovar distribution (i.e., frequencies). Correlations between variables of interest were determined using Pearson's correlation coefficient. For the purpose of analyzing serovar data, five categories were used: the first four categories corresponded to the four most prevalent serovars, and the remaining serovars were collapsed into one category (i.e., ''other serovars''), which included the 27 less frequently obtained serovars.

The MPN per carcass data were log transformed to approximate normal distribution. MPN per carcass data $\geq$ the limit of detection were used in the analysis. The MIXED procedure in SAS was used to evaluate associations between the variables of retail store, storage condition, socioeconomic stratum, chicken production system, and poultry company categories and log (MPN per carcass), including city as a random effect. A difference was considered statistically significant if $P < 0.05$.

The antimicrobial resistance outcomes (resistance or susceptible [i.e., binary]) and multidrug-resistant (MDR) isolates (those resistant to three or more antimicrobial classes) were cross tabulated with serovar, type of retail store, storage temperature, chicken production system, and poultry company using the chi-square or Fisher's exact test, where appropriate.

RESULTS

***Salmonella* prevalence and concentration.** From the total 301 samples of broiler carcasses collected, 110 (36.5%) were positive for *Salmonella*. *Salmonella* prevalence ranged from 29 to 53% among the cities sampled (Table 1). There were significant differences ($P < 0.05$) in *Salmonella* prevalence by (i) storage temperature, (ii) poultry company, and (iii) type of retail store (Table 2).

The overall mean count (log[MPN per carcass] and 95% confidence interval) of *Salmonella* was 2.1 (2.0 to 2.3), which ranged from 1.8 in Barranquilla to 3.0 in Sincelejo (Table 1). The overall distribution of the log MPN counts of *Salmonella* in carcass rinses is shown in Figure 1. There

TABLE 2. *Potential risk factors related to mean counts of* Salmonella *on broiler chicken carcasses at retail stores in Colombia*

Variable	Subvariable	No. (%) of *Salmonella*-positive samples	Mean log MPN/carcass (95% CI)[a]
Storage temperature	Chilled ($n = 186$)	73 (39) A	2.1 (1.9–2.2) A
	Frozen ($n = 94$)	22 (23) B	1.7 (1.5–2.0) A
	Ambient ($n = 21$)	15 (71) C	2.8 (2.1–3.4) B
Poultry company	Integrated ($n = 201$)	58 (29) A	1.9 (1.7–2.1) A
	Nonintegrated ($n = 100$)	52 (53) B	2.3 (2.1–2.6) B
Retail store type	Wet market ($n = 47$)	27 (57) A	2.4 (2.0–2.8) A
	Supermarket ($n = 123$)	34 (28) B	1.8 (1.6–2.0) B
	Independent ($n = 131$)	49 (37) C	2.2 (2.0–2.4) B
Chicken production system	Conventional ($n = 279$)	104 (37) A	2.1 (2.0–2.3) A
	Free-range ($n = 22$)	6 (27) A	1.7 (1.3–2.2) AA
SES[b]	2 ($n = 126$)	51 (40) A	2.0 (1.8–2.2) A
	3 ($n = 125$)	45 (36) A	2.2 (2.0–2.5) A
	4 ($n = 32$)	9 (28) A	1.9 (1.5–2.3) A
	5 ($n = 4$)	0 (0) A	—
	6 ($n = 5$)	2 (40) A	2.0 (1.1–2.9) A

[a] Mean log most probable number (MPN) of *Salmonella* per carcass and its 95% confidence interval (CI). Log MPN within each variable followed by a different letter are significantly different ($P < 0.05$).
[b] SES, social economic stratum. There were no *Salmonella*-positive samples from SES 5.

were significant differences ($P < 0.05$) in *Salmonella* mean count by (i) type of retail store, (ii) storage temperature, and (iii) poultry company (Table 2). Chickens from wet markets had significantly higher count of *Salmonella* compared with supermarkets and independent stores. Furthermore, chickens stored at ambient conditions had significantly higher count of *Salmonella* compared with those stored at chilled and frozen temperatures. Moreover, *Salmonella* counts on broiler carcasses produced by integrated poultry company were significantly less than those from nonintegrated poultry producers.

Distribution of *Salmonella* serovars. A total of 378 *Salmonella* isolates were serotyped. The distribution of *Salmonella* serovars by retail store type is shown in Table 3. Among the 378 isolates, 31 *Salmonella* serovars were identified. *Salmonella* Paratyphi B tartrate-positive (i.e., *Salmonella* Paratyphi B dT+) was the most prevalent serovar (44.7%), followed by Heidelberg (19%), Enteritidis (17.7%), Typhimurium (5.3%), Anatum (2.1%), Albany (1.6%), Braenderup (1.3%), Kentucky (1.3%), Mbandaka (0.5%), Bareilly (0.5%), and Subsp. r:1,2 (0.5%). The remaining 6% of the isolates corresponded to different serovars recovered from single samples (Table 3).

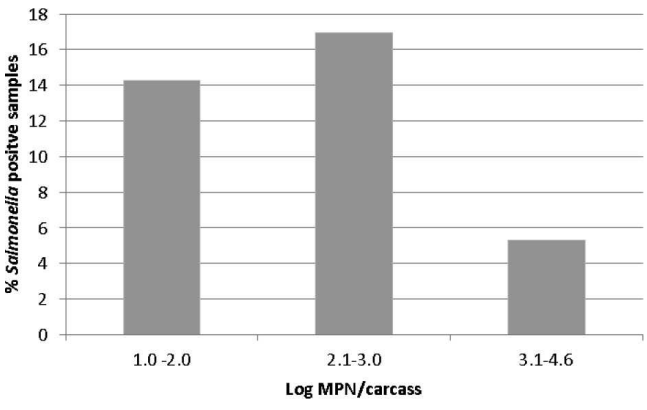

FIGURE 1. *Percentage bar chart illustrating the log most-probable-number (MPN) distribution of* Salmonella *on broiler chicken carcasses at retail stores in Colombia.*

There were significant differences ($P < 0.05$) in *Salmonella* serovar frequencies by (i) storage temperature, (ii) poultry company, (iii) type of retail store, (iv) chicken production system, and (v) city. *Salmonella* ParatyphiB dT+ was more frequently ($P < 0.05$) obtained from chicken sold at supermarkets and independent stores compared with wet markets, whereas, *Salmonella* Heidelberg was more frequent ($P < 0.05$) in chicken from independent stores and wet markets (Table 3). The ''other serovars'' group was most common ($P < 0.05$) for chicken from wet markets (19.2%) compared with supermarkets (10.2%) and independent stores (12.1%). *Salmonella* Paratyphi B dT+ was more prevalent ($P < 0.05$) on chilled and frozen chickens compared with the other serovars. For chickens stored at ambient temperature, *Salmonella* Heidelberg and ''other serovars'' were most prevalent ($P < 0.05$).

The prevalence of *Salmonella* Paratyphi B dT+ was significantly ($P < 0.05$) greater for chicken meat produced by integrated poultry companies (48%) compared with the other serovars. The distribution of *Salmonella* serovars by chicken production system revealed that Paratyphi B dT+ was the most frequent (45.1%) serovar on free-range produced chickens compared with the other serovars, whereas Paratyphi B dT+ (41.9%), Enteritidis (16.3%), and ''other serovars'' (25.6%) were most frequent for conventionally produced chickens ($P < 0.05$).

Antimicrobial resistance profiles. *Salmonella* isolates were tested for susceptibility to 22 antimicrobials of veterinary and human health importance. In general, isolates were frequently resistant to nitrofurantoin (72.1%), nalidixic acid (69.7%), streptomycin (66.8%), enrofloxacin (66.2%), tetracycline (57.3%), and trimethoprim-sulfamethoxazole (54.4%). The percentage of resistant isolates to each antimicrobial agent by serotype is shown in Table 4. In

TABLE 3. *Distribution of* Salmonella *serovars obtained from broiler carcass from independent, supermarket, and wet market stores in Colombia*

Salmonella serovar	No. (%) of isolates on samples[a]			Total no. (%) (n = 378)	P value[b]
	Independent (n = 157)	Supermarket (n = 127)	Wet markets (n = 94)		
Paratyphi B dT+	69 (43.9) A	65 (51.2) A	35 (37.2) B	169 (44.7)	<0.001
Heidelberg	33 (21.0) A	18 (14.2) B	21 (22.3) A	72 (19)	<0.001
Enteritidis	28 (17.8) A	28 (22) A	11 (11.7) B	67 (17.7)	<0.001
Typhimurium	8 (5.1) A	3 (2.49) A	9 (9.6) B	20 (5.3)	<0.001
Anatum	4 (2.5)	3 (2.49)	1 (1.1)	8 (2.1)	
Albany	0	4 (3.1)	2 (2.1)	6 (1.6)	
Braenderup	0	1 (0.8)	4 (4.3)	5 (1.3)	
Kentucky	4 (2.5)	1 (0.8)	0	5 (1.3)	
Bareilly	2 (1.3)	0	0	2 (0.5)	
Mbandaka	1 (0.6)	0	1 (1.1)	2 (0.5)	
Subsp. r:1,2	0	2 (1.6)	0	2 (0.5)	
Bardo	0	0	1 (1.1)	1 (0.3)	
Cuckmere	0	0	1 (1.1)	1 (0.3)	
Derby	0	0	1 (1.1)	1 (0.3)	
Essen	1 (0.6)	0	0	1 (0.3)	
Hillingdon		1 (0.8)	0	1 (0.3)	
Hoboken	1 (0.6)	0	0	1 (0.3)	
Hoghton	0	0	1 (1.1)	1 (0.3)	
Isangui	0	0	1 (1.1)	1 (0.3)	
Manhattan	1 (0.6)	0	0	1 (0.3)	
Muenchen	0	0	1 (1.1)	1 (0.3)	
Senftenber	0	0	1 (1.1)	1 (0.3)	
Stanley	0	0	1 (1.1)	1 (0.3)	
Tokoin	1 (0.6)	0	0	1 (0.3)	
Uganda	1 (0.6)	0	0	1 (0.3)	
Virchow	1 (0.6)	0	0	1 (0.3)	
Wagenia	0	0	1 (1.1)	1 (0.3)	
Yovokome	0	0	1 (1.1)	1 (0.3)	
Subsp. I, 4,5,12:1,2:–	1 (0.6)	0	0	1 (0.3)	
Subsp. I, 4,5,12:g,m:–	0	1 (0.8)	0	1 (0.3)	
Subsp. I, w,z_{28}:1,2	1 (0.6)	0	0	1 (0.3)	

[a] Percentages across retail store type followed by a different letter are significantly different ($P < 0.05$).
[b] P values are based on a likelihood ratio of chi-square test differences between retail store type for the top four most frequent serovars.

general, *Salmonella* Parathyphi B dT+ and *Salmonella* Heidelberg had significantly greater percentages of resistance compared with *Salmonella* Enteritidis and *Salmonella* Typhimurium.

Ninety-four percent (354 of 378) of the *Salmonella* isolates were resistant to at least one antimicrobial agent. The number of antimicrobial agents to which isolates were resistant ranged from 1 to 15. Among the *Salmonella* isolates, 35.2% (133 of 378) were resistant to 1 to 5 antimicrobial agents, 24.6% (95 of 378) from 6 to 10, and 33.9% (128 of 378) from 11 to 15 agents. The distribution of MDR *Salmonella* phenotypes cross tabulated by retail store type and storage temperature is shown in Table 5. There were no significant differences associated with multidrug resistance ($P > 0.05$), retail store type, and storage temperature (Table 5).

DISCUSSION

Our study determined the counts, serovar distribution, and antimicrobial resistance phenotypes of *Salmonella* on raw chicken meat at the retail level in Colombia. To the best of our knowledge, this is the first study that provides data on the quantification of *Salmonella* on broiler chicken meat in Colombia.

The overall *Salmonella* prevalence of 36.5% (n = 301) on broiler chicken meat determined in this study is higher than the prevalence of 27% (n = 1,003) determined in our previous work *(10)*. The difference in *Salmonella* prevalence between the two studies could be due to the inclusion of only the six cities having the highest *Salmonella* prevalence of the 22 principal cities previously sampled by Donado-Godoy et al. *(10)*. Nevertheless, the significant risk factors that we found in this study were consistent with the previous work *(10)*, confirming that the variables of storage temperature and poultry company were important factors for *Salmonella* contamination level and survival. Our findings verified that *Salmonella* prevalence and cell numbers on frozen chickens were lower than that on chilled chickens and chickens stored at ambient temperature, which supports the concept of storage temperature being an important risk factor for pathogen survival *(25)*. Both *Salmonella* prevalence and count were significantly less for

TABLE 4. *Antimicrobial resistance prevalence among* Salmonella *isolates obtained from broiler carcasses by serovar in Colombia*

Antimicrobial agent	Total no. (%)	No. (%) of resistant isolates in samples by serovar					*P* value[a]
		Paratyphi B dT+	Heidelberg	Enteritidis	Typhimurium	Other serovars[b]	
Aminoglycoside							
Amikacin	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Gentamicin	15 (4)	9 (5.4)	2 (2.8)	0 (0)	0 (0)	4 (8)	0.15
Tobramycin	15 (4)	10 (6)	1 (1.4)	0 (0)	0 (0)	4 (8)	0.07
Streptomycin	254 (67.2)	151 (92.1)	40 (55.6)	8 (12.1)	16 (80)	33 (67.4)	<0.0001
Penicillin β-lactamase inhibitor combination							
Amoxicillin–clavulanic acid	116 (30.8)	76 (45.2)	23 (31.9)	5 (7.5)	1 (5)	11 (22)	<0.001
Ampicillin	175 (46.4)	89 (53)	52 (72.2)	9 (13.4)	8 (40)	17 (34)	<0.001
Piperacillin/tazobactam	11 (2.9)	1 (0.6)	7 (10)	1 (1.5)	2 (10)	0 (0)	0.0004
Cephalosporin, first and second generation							
Cefazolin	164 (43.5)	89 (53)	47 (65.3)	7 (10.5)	6 (30)	15 (30)	<0.0001
Cephalosporin, third and fourth generation							
Cefepime	30 (7.9)	3 (1.8)	19 (26.4)	1 (1.5)	4 (20)	3 (6)	<0.0001
Cefotaxime	145 (38.5)	79 (47)	42 (58.3)	7 (10.5)	4 (20)	13 (26)	<0.0001
Cefoxitin	125 (33.2)	83 (49.4)	21 (29.2)	8 (11.9)	0 (0)	13 (26)	<0.0001
Ceftiofur	169 (44.7)	90 (55.2)	46 (63.9)	14 (21.5)	6 (30)	13 (26.5)	<0.0001
Ceftriazone	150 (39.8)	78 (46.4)	46 (63.9)	7 (10.4)	4 (20)	15 (30)	<0.0001
Fluoroquinolone							
Ciprofloxacin	238 (63.1)	151 (89.3)	54 (75)	7 (10.4)	8 (40)	18 (36)	<0.0001
Enrofloxacin	249 (65.6)	152 (89.9)	59 (81.9)	9 (13.6)	9 (45)	20 (40.8)	<0.0001
Levofloxacin	167 (44.2)	129 (76.3)	23 (31.9)	5 (7.5)	4 (20)	5 (10)	<0.0001
Carbapenem							
Ertapenem	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Imipenem	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	
Nitrofuran							
Nitrofurantoin	272 (72.1)	164 (97.6)	40 (55.6)	49 (73.1)	9 (45)	10 (20)	<0.0001
Quinolone							
Nalidixic acid	263 (69.6)	153 (90.5)	67 (94.4)	10 (15.2)	12 (60)	20 (40)	<0.0001
Tetracycline							
Tetracycline	216 (57.1)	114 (67.9)	68 (94.4)	4 (6)	12 (60)	18 (36)	<0.0001
Other							
Trimethoprim-sulfamethoxazole	205 (54.2)	146 (86.9)	35 (48.6)	4 (6)	8 (40)	12 (24)	<0.0001

[a] *P* values are based on the likelihood chi-square or Fisher's exact test of the differences in percentages by serotype, where appropriate.
[b] The ''other serovars'' category represents 13.3% of the total isolates ($n = 378$).

chickens from integrated poultry companies than those from nonintegrated companies. Moreover, both *Salmonella* prevalence and count were not significantly different between chickens raised as free range and those raised using conventional production systems. Research studies conducted at the retail level have yielded conflicting results, whereby some studies revealed higher *Salmonella* contamination prevalence on free-range chicken meat compared with conventionally raised chicken meat *(1, 8),* and another study *(17)* revealed lower *Salmonella* prevalence on organic poultry meat compared with conventionally grown poultry.

In this study, both *Salmonella* prevalence and count for chickens from wet markets were significantly greater than those for chickens from supermarkets and independent stores. Importantly, 47% of chickens collected from wet markets were stored at ambient temperature, which may contribute to the higher *Salmonella* prevalence and counts.

The overall mean count of *Salmonella* in chicken meat in Colombia in this study was 2.1 log MPN per carcass, which is slightly higher than findings in Australia (1.7 log MPN/cm^2) *(26),* The Netherlands (89% of fresh chicken had less than 1 log MPN per carcass) *(12),* New Zealand (0.61 log MPN/g) *(33),* and the United States (1.75 log MPN per carcass) *(28).* As for the log MPN distribution (Fig. 1), 14% of the samples in this study were contaminated with *Salmonella* counts ranging from 1.0 to 2.0 log MPN per carcass versus 3.7% in U.S. samples that fell in that interval, 17% of our samples were contaminated with 2.1 to 3.0 log MPN per carcass versus 1.2% of U.S. samples in the same interval, and 4.7 and 0.67% of our samples were in 3.1 to 4.0 and 4.1 to 4.6 log, respectively,

TABLE 5. *Phenotypic multidrug resistance of* Salmonella *isolates obtained from raw chicken by retail store type and storage temperature in Colombia*[a]

	No. (%) of isolate resistance						
		Retail store type			Storage temp		
Multidrug resistance	Total (n = 378)	Wet market (n = 94)	Large market (n = 127)	Small market (n=157)	Chilled (n = 263)	Frozen (n = 100)	Ambient (n = 15)
0	24 (6.4)	8 (8.5)	5 (3.9)	11 (7)	14 (5.3)	6 (6)	4 (26.7)
1	54 (14.3)	14 (14.9)	18 (14.2)	22 (14)	44 (16.7)	9 (9)	1 (6.7)
2	18 (4.8)	4 (4.3)	7 (5.5)	7 (4.5)	16 (6.1)	1 (1)	1 (6.7)
3	18 (4.8)	6 (6.4)	1 (0.8)	11 (7)	11 (4.2)	7 (7)	0 (0)
4	17 (4.5)	2 (2.1)	7 (5.5)	8 (5.1)	12 (4.6)	5 (5)	0 (0)
5	26 (6.9)	5 (5.3)	11 (8.7)	10 (6.4)	16 (6.1)	10 (10)	0 (0)
6	33 (8.7)	9 (9.6)	12 (9.5)	12 (7.6)	27 (10.3)	5 (5)	1 (6.7)
7	18 (4.8)	8 (8.5)	4 (3.2)	6 (3.8)	12 (4.6)	5 (5)	1 (6.7)
8	16 (4.2)	3 (3.2)	5 (3.9)	8 (5.1)	13 (4.9)	1 (1)	2 (13.3)
9	11 (2.9)	1 (1.1)	4 (3.2)	6 (3.8)	8 (3.0)	3 (3)	0 (0)
10	15 (4)	2 (2.1)	8 (6.3)	5 (3.2)	9 (3.4)	6 (6)	0 (0)
11	21 (5.6)	7 (7.5)	6 (4.7)	8 (5.1)	11 (4.2)	9 (9)	1 (6.7)
12	31 (8.2)	10 (10.6)	11 (8.7)	10 (6.4)	16 (6.1)	12 (12)	3 (20)
13	34 (9)	10 (10.6)	12 (9.5)	12 (7.6)	23 (8.8)	10 (10)	1 (6.7)
14	23 (6.1)	3 (3.2)	8 (6.3)	12 (7.6)	19 (7.2)	4 (4)	0 (0)
15	19 (5)	2 (2.1)	8 (6.3)	9 (5.7)	12 (4.6)	7 (7)	0 (0)

[a] Multidrug resistance phenotypes of *Salmonella* isolates (n = 378) from raw chicken meat samples in Colombia. The percentages of MDR *Salmonella* isolates are contrasted with retail store type and storage temperature. The overall differences in MDR *Salmonella* isolates by retail store type (P = 0.286) was not significant, whereas differences were significant by storage condition (P = 0.160) using the likelihood ratio chi-square test.

versus 0.27% of U.S. samples in the 3.1 to 4.0 interval *(28)*. The differences among the findings could be attributed to differences in sampling scheme and study design, sample type (whole chicken versus chicken parts and chilled versus frozen chickens), and *Salmonella* enumeration protocol.

Although the infective dose to cause salmonellosis in humans is still uncertain *(13),* findings from outbreak investigations have revealed that ingesting less than 100 salmonellae can cause illness in humans *(4, 19)*. Approximately 22% of the chickens had MPN log *Salmonella* counts between 2.1 to 4.6 (Fig. 1), which is more than this infective dose. Furthermore, improper handling of raw chicken can lead to higher counts that could be a significant contributing factor for salmonellosis in Colombia.

Salmonella Paratyphi B dT+ (variant Java) was the most frequently recovered serovar from chicken carcasses in this study. This finding is in agreement with previous studies conducted in Colombia at chicken farms and retail stores *(9),* as well as in slaughter houses in Venezuela *(3)*. *Salmonella* Paratyphi B dT+ (variant Java) is not a common serovar on chicken meat worldwide *(21, 24, 35)*. In Colombia, there are no reports of confirmed human cases associated with *Salmonella* Paratyphi B dT+ *(20)*; however, this serovar prevalence is increasing in some countries, such as The Netherlands, where it has increased from approximately 2% of all human *Salmonella* isolates in 1996 to 60% in 2002 *(21, 24, 35)*. In the United States, *Salmonella* Paratyphi B dT+ is ranked as the 15th most common *Salmonella* serotype isolated from human cases, although this serovar has not been isolated from chicken meat in the United States *(30)*. *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most prevalent serovars associated with human salmonellosis worldwide, including Colombia *(20)*. In the present study, these were the third and fourth most common serovars, and this high prevalence suggests that chicken meat is a potential vehicle of these types of *Salmonella* but not the only source to cause human salmonellosis in Colombia.

There was a high percentage of resistance to several antimicrobial agents among the *Salmonella* isolates in this study. Among the isolates, 35.2% were resistant to 1 to 5 antimicrobial agents, 24.6% from 6 to 10, and 33.9% from 11 to 15 agents. These highly MDR salmonellae can pose a signifcant public health risk in Colombia through chicken meat consumption as well as potential for isolate dissemination to other food production systems. *Salmonella* isolates in this study (94% resistance to at least one drug) were similar to results reported by Donado-Godoy et al. *(11)* for retail chicken *Salmonella* isolates from Colombia. In that study, it was determined that 98% of the *Salmonella* isolates (n = 200) on chicken meat from retail stores in Bogota were resistant to at least one antimicrobial agent. Also, MDR *Salmonella* isolates (resistant to three or more antimicrobial classes) were frequent, as was determined in this study. According to reports from the United States and Canada, percentages of *Salmonella* isolates resistant to at least one antimicrobial agent from retail chicken meat were 64% (n =171) and 47% (n = 382), respectively *(15, 31)*. Among all the serovars isolated in this study, *Salmonella* Paratyphi B dT+ and *Salmonella* Heidelberg had the

greatest level of antimicrobial resistance, which is also in agreement with results of previous studies *(9, 11)*.

These study findings revealed that *Salmonella* prevalence was high (36.5%), and 22% of samples had counts between 2 to 4.6 log per retail chicken samples. Furthermore, *Salmonella* Paratyphi B dT+ and *Salmonella* Heidelberg were not only the most commonly isolated serovars but also had the highest percentages of MDR phenotypes. Good management and good agriculture practices combined with implementation of hazard analysis and critical control points systems along the chicken production chain could reduce the risk of MDR *Salmonella* contamination of chicken meat in Colombia.

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