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Research Note

Antimicrobial Resistance and Genetic Diversity of *Campylobacter* spp. Isolated from Broiler Chicken at Three Levels of the Poultry Production Chain in Costa Rica

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ABSTRACT

Campylobacter spp. are considered the most common bacterial cause of human gastroenteritis, one of the four main causes of diarrheal disease worldwide, and they are one of the main foodborne pathogens causing hospitalizations and deaths. Here, 148 strains of *Campylobacter* spp. isolated from poultry at farms, processing plants, and retail stores in Costa Rica were examined for resistance to six antibiotics. An agar dilution test was used to determine the MIC and susceptibility profiles against doxycycline, ciprofloxacin, nalidixic acid, enrofloxacin, chloramphenicol, and erythromycin. In addition, a pulsed-field gel electrophoresis analysis was carried out to determine the genotype relatedness of a representative subset of the isolates. Approximately 136 (92%) of the 148 analyzed isolates showed resistance to the tested drugs. Nalidixic acid, ciprofloxacin, and enrofloxacin were the antibiotics for which resistance occurred most frequently (91.2, 85.8, and 85.8%, respectively); followed by doxycycline (25.0%), chloramphenicol (5.4%), and erythromycin (2.7%). The profile conferring only resistance to quinolones was the most frequently found, and only 2.0% of the isolates showed resistance to quinolones and macrolides simultaneously. Results showed a high frequency of resistant *Campylobacter* spp. strains and evidenced the distribution, selection, and circulation of resistant strains along the poultry chain from farms to consumers. Cross-contamination and resistance seem to play important roles in the dissemination of these strains at specific points of the poultry chain, even when control measures are being taken. The establishment of effective surveillance and control strategies represents an essential tool for foodborne diseases mitigation. The rational use of antibiotics, especially those still showing efficacy, should be a priority in both human and veterinary medicine to contain the progress of this phenomenon and its consequences.

HIGHLIGHTS

- Approximately 92% of *Campylobacter* isolates were resistant to at least one antibiotic.
- Five different susceptibility profiles were detected among 148 isolates.
- Resistance to quinolones was the most frequent profile.
- Differences were observed for resistance to ciprofloxacin and enrofloxacin along the chain.
- PFGE analysis suggests circulation of resistant strains along the chain.

Key words: Antimicrobial resistance; *Campylobacter*; Chicken meat; Foodborne pathogen; Public health; Pulsed-field gel electrophoresis

The genus *Campylobacter* comprises 27 species and 8 subspecies of bacteria *(31),* many of which are capable of causing campylobacteriosis in humans *(4)*. *Campylobacter* is considered the most common bacterial cause of human gastroenteritis, and it is one of the four major causes of diarrheal disease worldwide *(37, 48)*. Because of its survival ability in the animal gut, *Campylobacter* can contaminate raw meat during the slaughter process. Hence, eating undercooked chicken meat or ready-to-eat foods that have been in contact with raw chicken is the most common source of infection *(21, 40)*.

Campylobacter infections do not usually require therapeutic intervention *(41)*. However, there are specific clinical circumstances in which the use of antibiotics is indicated *(1, 34)*. Ciprofloxacin, an antibiotic of the fluoroquinolone group, has been widely used for the treatment of gastroenteritis caused by *Campylobacter* and

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FIGURE 1. *Frequency of resistant* Campylobacter *isolates (*n = *148) according to the MIC against six antibiotics.*

ATB*	MIC µg/mL[1]											n resistant (%)
	0.25	0.50	1.00	2.00	4.00	8.00	16.00	32.00	64.00	128.00	256.00	
ERY[2]					144	0	**3**	**1**	**0**	**0**		4 (2.7)
CIP[2]		17	4	**1**	**3**	**7**	**116**					127 (85.8)
ENR[3]	21	0	**0**	**35**	**58**	**34**						127 (85.8)
NAL[4]						11	2	**1**	**2**	**12**	**120**	135 (91.2)
DOX[2]			103	8	**3**	**2**	**10**	**22**				37 (25)
CHL[4]					117	23	8	**0**	**0**	**0**		0 (0)

[1]Vertical lines represent susceptible breakpoints.
[2]Based on breakpoints recommended by CLSI M45-A3 (2016).
[3]*Enterobacteria* cut-off values in CLSI VET01S 5th ed (2020).
[4]Based on cut-off values adopted by CDC NARMS (2019).
*Antibiotics (ATB) abbreviation: ERY (erythromycin), CIP (ciprofloxacin), ENR (enrofloxacin), NAL (nalidixic acid), DOX (doxycycline), CHL (chloramphenicol).

other bacterial pathogens *(26)*. Nevertheless, due to the rapid appearance and spread of resistant strains in the 1990s, macrolides were positioned as the drugs of choice for the treatment of severe cases of campylobacteriosis *(1, 19)*. As a result, an increasing trend in macrolide resistance has been observed in the last decade. Currently, the prescription of alternative antibiotics, as well as their extensive use in food production animals, has favored the development and spread of resistant strains to other drugs, triggering an alert for health authorities worldwide because campylobacteriosis is considered an emerging human disease *(7, 14, 47, 49)*.

In Costa Rica, the overall prevalence of *Campylobacter* in poultry for human consumption at farm, in processing plants, and in retail meat is 59.4% *(54)*. In addition, the consumption of poultry products and by-products nationwide is more than 23 kg per capita per year, surpassing the consumption reported by all other Central American countries *(50)*. Given these conditions, this study aimed to determine the antimicrobial resistance profiles to six antibiotics and the genetic diversity of *Campylobacter* strains isolated from broiler chicken at three levels of the poultry production chain in Costa Rica.

MATERIALS AND METHODS

Sample selection. In total, 148 *Campylobacter* isolates (n = 122 *C. jejuni,* n = 10 *C. coli,* n = 16 *Campylobacter* spp.) recovered from poultry at different establishments nationwide between March and July 2015 were analyzed. Samples were collected from cecal content (CC) of broilers from 37 farms (n = 50), carcass rinses (CR) from poultry after the spin chiller (treated with chlorine and/or peracetic acid) at six processing plants (n = 55), and CR of whole clean chicken at 43 retail stores (RS) (n = 43). Buffered peptone water (Oxoid Ltd., Ogdensburg, NY) was used for the rinsing process. Both CR and CC were enriched in Preston enrichment broth base (Oxoid Ltd.) and later incubated in modified charcoal cefoperazone deoxychocolate agar plates (Oxoid Ltd.) for 48 h at 42 ± 1°C under microaerophilic conditions (10% O_2, 5% CO_2, and N_2 for balance) *(54)*. Next, all isolates were analyzed by enzymatic tests (oxidase and catalase), microscopic morphology, and specific-species PCR, as described by Zumbado et al. *(54)*. Confirmed *Campylobacter* isolations were stored at −80°C at the Bacteriology Laboratory, Escuela de Medicina Veterinaria, Universidad Nacional *(54)*.

Antimicrobial resistance assay. Antibiotic susceptibility profiles were determined using a dilution technique in Mueller-Hinton agar (Oxoid Ltd.), as described in Clinical and Laboratory Standards VET01-A4 *(10)*. Five concentrations of erythromycin, nalidixic acid, ciprofloxacin, enrofloxacin, doxycycline, and chloramphenicol (Oxoid Ltd.) were tested in duplicated Mueller-Hinton agar plates by using serial dilutions in a base 2 logarithm (Fig. 1). Concentrations were defined based on the cut-off values published in Clinical and Laboratory Standards Institute M45 for erythromycin, ciprofloxacin, and doxycycline *(12)*; Centers for Disease Control and Prevention-National Antimicrobial Resistance Monitoring System for nalidixic acid and chloramphenicol *(9)*; and Clinical and Laboratory Standards Institute VET01-S5 for enrofloxacin *(13)*. Once inoculated, the plates were incubated for 48 h at 37°C under microaerophilic conditions *(11),* using a Campygen generating system (Oxoid Ltd.). *C. jejuni* ATCC 33560 was used as a control strain considering the ranges established in the VET01-S3 protocol *(11)*. The MIC was defined as the antibiotic concentration (in micrograms per milliliter) at the lowest dilution that completely inhibited bacterial growth *(10)*. Furthermore, the MIC values obtained for each isolate were compared with breakpoints depicted in Figure 1. All strains were classified as susceptible or resistant. The resistant group included intermediate and resistant isolates, because the intermediate profile has a MIC above the limit required to be considered susceptible and bacteria may not respond adequately to an antibiotic treatment under the usual therapeutic conditions *(10)*. Isolates classified as resistant to certain antibiotics were classified as resistant to the group of antibiotics to which the drug belongs. Likewise, those classified as resistant to three or more groups of antibiotics according to their MICs were considered multiresistant *(29)*.

Statistical analysis. A nonconditional logistic regression model was used to determine the association of isolates recovered at three points of the poultry chain with the susceptibility profiles. The rates of susceptibility and resistance were used as dependent variables, and the origin of the isolates (CC, CR, or RS) as the independent variable. Comparisons were assessed by a chi-square test and odd ratios (OR), using MINITAB 17 statistical software (Minitab, LLC, State College, PA). Associations considered a P value <0.05 as significant if the value of 1 was not included in the 95% confidence interval.

Molecular genotyping. A pulsed-field gel electrophoresis (PFGE) was performed to determine the DNA fingerprints of 68 isolates, following the protocol described in the Standard Operating Procedure for PulseNet PFGE of *C. jejuni,* developed by the Centers for Disease Control and Prevention *(8)*. The aim of this procedure was to determine the genetic relatedness of representative samples based on their origin (CC = 22, CR = 24, and RS = 22) and susceptibility profiles. Thus, different samples were selected based on the sample type, date of collection, and antimicrobial resistance profiles. Samples coming from same

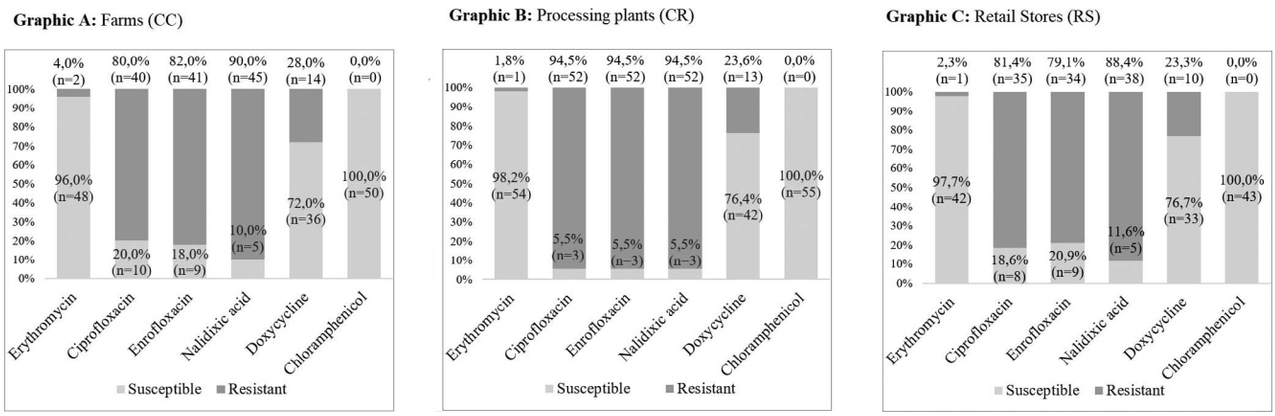


FIGURE 2. *Antimicrobial susceptibility frequencies displayed by 148* Campylobacter *spp. isolates, according to antibiotic and poultry chain point.*

sampling date and source with equal resistance profiles were considered as similar. In brief, bacterial cell suspensions in 0.85% saline solution, adjusted to a density of 0.8 to 1.2 McFarland (DensiCHECK plus, Biomérieux, Inc., Durham, NC), were used to prepare each of the plugs in 1% SeaKem Gold agarose (Lonza Rockland, Inc., Rockland, ME). Once molded, the plugs were lysed for a 2-h period before the washing phase. A prerestriction step with Tango buffer (Thermo Fisher Scientific, Waltham, MA) was executed before digestion with *Sma*I 40U (Thermo Fisher Scientific), during 3 h at 30°C. *Salmonella enterica* ser. Braenderup H9812 was used as control strain and digested with *Xba*I (Thermo Fisher Scientific). Electrophoresis was performed in SeaKem Gold 1% agarose gels of 10 and 15 lanes on a CHEF Mapper XA System (Bio-Rad Laboratories, Hercules, CA) at 13.5°C, 130 mA (15 lanes) and 112 mA (10 lanes), and a run time of 18 h. Gels were stained with a solution of GelRed 3X for 30 min and then washed with deionized water. Documentation of the images was performed using a GelDoc XR UV transilluminator (Bio-Rad Laboratories).

PFGE patterns were analyzed using the BioNumerics 5.1 software (Applied Maths NV, Sint-Martens-Latem, Belgium). The similarity levels were calculated using the Dice coefficient with a 1.5% optimization and a 1.5% band positioning tolerance to cluster the banding patterns. Dendogram construction was performed using the simple agglomerative hierarchical clustering method unweighted pair group method with arithmetic mean.

RESULTS

General features. In total, 136 (91.9%) of the 148 *Campylobacter* isolates showed resistance to at least one of the six antibiotics included in this study. Resistant *Campylobacter* was recovered in CC of 45 (90.0%) of 50 farm isolates, 52 (94.5%) of 55 CR at processing plants, and 39 (90.7%) of 43 carcasses from RS. Most of the isolations displayed resistance to nalidixic acid, enrofloxacin, and ciprofloxacin, whereas frequencies of resistance to doxycycline and erythromycin were lower. None of the isolates displayed resistance to chloramphenicol (Fig. 1).

Susceptibility profiles according to the poultry chain point. Resistant strains displayed a wide distribution along the three points of the chain (Fig. 2). Nevertheless, an association between resistant strains to ciprofloxacin and enrofloxacin and the origin of the isolations was observed (Table 1). For resistance to ciprofloxacin, a decreased risk was observed for the point of the poultry chain CC (i.e., isolates recovered from farms) compared with the point CR (i.e., isolates recovered from processing plants) (OR = 0.23; 95% confidence interval = 0.06 to 0.89; $P = 0.02$). For enrofloxacin, the strains from RS showed significantly lower frequencies of resistance than those from processing plants (OR = 0.22; 95% confidence interval = 0.06 to 0.86; $P = 0.02$). According to the statistical analysis criteria, there were no other significant comparisons.

Susceptibility profiles by antibiotic groups. Resistance to quinolones was the unique pattern most frequently observed (64.9%), followed by resistance to both quinolones and tetracyclines (24.3%). The third most observed pattern was susceptibility to the four groups of antibiotics (8.1%; Fig. 3). None of the analyzed isolates depicted a multiresistantce profile against the tested antibiotics.

PFGE patterns. In total, 58 *C. jejuni* and 11 *C. coli* isolates from the three levels of the poultry chain were analyzed by PFGE, and 39 different PFGE profiles were generated (25 unique patterns and 14 clusters of indistinguishable strains), with a similarity level ranging from 37.6 to 100.0% (Fig. 4). Strains isolated from the three levels of the poultry chain were widely distributed among the different clusters. Moreover, some isolates from different sources shared the same PFGE profile: PFGE patterns 004 (12.1%), 027 (10.3%), and 012 (8.6%) were the most frequently observed. Finally, isolates with varied susceptibility patterns were widely distributed throughout all the clusters. However, in some cases, the same antibiotic susceptibility profile was observed in genotypically indistinguishable strains, mostly sharing resistance to the three quinolones included in this research.

DISCUSSION

The results showed a high prevalence of resistance, mainly to quinolones and doxycycline, and to a lesser extent, to erythromycin. A similar trend has been observed

TABLE 1. *Comparisons of antimicrobial susceptibility frequencies to six antibiotics according to the poultry chain point*[a]*Author: A designated footnote should explain the meaning of the dashes in Table 1 body. Please provide/complete text for this new footnote* c. *Copy editor*

Antimicrobial agent	*n*	Poultry chain point	Absolute frequency of isolates		Measure of association A and B	
			n susceptible (%)	*n* resistant (%)	A-B: OR (*P* value)[b]	95% confidence interval
Erythromycin	50	CC	48 (96.0)	2 (4.0)	CC-CR: 2.25 (0.25)	0.20–25.60
	55	CR	54 (98.2)	1 (1.8)	RS-CR: 1.29 (0.40)	0.08–21.16
	43	RS	42 (97.7)	1 (2.3)	RS-CC: 0.57 (0.32)	0.05–6.53
Ciprofloxacin	50	CC	10 (20.0)	40 (80.0)	CC-CR: 0.23 (0.02)	0.06–0.89
	55	CR	3 (5.5)	52 (94.5)	RS-CR: 0.25 (0.04)	0.06–1.02
	43	RS	8 (18.6)	35 (81.4)	RS-CC: 1.09 (0.87)	0.39–3.08
Enrofloxacin	50	CC	9 (18.0)	41 (82.0)	CC-CR: 0.26 (0.04)	0.07–1.03
	55	CR	3 (5.5)	52 (94.5)	RS-CR: 0.22 (0.02)	0.06–0.86
	43	RS	9 (20.9)	34 (79.1)	RS-CC: 0.83 (0.72)	0.30–2.33
Nalidixic acid	50	CC	5 (10.0)	45 (90.0)	CC-CR: 0.52 (0.19)	0.12–2.30
	55	CR	3 (5.5)	52 (94.5)	RS-CR: 0.44 (0.27)	0.10–1.95
	43	RS	5 (11.6)	38 (88.4)	RS-CC: 0.84 (0.80)	0.23–3.14
Doxycycline	50	CC	36 (72.0)	14 (28.0)	CC-CR: 1.26 (0.61)	0.52–3.02
	55	CR	42 (76.4)	13 (23.6)	RS-CR: 0.98 (0.97)	0.38–2.51
	43	RS	33 (76.7)	10 (23.3)	RS-CC: 0.78 (0.60)	0.31–1.99
Chloramphenicol	50	CC	50 (100.0)	0 (0.0)	—[c]	—
	55	CR	55 (100.0)	0 (0.0)	—	—
	43	RS	43 (100.0)	0 (0.0)	—	—

[a] $n = 148$: farm (CC) = 50, abattoir (CR) = 55, and retail (RS) = 43.
[b] Odds ratio (OR) comparing the frequencies between poultry chain points (point A vs point B) for each tested antibiotic. Significant associations considered a P value <0.05 if value =1 was nonincluded in the 95% confidence interval.
[c] —, .

in multiple investigations worldwide *(5, 22, 24, 27, 38, 39, 44, 51)*. However, some reports have shown results that contrast with this pattern *(6, 33),* and a worldwide homogeneity among *Campylobacter* susceptibility profiles has not been observed. Comparisons among studies using similar methodology to that of the present research showed dissimilar results that could be attributed to differences in the regional use of antimicrobials in food animals, particularly in poultry production *(24)*. Traditionally, the emergence of resistant strains has been associated with the use of antimicrobial drugs in production animals *(16, 20, 43)*. In Costa Rica, information about the use of antibiotics in both agriculture and animal husbandry is scarce. At the national level, these drugs are commonly used indiscriminately, mediated by a poor technical criterion in the agricultural sector *(17)*. In species intended for human consumption, the current legislation demands compliance with withdrawal and discarding periods established in the *Codex Alimentarius*. However, no prescription is required to dispense antibiotics for animal husbandry *(2)*. Consequently, there is a high use of these drugs in the agricultural sector, and data reveal a use of from 1,169 to 109,908 g/ha yearly in some country regions *(17)*. The consequences of this antimicrobial overuse and the impact on human infections remain unclear. In 2014, the National Bacteriology Reference Center analyzed 27 *Campylobacter* human isolates; 55% ($n = 15$) displayed resistance to ciprofloxacin, 7.4% ($n = 2$) were resistant to tetracycline, and none were resistant to macrolides *(45),* a similar tendency to that observed in the present study. In people, in addition to the implications related to the difficulty in infections treatment, resistant strains are associated to hospitalizations, extended clinical symptoms, and higher risk of adverse events (death or invasive disease) *(25, 28, 30, 42)*.

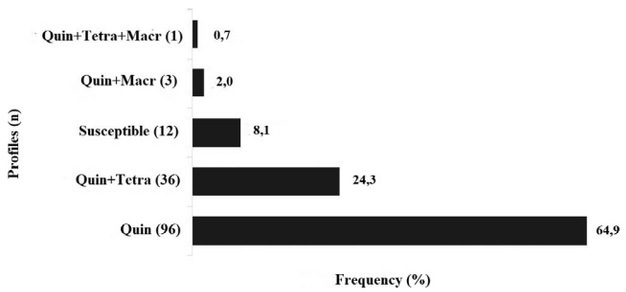
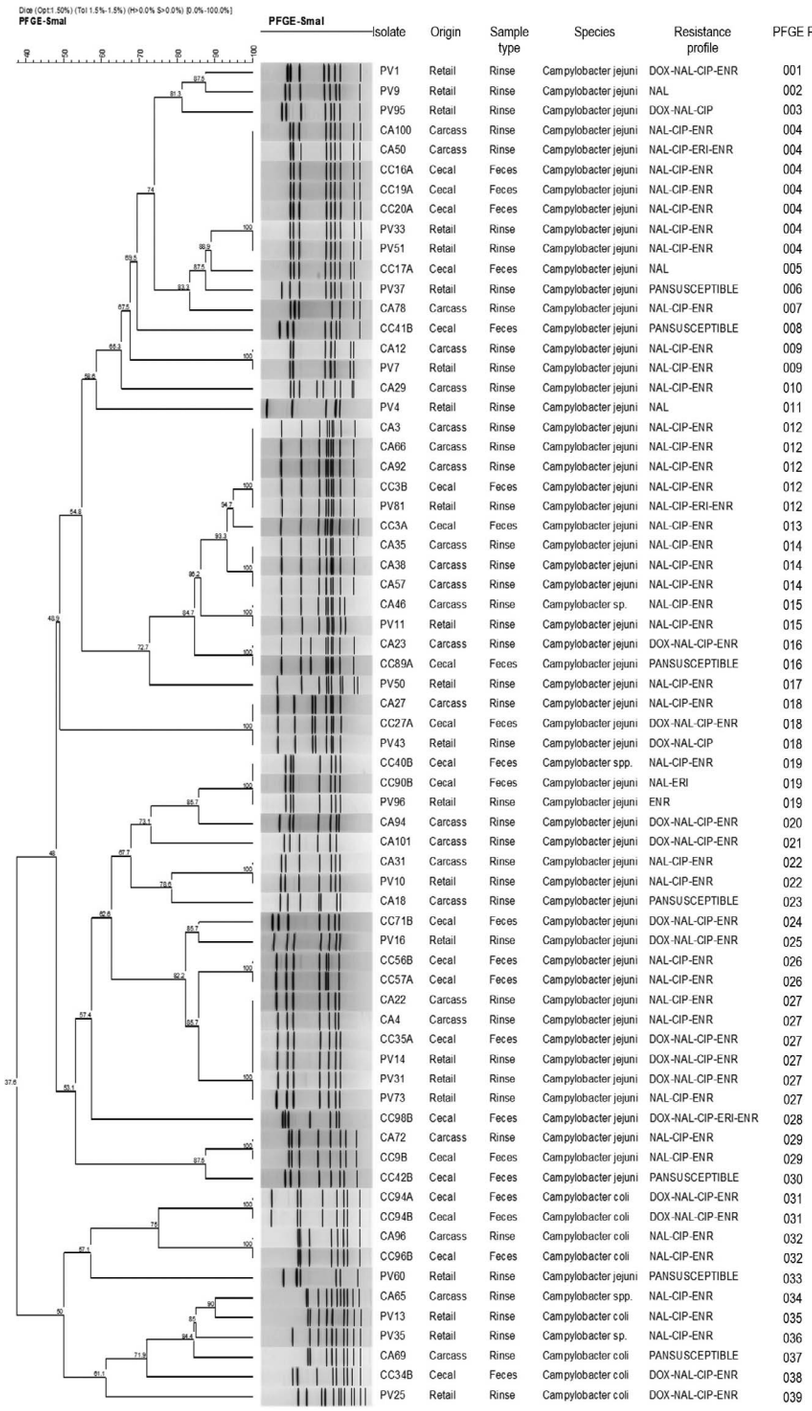


FIGURE 3. *Antimicrobial susceptibility profiles displayed by 148* Campylobacter *spp. isolates recovered from broiler chicken at three levels of the poultry production chain.*

From a public health perspective, simultaneous resistance to macrolides and quinolones is considered a highly undesirable pattern because these antibiotics are the first and second line for infections treatment in humans *(20)*. In this research, the single profile conferring resistance to quinolones was the most common. By contrast, a low percentage of isolates displayed the highly undesirable pattern of resistance to quinolones and macrolides concurrently. Another remarkable finding was the absence of multiresistant strains, contrasting with the data from studies in various geographical areas where multiresistance rates are much higher *(24, 32, 39, 46, 52)*.



Isolate	Origin	Sample type	Species	Resistance profile	PFGE Pattern
PV1	Retail	Rinse	Campylobacter jejuni	DOX-NAL-CIP-ENR	001
PV9	Retail	Rinse	Campylobacter jejuni	NAL	002
PV95	Retail	Rinse	Campylobacter jejuni	DOX-NAL-CIP	003
CA100	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	004
CA50	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ERI-ENR	004
CC16A	Cecal	Feces	Campylobacter jejuni	NAL-CIP-ENR	004
CC19A	Cecal	Feces	Campylobacter jejuni	NAL-CIP-ENR	004
CC20A	Cecal	Feces	Campylobacter jejuni	NAL-CIP-ENR	004
PV33	Retail	Rinse	Campylobacter jejuni	NAL-CIP-ENR	004
PV51	Retail	Rinse	Campylobacter jejuni	NAL-CIP-ENR	004
CC17A	Cecal	Feces	Campylobacter jejuni	NAL	005
PV37	Retail	Rinse	Campylobacter jejuni	PANSUSCEPTIBLE	006
CA78	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	007
CC41B	Cecal	Feces	Campylobacter jejuni	PANSUSCEPTIBLE	008
CA12	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	009
PV7	Retail	Rinse	Campylobacter jejuni	NAL-CIP-ENR	009
CA29	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	010
PV4	Retail	Rinse	Campylobacter jejuni	NAL	011
CA3	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	012
CA66	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	012
CA92	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	012
CC3B	Cecal	Feces	Campylobacter jejuni	NAL-CIP-ENR	012
PV81	Retail	Rinse	Campylobacter jejuni	NAL-CIP-ERI-ENR	012
CC3A	Cecal	Feces	Campylobacter jejuni	NAL-CIP-ENR	013
CA35	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	014
CA38	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	014
CA57	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	014
CA46	Carcass	Rinse	Campylobacter sp.	NAL-CIP-ENR	015
PV11	Retail	Rinse	Campylobacter jejuni	NAL-CIP-ENR	015
CA23	Carcass	Rinse	Campylobacter jejuni	DOX-NAL-CIP-ENR	016
CC89A	Cecal	Feces	Campylobacter jejuni	PANSUSCEPTIBLE	016
PV50	Retail	Rinse	Campylobacter jejuni	NAL-CIP-ENR	017
CA27	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	018
CC27A	Cecal	Feces	Campylobacter jejuni	DOX-NAL-CIP-ENR	018
PV43	Retail	Rinse	Campylobacter jejuni	DOX-NAL-CIP	018
CC40B	Cecal	Feces	Campylobacter spp.	NAL-CIP-ENR	019
CC90B	Cecal	Feces	Campylobacter jejuni	NAL-ERI	019
PV96	Retail	Rinse	Campylobacter jejuni	ENR	019
CA94	Carcass	Rinse	Campylobacter jejuni	DOX-NAL-CIP-ENR	020
CA101	Carcass	Rinse	Campylobacter jejuni	DOX-NAL-CIP-ENR	021
CA31	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	022
PV10	Retail	Rinse	Campylobacter jejuni	NAL-CIP-ENR	022
CA18	Carcass	Rinse	Campylobacter jejuni	PANSUSCEPTIBLE	023
CC71B	Cecal	Feces	Campylobacter jejuni	DOX-NAL-CIP-ENR	024
PV16	Retail	Rinse	Campylobacter jejuni	DOX-NAL-CIP-ENR	025
CC56B	Cecal	Feces	Campylobacter jejuni	NAL-CIP-ENR	026
CC57A	Cecal	Feces	Campylobacter jejuni	NAL-CIP-ENR	026
CA22	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	027
CA4	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	027
CC35A	Cecal	Feces	Campylobacter jejuni	DOX-NAL-CIP-ENR	027
PV14	Retail	Rinse	Campylobacter jejuni	DOX-NAL-CIP-ENR	027
PV31	Retail	Rinse	Campylobacter jejuni	DOX-NAL-CIP-ENR	027
PV73	Retail	Rinse	Campylobacter jejuni	NAL-CIP-ENR	027
CC98B	Cecal	Feces	Campylobacter jejuni	DOX-NAL-CIP-ERI-ENR	028
CA72	Carcass	Rinse	Campylobacter jejuni	NAL-CIP-ENR	029
CC9B	Cecal	Feces	Campylobacter jejuni	NAL-CIP-ENR	029
CC42B	Cecal	Feces	Campylobacter jejuni	PANSUSCEPTIBLE	030
CC94A	Cecal	Feces	Campylobacter coli	DOX-NAL-CIP-ENR	031
CC94B	Cecal	Feces	Campylobacter coli	DOX-NAL-CIP-ENR	031
CA96	Carcass	Rinse	Campylobacter coli	NAL-CIP-ENR	032
CC96B	Cecal	Feces	Campylobacter coli	NAL-CIP-ENR	032
PV60	Retail	Rinse	Campylobacter jejuni	PANSUSCEPTIBLE	033
CA65	Carcass	Rinse	Campylobacter spp.	NAL-CIP-ENR	034
PV13	Retail	Rinse	Campylobacter coli	NAL-CIP-ENR	035
PV35	Retail	Rinse	Campylobacter sp.	NAL-CIP-ENR	036
CA69	Carcass	Rinse	Campylobacter coli	PANSUSCEPTIBLE	037
CC34B	Cecal	Feces	Campylobacter coli	DOX-NAL-CIP-ENR	038
PV25	Retail	Rinse	Campylobacter coli	DOX-NAL-CIP-ENR	039

FIGURE 4. *Pulsed-field gel electrophoresis (PFGE) patterns of 148* Campylobacter *spp. isolates recovered from poultry production at farms (cecal), abattoirs (carcass), and retail stores (retail) in Costa Rica.*

Other important findings in this investigation are the differences in antibiotic susceptibility according to the poultry production chain point. Carcasses were kept together in cold water during the cooling process at abattoirs. Under these cooling conditions, cross-contamination with strains of *Campylobacter* spp. can occur between different batches of birds, even when preventive measures are taken, such as the use of chlorinated water and peracetic acid *(15, 53, 54)*. This might be the reason for some isolates from the same batches of birds at farms and processing plants displayed different PFGE profiles, that is, strains CC94B and CA94 (both nonsusceptible to quinolones). The persistence of resistance strains seems to play an important role in this regard. Previous studies have shown that antimicrobial resistance could confer bacteria a better response to stress causes by cold *(3)*. González and Hänninen *(23)* also showed higher survival of ciprofloxacin-resistant *Campylobacter* ATCC33560 in 4°C water than susceptible strains. This selection and dissemination of resistant strains could explain the higher risk of resistance to ciprofloxacin observed for isolates recovered from abattoirs (CC) compared with those from farms (CR). PFGE analysis

also provides information that suggests the circulation of resistant strains along the poultry production chain. For example, indistinguishable strains CC96B and CA96, collected from CC (farms) and postslaughter CR (processing plants) from the same batch of birds, also shared the same antibiotic susceptibility profile.

Cross-contamination could play an important role in those significant differences observed in the resistance to enrofloxacin between isolates from RS and processing plants (CR). As part of this study, previously published results of the prevalence and risk factors showed a positive association between the nonphysical separation of meats from different animals and the presence of *Campylobacter* spp. in retail chicken *(54)*. This could imply that some of the strains obtained from RS could be originated from products other than chicken meat *(54),* such as vegetables, uncooked processed products, or unpasteurized dairy products. Nevertheless, the prevalence and resistance profiles of *Campylobacter* spp. recovered from other food products in Costa Rica have not been investigated; thus, more studies are required to determine the validity of this hypothesis.

PFGE patterns displayed high diversity, a common finding in *Campylobacter* genotypic analyzes *(18, 24)*. Nevertheless, some strains isolated from the same level of the poultry chain point, but from different establishments, displayed the same genotypic pattern and susceptibility profile. This might indicate a wide distribution of genetically related strains. However, given the discrimination power of this genotyping technique, it is not possible to ensure that these strains were identical *(35)*. Whole genome sequencing should be considered in subsequent studies to assess the findings of the current investigation *(36)*.

This study represents the first report of antimicrobial resistance and genetic diversity of *Campylobacter* isolated from the poultry production chain in Costa Rica. The results show a high frequency of resistance, especially to quinolones, and evidence of the distribution, selection, and circulation of resistant strains along the poultry chain from farms to consumers. Cross-contamination seems to play an important role in the dissemination of these strains at specific points of the poultry chain, even when some control measures are in place. Given this scenario, the establishment of effective surveillance and control strategies to prevent *Campylobacter* dissemination along the production chain represents an essential tool for foodborne diseases mitigation. The rational use of antibiotics, especially those that still show efficacy, should be a priority issue in both human and veterinary medicine to contain the progress of this phenomenon and its consequences.

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