Journal of Food Protection, Vol. 78, No. 4, 2015, Pages 751–759
doi:10.4315/0362-028X.JFP-14-349

Prevalence, Resistance Patterns, and Risk Factors for Antimicrobial Resistance in Bacteria from Retail Chicken Meat in Colombia

PILAR DONADO-GODOY,[1]* BARBARA A. BYRNE,[2] MARIBEL LEÓN,[3] RICARDO CASTELLANOS,[1] CONSUELO VANEGAS,[4] ADRIANA CORAL,[5] ALEJANDRA AREVALO,[1] VIVIANA CLAVIJO,[1] MERCEDES VARGAS,[6] JUAN J. ROMERO ZUÑIGA,[7] McALLISTER TAFUR,[3] ENRIQUE PÉREZ-GUTIERREZ,[8] AND WOUTRINA A. SMITH[2]

[1]*Corporación Colombiana de Investigación Agropecuaria (CORPOICA), Centro de Biotecnología y Bioindustria (CBB), Kilometro 14, Vía Mosquera, Cundinamarca, Colombia;* [2]*School of Veterinary Medicine, University of California, Davis, One Shields Avenue, Davis, California 95616, USA;* [3]*Instituto Colombiano Agropecuario (ICA), Carrera 41 No. 17-81, Bogotá D.C., Colombia;* [4]*Universidad de los Andes, Laboratorio de Ecología Microbiana y de Alimentos (LEMA), Carrera 1 No. 18A-12, Bogotá D.C., Colombia;* [5]*Carulla S.A. Laboratorio de Calidad, Carrera 68 D No. 21-35, Bogotá D.C., Colombia;* [6]*Instituto Nacional de Vigilancia de Medicamentos y Alimentos, Carrera 68 D No. 17-11/21, Bogotá D.C., Colombia;* [7]*Universidad Nacional de Costa Rica, Programa de Investigación en Medicina Poblacional, Escuela de Medicina Veterinaria, P.O. Box 304-3000, Heredia, Costa Rica; and* [8]*Pan American Health Organization, Regional Office for the Americas of the World Health Organization, 525 Twenty-third Street N.W., Washington, D.C. 20037, USA*

MS 14-349: Received 22 July 2014/Accepted 4 December 2014

ABSTRACT

As a step toward implementing the Colombian Integrated Program for Antimicrobial Resistance Surveillance (COIPARS), this study aimed to establish the baseline antimicrobial resistance patterns of *Salmonella* serovars, *Escherichia coli,* and *Enterococcus* spp. isolates in retail poultry meat from independent stores and from a main chain distributor center. MICs of the isolates were determined for antimicrobials used both in humans and animals, using an automated system. *Salmonella* serovars were isolated from 26% of the meat samples and *E. coli* from 83%, whereas *Enterococcus faecalis* and *Enterococcus faecium* were detected in 81 and 13% of the meat samples, respectively. A principal finding of concern in this study was that almost 98% of isolates tested were multidrug resistant. Ceftiofur, enrofloxacin, nalidixic acid, and tetracycline were the antimicrobials that showed the highest frequency of resistance among *Salmonella* and *E. coli* isolates. For enterococci, 61.5% of *E. faecium* isolates were found to be resistant to quinupristin-dalfopristin; this is significant because it is used to treat nosocomial infections when vancomycin resistance is present. Vancomycin resistance was detected in 4% of the *E. faecalis* isolates. The results of our study highlight the need for rapid implementation of an integrated program for surveillance of antimicrobial resistance by the Colombian authorities in order to monitor trends, raise awareness, and help promote practices to safeguard later generation antimicrobial agents.

In recent years, antimicrobial resistance (AMR) has emerged as a global public health problem; it threatens to narrow the potential uses of antimicrobials to treat infectious diseases *(16).* The implications of AMR raise concerns not only among public health authorities but also among animal and food safety authorities *(16, 32, 48, 49),* partly because antimicrobial-resistant bacteria can be transmitted from food animals to humans through the food chain *(45).* Modern food animal production systems commonly use antimicrobial agents to prevent, control, and treat bacterial infections *(45),* and some of these agents are also used as growth promoters in poultry and swine production systems *(3, 44).* Although widespread use of antimicrobials in the primary sector has benefits for producers, it also contributes to the increasing emergence of antimicrobial-resistant bacteria *(1, 3).*

* Author for correspondence. Tel: +57-313-366-0458; Fax: +57 (1) 422-7300; E-mail: pidonado@corpoica.org.co.

Meat products are sources of human infection from antimicrobial-resistant pathogens such as *Salmonella* serovars *(25, 29, 34, 36).* These bacteria can carry resistance genes that are transferable to humans *(8).* Similarly, because commensal bacteria, such as *Escherichia coli* and enterococci, can transfer resistance genes to pathogens *(38, 50),* they may also be a threat to human health *(10).*

Animal protein consumption is rapidly increasing in Colombia, similar to the trend in other developing countries. It is estimated that a person on minimum wage in Colombia purchases almost three times more chicken meat and twice as much bovine meat annually than swine meat (http://www.indexmundi.com). In such dynamic conditions, the food safety considerations, particularly in the poultry chain, are an increasing challenge.

Colombia is free of avian influenza, and the poultry industry has attempted to reach international food safety and animal health standards in order to take advantage of access to international markets. However, there are still limiting

factors, including inadequate knowledge of the baseline prevalence of foodborne pathogens such as *Salmonella* and their AMR profiles, in retail meat of different origins, which prevent Colombia from fully benefiting from international commerce. There are also limited data about the AMR in commensal bacteria such as *E. coli* and enterococci in retail chicken meat in Colombia.

To better assess the risk factors linked to foodborne pathogens and to the development of AMR, several countries, including Canada *(13),* the United States *(26),* and Denmark *(7),* have initiated integrated programs for the surveillance of AMR along meat chains (farm production, abattoirs, and retail sectors) and humans *(28).* As part of a pilot initiative to set up such integrated systems in Colombia, namely COIPARS (Colombian Integrated Program for Antimicrobial Resistance Surveillance), a survey was conducted to determine the prevalence, resistance patterns, and risk factors for antimicrobial-resistant *Salmonella* serovars, *E. coli,* and *Enterococcus* spp. in retail poultry meat.

MATERIALS AND METHODS

Study approach. Samples were collected in Bogota between March and October 2009, from two different retail facilities. The sample size ($n = 200$) was divided evenly between independent retail stores and the distribution center of the main retail chain market group of the country. Independent retail stores were those that belonged either to integrated poultry companies or small-scale nonintegrated poultry companies and included butchers, supermarkets, wet markets, company stores, and small neighborhood stores. Stores were selected by convenience based on proximity within localities. For the independent stores, samples were placed in insulated containers with cold gel packs, following the methodology of the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) *(11),* and then were transported in less than 4 h to the laboratory for processing. In the main distribution center, samples were selected upon arrival and were transported immediately to the laboratory for processing the same day.

Date and time of sample collection, ambient temperature, company, store type, store name, location, type of sample, origin (including whether the sample came from an organic or antimicrobial-free farm), sell-by date, and price per kilogram were recorded, as well as the date and sample temperature upon arrival at the laboratory for processing. The socioeconomic status of each locality where the sample was collected was also noted in order to represent the AMR situation through the political and economic divisions of the city. 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 the highest *(21).*

Microbiological isolation and identification. Sampling of chicken meat was done following CIPARS 2007 protocols *(11)*; each sample consisted of one package of thighs with skin on a Styrofoam tray. This sample type was chosen because it is the most popular chicken product in supermarkets. Isolation and identification were done at the Laboratory of Microbiological and Food Ecology, Universidad de los Andes, Bogotá, and at the Laboratory for Quality Control of the main chain market. Serotyping of *Salmonella* was done at the Microbiological Laboratory of the National Institute for Drug and Food Surveillance, the national reference laboratory for bacteria isolated from food.

Briefly, the microbiological isolation was done following CIPARS 2007 protocols for isolating *Salmonella* serovars, *E. coli,* and *Enterococcus* spp. from meat and poultry samples *(40–42).* The confirmation of the bacterial genus of the isolates was done using the automated microbiological system BD Phoenix according to the manufacturer's instructions (Difco, BD, Sparks, MD). Serovars were identified using the Kauffman-White scheme for classification of somatic O and flagellar H antigen type *(39)* and manufacturer's instructions (Difco, BD). All the isolates were frozen at −70°C for further study.

The Phoenix automated microbiological system was used to determine the antimicrobial MIC for isolates. The BD Phoenix is an automated microbroth dilution system for gram-positive and gram-negative bacteria *(14).* The Phoenix NMIC/ID-121 panel was selected for gram-negative bacteria and the NMIC/ID-53 panel for gram-positive bacteria *Enterococcus* because they involved antimicrobial agents used in animal and human health. The panels for *Salmonella* and *E. coli* included the following 21 agents: amoxicillin–clavulanic acid (AMC), amikacin (AMK), ampicillin (AMP), aztreonam (ATM), cefazolin (CZO), cefepime (FEP), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ), ceftriazone (CRO), ciprofloxacin (CIP), ertapenem (ETP), gentamicin (GEN), imipenem (IPM), levofloxacin (LVX), meropenem (MEM), nitrofurantoin (NIT), piperacillin-tazobactam (TZP), tetracycline (TCY), tobramycin (TOB), and trimethoprim-sulfamethoxazole (SXT). Interpretative criteria were based on Clinical and Laboratory Standards Institute (CLSI) standards *(15).* Specifications for the concentration range of each antimicrobial in the BD Phoenix NMIC/ID-121 panel are published elsewhere *(18).* For *Enterococcus* spp., the panel included amoxicillin–clavulanic acid (AMC), ampicillin (AMP), cefazolin (CZO), cefoxitin (FOX), chloramphenicol (CHL), ciprofloxacin (CIP), clindamycin (CLI), erythromycin (ERY), fosfomycin (FOS), fusidic acid (FUS), gentamicin (GEN), gentamicin high (GEH), levofloxacin (LVX), linezolid (LNZ), mupirocin (MUP), nitrofurantoin (NIT), oxacillin (OXA), penicillin G (PEN), quinupristin-dalfopristin (QDA), rifampin (RA), streptomycin high (STH), teicoplanin (TEC), trimethoprim-sulfamethoxazole (SXT), and vancomycin (VAN). Interpretative criteria were based on CLSI standards *(15).* Specifications for the concentration range of each antimicrobial agent in the BD Phoenix NMIC/ID-53 panel are listed elsewhere *(18).*

Additionally, when not present in the panel, ceftiofur (XNL), enrofloxacin (ENR), streptomycin (STR), chloramphenicol (CHL), nalidixic acid (NAL), and tilmicosin (TIL) were evaluated for all bacteria by use of the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Difco), and results were interpreted based on criteria stated by the CLSI *(15).*

E. coli ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as control organisms in both methodologies for each assay.

Categorization of antimicrobials. Following the methodology of CIPARS *(12),* antimicrobials were categorized based on their critical importance to human health. Antimicrobials were classified as category I (very high importance in human medicine), category II (high importance), category III (medium importance), and category IV (low importance). Antimicrobials in category IV were not included in this study.

Resistance markers. Extended-spectrum β-lactamase was tested in *E. coli* using the Phoenix ESBL test, based on growth in the wells of cefpodoxime, ceftazidime, ceftriaxone, and cefotaxime, with or without clavulanic acid *(47).* For enterococci, resistant

TABLE 1. *Distribution of poultry meat* Salmonella *serovars by type of store in Bogota, Colombia*

	Independent stores ($n = 23$)		Main chain distributor center ($n = 28$)	
Serovar	Frequency	Prevalence (%)	Frequency	Prevalence (%)
Paratyphi B	9	39.1	16	57.1
Heidelberg	4	17.4	4	14.3
Enteritidis	3	13.0	6	21.4
Typhimurium	3	13.0		
Muenster	1	4.3	0	0
Lome			1	3.6
Rough (b:1,2)	2	8.7	1	3.6
Rough (g,m:−)	1	4.3		

markers included high-level gentamicin resistance (HLGR), high-level streptomycin resistance (HLSR), and vancomycin resistance (VRE) in accordance with Becton Dickinson's description of the protocol for detection of resistance markers *(9)*.

Data management. Data entry and error checking were done using Microsoft Access 2007 *(30)*.

Statistical analysis. The statistical package SPSS 16 (SPSS, Chicago, IL) *(6)* was used for comparing antimicrobial patterns and for univariable and multivariable analysis.

Descriptive analysis. Chi-square or Fisher's exact test were used for comparing categorical variables and independent *t* tests for continuous variables between store types and socioeconomic category. Correlation between variables of interest was done using Pearson's correlation coefficients. A difference was considered statistically significant at $P < 0.05$.

An isolate was defined as resistant if it was not susceptible to one or more of the antimicrobials that were tested. Isolates with intermediate results were classified as susceptible. Multidrug-resistant (MDR) isolates were those resistant to two or more antimicrobials. The frequency and percentage distribution of AMR was calculated by bacterium and by type of store and, in the case of *Salmonella,* also by serovar. The AMR pattern for each isolate was determined, was categorized on the basis of its antimicrobial susceptibility status, and was classified by serovar and store type.

RESULTS

Samples and retail stores. A total of 200 chicken meat samples were analyzed. The samples from independent stores ($n = 100$) came from 17 (85%) of 20 localities and covered five of Bogota's six socioeconomic strata. Among the independent stores, 30% of the samples were from butchers, 10% were from supermarkets, and the remaining 60% were from a combination of stores, including company stores, wet markets (open food markets), and small neighborhood stores. The mean sample temperature in stores was 4°C (range, 0 to 12°C) and, upon arrival in the laboratory, was 7°C (0 to 14°C). The mean sample weight was 530 g (range, 210 to 1,100 g). The mean price per kilogram of meat ranged from 2.3 to 5.7 U.S. dollars.

All samples from the large chain distribution center ($n = 100$) were processed following hazard analysis and critical control point methodology in the center. After slaughter, chickens were immersed in iced, chlorinated water. Only 8 (4%) samples were classified as antimicrobial-free samples, which came from organic production systems that did not use antimicrobials as growth promoters in the feed or as disease treatments.

***Salmonella* serovars.** *Salmonella* was found in 51 (26%) of 200 cultured chicken meat samples. There was no statistically significant difference between the prevalence in independent store samples (23%) and the chain distribution center samples (28%). Table 1 shows that *Salmonella* Paratyphi B was the most prevalent *Salmonella* serovar (49%), followed by Heidelberg (15.7%), Enteritidis (17.7%), and Typhimurium (5.9%). *Salmonella* serovars Lome and Muenster were each found in a single sample (4%). The remaining *Salmonella* serovars were classified as Rough (7.9%). No association was found among the types of stores or socioeconomic strata and the prevalence of bacteria isolated or their resistance patterns ($P > 0.1$).

The prevalences of resistant *Salmonella* isolates for each antimicrobial agent are presented in Table 2. All *Salmonella* isolates were resistant to TIL, and antimicrobials that showed significant differences ($P \leq 0.05$) in the percentage of resistance among independent stores and the main chain distributor center were CIP, NIT, and NAL. Resistance percentages were also significantly different among serovars for CIP, NIT, STX, ENR, STR, and NAL ($P < 0.01$); and, specifically for *Salmonella* Paratyphi B, the percentages of resistant isolates for these antimicrobials were higher.

In this study, one isolate of *Salmonella* was susceptible to all of the antimicrobials tested (2%), and the number of antimicrobials in the resistance patterns in the study ranged from 1 to 17. Among the 51 *Salmonella* isolates, 18 (35.3%) were resistant to 1 to 4 antimicrobials, 13 (25.5%) to 5 to 8, and 20 (39.2%) to 9 to 14 antimicrobials. The median number of antimicrobials to which *Salmonella* had full resistance was 7. The antimicrobials tetracycline and nalidixic acid showed the highest frequency of resistance. AMR patterns grouped according to serovar are presented in Table 3. Ceftiofur, nalidixic acid, and tetracycline (XNL-NAL-TCY) constituted the most prevalent MDR profile, with antimicrobials found in four core patterns as follows: pattern 1, XNL-NAL-TCY (51%); pattern 2, XNL-NAL-TCY-ENR-NIT-STR (35.5%); pattern 3, XNL-NAL-CIP-ENR-NIT-STR-STX (31.4%); and pattern 4, XNL-NAL-TCY-CIP-ENR-NIT-STR-STX (23.5%). These patterns were only found in *Salmonella* Paratyphi B and *Salmonella* Rough. Significant differences ($P < 0.05$) in the percentages of pattern 3 (XNL-NAL-CIP-ENR-NIT-STR-STX) were found between the two origin sources of the isolates. Seventy-five percent of isolates from the chain distribution center showed pattern 3 compared with 25% from the independent stores.

The resistance of *Salmonella* to antimicrobials based on categories of importance to human health is shown in Tables 4 and 5. Comparisons of the resistance to antimicrobials for *Salmonella* serovars between farms and retail stores showed that nearly all the antimicrobials in categories II and III had 40% or more resistance, irrespective of farm or retail source.

TABLE 2. *Prevalence of antimicrobial-resistant bacterial isolates in Colombian poultry meat*

Antimicrobial agent	Abbreviation	Prevalence (%)			
		Salmonella serovars ($n = 51$)	*Escherichia coli* ($n = 165$)	*Enterococcus faecalis* ($n = 161$)	*Enterococcus faecium* ($n = 26$)
Amikacin	AMK	0	0.6	—[a]	—
Amoxicillin-clavulanate	AMC	31.4	8.5	0	0
Ampicillin	AMP	33.3	40.0	0	0
Aztreonam	AZT	0	18.5	—	—
Cefazolin	CZO	35.3	31.6	100	100.0
Cefepime	FEP	0	1.5	—	—
Cefotaxime	CTX	31.4	20	—	—
Cefoxitin	FOX	31.4	9.1	—	—
Ceftazidime	CAZ	0	4.8	—	—
Ceftiofur	XNL	25.5	16.4	100	92.3
Ceftriaxone	CRO	31.4	17	—	—
Cephalothin	CEP	—	59.3	—	—
Chloramphenicol	CHL	7.8	34.5	23.1	15.4
Ciprofloxacin	CIP	41.2	32.1	27.3	11.5
Clindamycin	CLI	—	—	100	100
Enrofloxacin	ENR	54.9	50.3	80.6	76.9
Erythromycin	ERY	—	—	82.0	73.1
Ertapenem	ETP	0	3	—	—
Fosfomycin	FOS	—	—	0	0
Gentamicin	GEN	0.0	8.5	34.4	15.4
Gentamicin-synergy	GEH	—	—	21.1	15.4
Imipenem	IPM	0	0	—	—
Levofloxacin	LVX	2.0	29.1	18.0	3.8
Linezolid	LNZ	—	—	1.2	0
Meropenem	MEM	0	0	—	—
Nalidixic acid	NAL	66.0	64.0	—	—
Nitrofurantoin	NIT	51.0	7.9	1.2	34.6
Oxacillin	OXA	—	—	—	—
Penicillin	PEN	—	—	0.6	11.5
Piperacillin-tazobactam	TZP	2.0	0	—	—
Quinupristin-dalfopristin	QDA	—	—	—	61.5
Streptomycin	STR	56.9	71.5	100	92.3
Streptomycin-synergy	STH	—	—	56.5	57.7
Teicoplanin	TEC	—	—	1.9	0
Tetracycline	TCY	60.8	92.5	98.1	88.5
Tilmicosin	TIL	100.0	94.5	97.5	96.2
Tobramycin	TOB	2.0	7.3	—	—
Trimethoprim-sulfamethoxazole	STX	49.0	32.7	2.5	3.8
Vancomycin	VAN	—	—	3.7	0

[a] —, not applicable.

***E. coli*.** *E. coli* was recovered from 165 (82.5%) of 200 samples and was present in 69% of independent store samples compared with 96% of samples from the chain distributor center. The prevalence of resistant *E. coli* isolates for each antimicrobial agent is presented in Table 2. The resistance of *E. coli* to antimicrobials categorized on the importance to human health is presented in Table 5. The extended-spectrum β-lactamase marker was present in 26 (15.8%) of the isolates.

***Enterococcus* spp.** Enterococci were recovered from 188 (94.0%) of the 200 samples. Three species of enterococci were found in this study: *E. faecalis* was the most common (85.6%), followed by *Enterococcus faecium* (13.8%); there was a single isolate of *Enterococcus rafinosus* (0.5%). The prevalence of *E. faecalis* was higher in the samples that came from the chain distributor center (55.6%) compared with the independent stores ($P = 0.01$), whereas *E. faecium* prevalence was higher in samples from independent stores (73.1%) ($P < 0.01$).

E. faecalis was resistant to many antimicrobials. Table 2 shows that the highest prevalence of resistance was associated with tilmicosin (97.5%), followed by tetracycline (98.1%), erythromycin (82.0%), and enrofloxacin (80.6%). *E. faecalis* showed a significantly higher resistance prevalence ($P < 0.05$) to ciprofloxacin, gentamicin, levofloxacin, and tetracycline, whereas *E. faecium* presented higher significant resistance ($P < 0.05$) to nitrofurantoin and penicillin (Table 2). The prevalences of resistant *E. faecalis* and *E. faecium* to vancomycin were 3.7 and 0%, respectively.

The distribution of resistance based on importance in human medicine is presented in Tables 4 and 5. The most

TABLE 3. *Antimicrobial resistance pattern distribution for the most prevalent* Salmonella *serovars from poultry meat in Colombia*

Serovar	Pattern	Frequency	Prevalence (%)
Paratyphi B	AMC-AMP-CZO-FOX-CRO-CTX-XNL-ATM-NAL-CIP-ENR-STR-NIT-TCY-SXT-TIL-CHL	1	4.0
	AMC-AMP-CZO-FOX-CRO-CTX-XNL-ATM-NAL-CIP-ENR-STR-NIT-TCY-SXT-TIL	1	4.0
	AMC-AMP-CZO-FOX-CRO-CTX-XNL-NAL-CIP-ENR-STR-NIT-TCY-SXT-TIL	2	8.0
	AMC-AMP-CZO-FOX-CRO-CTX-NAL-CIP-ENR-STR-NIT-TCY-SXT-TIL-CHL	1	4.0
	AMC-AMP-CZO-FOX-CRO-CTX-NAL-CIP-ENR-STR-NIT-TCY-SXT-TIL	2	8.0
	AMC-AMP-CZO-FOX-CRO-CTX-XNL-STR-NIT-TCY-SXT-TIL	1	4.0
	AMC-AMP-CZO-FOX-CRO-CTX-XNL-NAL-TCY-TIL	1	4.0
	NAL-CIP-ENR-STR-TOB-NIT-TCY-SXT-TIL-CHL	1	4.0
	NAL-CIP-ENR-STR-NIT-TCY-SXT-TIL-CHL	1	4.0
	AMP-NAL-CIP-ENR-STR-NIT-TCY-SXT-TIL	1	4.0
	NAL-CIP-LVX-ENR-STR-NIT-TCY-SXT-TIL	1	4.0
	NAL-CIP-ENR-STR-NIT-TCY-SXT-TIL	3	12.0
	NAL-TZP-CIP-ENR-STR-NIT-SXT-TIL	1	4.0
	NAL-CIP-ENR-STR-NIT-SXT-TIL	4	16.0
	NAL-CIP-ENR-STR-NIT-TCY-TIL	1	4.0
	NAL-CIP-ENR-NIT-TIL	1	4.0
	CZO-TIL	1	4.0
	TIL	1	4.0
	Total	25	100.0
Heidelberg	AMC-AMP-CZO-FOX-CRO-CTX-XNL-NAL-CIP-ENR-TCY-TIL	2	25.0
	AMC-AMP-CZO-FOX-CRO-CTX-XNL-NAL-TCY-TIL	3	37.5
	NAL-CIP-ENR-STR-TCY-TIL	1	12.5
	NAL-CIP-ENR-TCY-TIL	1	12.5
	NAL-CIP-TCY-TIL	1	12.5
	Total	8	100.0
Enteritidis	TIL-NIT	1	11.1
	TIL-CZO	1	11.1
	TIL-GEN	1	11.1
	TIL	6	66.7
	Total	9	100.0
Typhimurium	STR-TCY-SXT-TIL	1	33.3
	STR-TCY-TIL	1	33.3
	STR-TIL	1	33.3
	Total	3	100.0

common AMR pattern observed for *E. faecalis* was CLI-ERY-ENR-XNL-STH-STR-TIL-TCY (23 of 160) and, for *E. faecium,* CLI-ERY-ENR-NIT-STH-STR-TIL-TCY-XNL (3 of 26). The median number of antimicrobials in the resistance pattern for all the enterococci was 11. Overall, 80% of *E. faecium* and 96.2% of *E. faecalis* were resistant to more than 9 antimicrobials (Table 6).

Resistance markers were present in 151 (93.8%) of 161 isolates of *E. faecalis* and in 23 (88.5%) of 26 *E. faecium* isolates (Table 7). The resistance markers presented by the enterococci isolates were high-level gentamicin resistance (MIC $>$ 500 μg/ml), high-level streptomycin resistance (MIC $>$ 1,000 μg/ml), a combination of high-level gentamicin resistance and high-level streptomycin resistance, vancomycin resistance, and the combination of high-level streptomycin resistance and vancomycin resistance.

DISCUSSION

This study was conducted as a step toward implementation of the Integrated Antimicrobial Surveillance Program for AMR in Colombia (COIPARS). The initial phase was to determine the prevalence (41%, $n = 70$), risk factors, and AMR profiles of *Salmonella* in broiler farms *(18).* Most of the previous studies in Colombia *(5, 24, 31)* evaluated *Salmonella* serovars, *E. coli,* or enterococci isolated from either foodborne diseases or from nosocomial infections in hospital settings, whereas our study presents the first set of baseline data for AMR in retail market chicken for these target microbes. One of the strengths of the study is its adaptation of methodologies from CIPARS that were not only easy to implement but that also provided standardized procedures to assess the prevalence of target bacteria. It also allowed for comparisons with other integrated surveillance programs utilizing the same methods *(12).*

As in every country, the occurrence of *Salmonella* serovars in food products in Colombia is a risk for human health. The prevalence of *Salmonella* (25.5%) in this study was different from the reports in retail market surveys from other countries, which ranged from 3% in New Zealand *(51)* to as high as 39% in Brazil *(43)* and 42% in Australia *(37).* However, the methodology used by these studies differed from ours, and thus it is difficult to make conclusive inferences. Our

TABLE 4. *Prevalence of antimicrobial-resistant* Salmonella *serovars,* Escherichia coli, *and* Enterococcus *isolates of very high importance (category I) to human medicine in poultry meat*

Antimicrobial agent	Abbreviation	Resistance prevalence (%)			
		Salmonella serovars ($n = 51$)	*E. coli* ($n = 165$)	*Enterococcus faecalis* ($n = 161$)	*Enterococcus faecium* ($n = 26$)
Carbapenems					
Ertapenem	ETP	0	3	—[a]	—
Imipenem	IPM	0	0	—	—
Meropenem	MEM	0	0	—	—
Cephalosporins, 3rd and 4th generation					
Cefepime	FEP	0	1.5	—	—
Cefotaxime	CTX	31.4	20	—	—
Cefoxitin	FOX	31.4	9.1	—	—
Ceftazidime	CAZ	0	4.8	—	—
Ceftiofur	XNL	25.5	16.4	100	92.3
Ceftriaxone	CRO	31.4	17	—	—
Fluoroquinolones					
Ciprofloxacin	CIP	41.2	32.1	27.3	11.5
Enrofloxacin	ENR	54.9	50.3	80.6	76.9
Levofloxacin	LVX	2.0	29.1	18.0	3.8
Glycopeptides					
Teicoplanin	TEC	—	—	1.9	0
Vancomycin	VAN	—	—	3.7	0
Monobactams					
Aztreonam	AZT	0	18.5	—	—
Ozazolidinones					
Linezolid	LND	—	—	1.2	0
Penicillin β-lactamase inhibitor combinations					
Amoxicillin-clavulanate	AMC	31.4	8.5	0	0
Ampicillin	AMP	33.3	40.0	0	0
Piperacillin-tazobactam	TZP	2.0	0	—	—
Streptogramins					
Quinupristin-dalfopristin	SYN	—	—	—	61.5

[a] —, not applicable.

study demonstrated the ease with which the CIPARS protocol can be adapted by official, as well as private, laboratories in a developing country such as Colombia.

Even though differences in prevalence of AMR between independent stores and main distributor centers were expected due to different processing plants as well as diverse handling environments, no significant differences between these two types of establishments were observed. In comparison with our first study at the farm level *(18),* an increase in the number of serovars in retail stores was established compared to broiler farms. Specifically, only two *Salmonella* serovars (Paratyphi B and Heidelberg) were detected at broiler farms; whereas, in the current study, four additional serovars were found. This increase in serovars indicates that additional contamination may be taking place anywhere between the farms and the retail stores and should be addressed *(34).* Alternatively, these serovars could have arisen from farms not sampled in our previous study.

The presence of AMR to several antimicrobials in almost all the isolates of the three bacteria evaluated in this study poses a risk to the human and animal population in Colombia. This is an important finding, considering the ability of these bacteria to cause foodborne diseases and to disseminate resistance genes *(2, 23, 35).* An important finding of concern in this study is that almost 98% of all the isolates tested were MDR. Again, these results are in concordance with the results in our previous study of poultry farms, in which 100% of the *Salmonella* isolates were MDR and none of the isolates were susceptible to all drugs tested *(18).* This is one of the highest reported prevalences of MDR *Salmonella* serovars in retail market meat *(25, 27).* Knowing that multidrug resistance is multifactorial, the importance of initiating the integrated AMR surveillance program is even greater.

Regarding resistance to first- and second-generation quinolones, which are the principal agents used in the treatment of human salmonellosis, our study found the prevalence of resistance to ciprofloxacin (41.2%), enrofloxacin (54.9%), and nalidixic acid (66.0%) to be much

TABLE 5. *Prevalence of resistant* Salmonella *serovars,* Escherichia coli, *and* Enterococcus *of high and medium importance in human medicine from poultry meat in Colombia*

Category	Antimicrobial agent	Abbreviation	Resistance prevalence (%)			
			Salmonella serovars ($n = 51$)	*E. coli* ($n = 165$)	*Enterococcus faecalis* ($n = 161$)	*Enterococcus faecium* ($n = 26$)
II—high importance	Aminoglycosides					
	Amikacin	AMK	0	0.6	—[a]	—
	Gentamicin	GEN	0.0	8.5	3.4	15.4
	Gentamicin-synergy[b]	GEH	—	—	21.1	15.4
	Tobramycin	TOB	2.0	7.3	—	—
	Streptomycin	STR	56.9	71.5	100	92.3
	Streptomycin-synergy	STH	—	—	56.5	57.7
	Cephalosporins, 1st and 2nd generation					
	Cefazolin	CZO	35.3	31.6	100	100
	Cephalothin	CEP	—	59.3	—	—
	Lincosamides					
	Clindamycin	CLI	—	—	100	100
	Macrolides					
	Erythromycin	ERY	—	—	82.0	73.1
	Tilmicosin	TIL	100.0	94.5	97.5	96.2
	Penicillins					
	Penicillin	PEN	—	—	0.6	11.5
	Oxacillin	OXA	—	—	100	100
	Quinolones					
	Nalidixic acid	NAL	66.0	64.0	—	—
	Trimethoprim-sulfamethoxazole	STX	49.0	32.7	2.5	3.8
III—medium importance	Fosfomycin	FOS	—	—	0	0
	Nitrofurans					
	Nitrofurantoin	NIT	51.0	7.9	1.2	34.6
	Tetracycline	TCY	60.8	92.5	98.1	88.5
	Chloramphenicol	CHL	7.8	34.5	23.1	15.4

[a] —, not applicable.
[b] Gentamicin-streptomycin (500 to 1,000 μg/ml).

higher than that reported in Canada *(12),* the United States *(25, 34),* and Denmark *(17).* To control this increase in resistance, the Colombian Ministry of Agriculture may consider mandating the use of prescriptions as a requirement for purchase of antimicrobials for use in the animal production sector.

Another important finding in this study is the high prevalence of resistance to ceftiofur (25.5%), which suggests the possibility of an association between the use of antimicrobials like ceftiofur at primary production systems and the presence of resistance in retail market bacteria. Extended-spectrum cephalosporins are used to treat many human infections, including septicemia in pregnant women and children, and the use of ceftiofur in primary production systems could lead to resistance to other cephalosporins *(19).* To diminish the risk of AMR for public health, actions could be taken by the official sector in Colombia in order to control the use of ceftiofur, as was done in Canada based on the results of the reports of CIPARS *(12).*

E. coli is abundant in the gastrointestinal tract and can cause disease in its own right under some circumstances; it was used in this study as an indicator of AMR of gram-negative bacteria. Given that the prevalence of resistant

TABLE 6. *Distribution of* Salmonella *serovars,* Escherichia coli, *and* Enterococcus *in poultry meat by the number of antimicrobial drugs to which they were resistant*

No. of antimicrobials	Prevalence of resistance (%)			
	Salmonella serovars ($n = 51$)	*E. coli* ($n = 165$)	*Enterococcus faecalis* ($n = 161$)	*Enterococcus faecium* ($n = 26$)
0	0	0.6	0	0
1–4	35.3	30.3	0	0
5–8	25.5	38.8	4.3	23.1
9–17	39.2	30.3	95.7	76.9

TABLE 7. *Resistance markers present in* Enterococcus *spp.*[a]

Markers	No. (%) resistant	
	Enterococcus faecalis ($n = 161$)	*Enterococcus faecium* ($n = 26$)
HLGR	34 (21.1)	4 (15.4)
HLSR	91 (56.5)	15 (57.7)
HLSR-HLGR	17 (10.6)	4 (15.4)
HLSR-VRE	3 (1.9)	0 (0)
VRE	6 (3.7)	0 (0)

[a] HLGR, high-level gentamicin resistance; HLSR, high-level streptomycin resistance; VRE, vancomycin resistance.

isolates found for most of the antimicrobials was significantly different in *E. coli* ($P < 0.05$) compared to *Salmonella,* this microorganism should be used in an integrated AMR surveillance program.

Of *E. coli* isolates, 99% were resistant to at least one antimicrobial drug. This is higher than found in prior studies *(20),* and resistance could be developing at the farm level or after. The highest prevalence of resistance, to tetracycline (92.5%), could be associated with the use of chlortetracycline *(4)* in feed as a growth promoter; this use is allowed in broiler farms in Colombia, in contrast with other European countries, such as Denmark, where it has been well documented that a substantial decrease of AMR occurred after growth promoter bans *(22).* Based on the AMR patterns found in our study, more research should be done to establish the types of growth promoters used in animal feed in Colombia and their impact on acquiring AMR. Furthermore, the use of antimicrobials in animal feed should also be closely evaluated.

Similar to findings in the United States *(23, 46),* a high prevalence of resistance in *E. faecium* isolates to the important antimicrobial quinupristin-dalfopristin was found, which is used when vancomycin resistance is present. This finding is especially puzzling because this streptogramin is not yet available in Colombia and the use of virginamycin, a suspected contributor to human carriage of *E. faecium (23),* is prohibited on Colombian poultry farms. The use of avoparcin is also prohibited, and the detection of vancomycin-resistant (VR) *E. faecalis* isolates poses a risk to humans due to the potential spread of VR genes among enterococci and to staphylococci, which are major causes of nosocomial infections *(33).*

One limitation of this study was that, due to financial constraints, evaluation of abattoirs was not possible, although these are an important link between the farm and the retail stores. This prevented estimation of the quantitative contribution of the primary sector in the presence of antimicrobial-resistant bacteria in retail stores. Future studies should take into account the entire poultry food chain using a similar study protocol.

In conclusion, our findings suggest the importance of assessing the classes of antimicrobials, the amounts, and the type of uses in the poultry industry in Colombia as a final step for the implementation of COIPARS. Furthermore, with the high prevalence of multidrug resistance reported in our study, Colombian authorities should endeavor to facilitate the implementation of an integrated AMR surveillance program.

ACKNOWLEDGMENTS

We notably thank Ian Gardner for his scientific guidance, essential for the completion of this research. We remarkably thank Dr. Naila Baig-Ansari, consultant epidemiologist for her advice in finalizing this manuscript. We thank Maria Victoria Ovalle for the validation of the BD Phoenix methodology. Finally, we extend our gratitude to Fabian Torres and Claudia Carrillo from Carulla and to Andres Valderrama from Universidad de los Andes, for their technical support during the execution of this project.

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