Veterinary Integrative Sciences 2024; 22(2): 501 - 510 DOI; 10.12982/VIS.2024.035

Vet Integr Sci

Veterinary Integrative Sciences

Faculty of Veterinary Medicine
Chiang Mai University

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj



Research article

Prevalence of *Campylobacter* and antibiotic susceptibility in chickens at slaughterhouses and retail markets in the Mekong Delta

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Abstract

This study aims to determine the prevalence of *Campylobacter* and its antibiotic susceptibility at slaughterhouses and live poultry markets in the Mekong Delta, Vietnam. A total of 177 samples, including 112 fecal samples, 28 cecal samples, and 37 rectal swab samples were collected. Thirty-nine samples tested positive for *Campylobacter* spp. by culture method, accounting for 22%. Isolation rates of *Campylobacter* in rectal swab samples were highest at 29.7% (11/37), followed by cecal samples at 25% (7/28) and fecal dropping samples at 18.8% (21/112). Species identification was performed by multiplex PCR and the result showed that the prevalence of *Campylobacter coli* (12.4%) was higher than *Campylobacter jejuni* (3.4%). Antibiotic susceptibility test showed that all isolated *Campylobacter* were susceptible to imipenem and meropenem (100%). Most *Campylobacter* were resistant to tetracycline (96.4%), ciprofloxacin (92.9%), nalidixic acid (89.3%), and ampicillin (67.9%). This study highlights the high contamination of *Campylobacter* and its antibiotic resistance in poultry in the Mekong Delta, Vietnam.

Keywords: Antimicrobial resistance, *Campylobacter*, Chicken, Mekong Delta

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Article history; *received manuscript: 29 April 2023*
revised manuscript: 25 September 2023
accepted manuscript: 02 October 2023
published online: 1 November 2023
Academic editor: *Korakot Nganvongpanit*

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INTRODUCTION

Campylobacter is a genus of Gram-negative bacteria with variable morphology with spiral, curved, or rod-shaped and are motile (Gilbreath et al., 2011). The bacteria is an etiological agent for Campylobacteriosis, which is a foodborne zoonotic disease. *Campylobacter* spp., particularly *C. jejuni* and *C. coli*, are among the most important pathogens causing human gastroenteritis likely more frequent than others by *Salmonella*, *Shigella*, or *Escherichia* (Acheson and Allos, 2001). Domestic and wild animals have a role to play in the widespread nature of human infection. Poultry is among the primary reservoirs of *Campylobacter* spp. without clinical symptoms, which reside in the lower gastrointestinal tracts of these animals. However, people with *Campylobacter* infection may undergo a variety of symptoms, including bloody diarrhea, abdominal pain, fever, headache, nausea, and/or vomiting. These symptoms might last 3 to 6 days without antibiotic treatment. However, more severe conditions can lead to autoimmune sequelae, e.g., Guillain Barré syndrome, especially in those already suffering from a weakened immune system (WHO, 2022).

Although *Campylobacter* infections are usually mild, self-limiting symptoms, severe or prolonged should need antimicrobial therapy. Fluoroquinolone, macrolides, and aminoglycosides are considered among the first-line antibiotics for treatment (Dai et al., 2020). Recently, there has been a significant rise in the number of *Campylobacter* strains worldwide that are resistant to antibiotics, posing a threat to both veterinary and human medicine (Kaakoush et al., 2015).

The strong evidence suggests the incidence of Campylobacteriosis has been on the rise since the early 2000s. The acceleration in the incidence and number of cases have been reported in several countries or regions (Kaakoush et al., 2015). Additionally, there is an increasing concern about antimicrobial resistance in *Campylobacter* isolates found in both humans and animals. However, in Vietnam to date, there are few published studies regarding the prevalence of *Campylobacter* spp. in animals (Luu et al., 2006; Carrique-Mas et al., 2014; Nguyen et al., 2014; Nguyen et al., 2016c; Minh et al., 2023). Previous studies found high proportions of *Campylobacter* in poultry and pigs resistant to a variety of antimicrobial agents, including fluoroquinolones (nalidixic acid and ciprofloxacin) (Nguyen et al., 2016a; Nguyen et al., 2016c). The objectives of this study were 1) to determine the prevalence of *Campylobacter* at slaughterhouses and retail markets in the Mekong Delta, Vietnam; 2) to determine antibiotic susceptibility of isolated *Campylobacter.*

MATERIALS AND METHODS

Sample collection

All experimental protocols followed the Animal Welfare Assessment (DT2022-03/KNN). The survey was conducted from June 2022 to October 2022 in An Giang, Can Tho, and Soc Trang provinces, which are representatives of thirteen provinces in the Mekong Delta, Vietnam. Sampling sites were at six slaughterhouses (one for An Giang, four for Can Tho and one for Soc Trang) and seven local fresh meat markets (two for An Giang, three for Can Tho

and two for Soc Trang). Chicken fecal, cecal, and rectal swab samples were collected from 112 live chickens after de-feathering and chilling.

Sample collection was performed following the regional antimicrobial resistance monitoring and surveillance guidelines (FAO, 2019). Immediately after collection, the samples were placed in individually sealed containers and transferred to the laboratory at 20°C within 12 h.

The information of each sample was recorded to track animals from the slaughterhouse to the markets. Data on slaughterhouse management practices, information on the flocks/herds, and other variables were collected using questionnaires. Both interviews and sample collections were conducted by trained veterinarians.

Isolation of *Campylobacter*

Detection and isolation of *Campylobacter* based on ISO 10272-1: 2017 (International Organization for Standardization) procedures. All specimens were inoculated directly on modified Charcoal Cefoperozone Deoxycholate (mCCD) agar (Oxoid, UK) and *Campylobacter* selective agar (Preston agar) (Oxoid, UK) for isolated colonies. Selective media plates were incubated at 41.5°C for 44±4 h under microaerophilic conditions (5% O_2, 10% CO_2, and 85% H_2) using CampyGen gas packs (Oxoid Ltd, UK).

A typical colony of *Campylobacter* on mCCD agar is greyish, flat and moist, often with a metallic sheen. On Preston agar, it is small and grey-brown in color. Suspicious colonies were selected from each media for biochemical tests. Gram-negative, seagull wings shape, spiraling corkscrew motility, oxidase-positive, and inhibited at 25°C under an aerobic atmosphere were identified as *Campylobacter*. *Campylobacter* spp. were differentiated based on the detection of catalase activity, hippurate hydrolysis, and indoxyl acetate hydrolysis.

Identification of *Campylobacter* spp. using PCR detection

Whole presumptive *Campylobacter* isolates were identified to the species (*C. jejuni* and *C. coli*) by PCR. DNA template for PCR of a single *Campylobacter* strain was performed by boiling method. A loopful of a colony was picked from the blood agar plated (Oxoid, UK) and mixed well into 1 mL sterile deionized distilled water. The suspension of bacteria was placed on a thermal block at 99°C for 15 min and centrifuged at 13,000 rpm for 5 min to lyse the cells. The supernatant from this suspension was used as the template for PCR.

Amplification of the DNA template was performed using multiplex PCR (mPCR) with four primer pairs that were previously developed (Table 1). A total 14 µl reaction mixture contains 3 µl of DNA template, 7 µl of the 2x MyTaq Mix master mix that included $MgCl_2$, 10x PCR buffer, dNTPs, 10 units of Taq DNA polymerase (Cat# BIO-25041, Meridian-Bioline, Germany) and 1 µl of mixture forward primers (10 µM of each primer 16S rRNA-F, *hipO*-F and *glyA*-F) and 1 µl of reverse ones (10 µM of each primer 16S rRNA-R, *hipO*-R and *glyA*-R). The initial denaturation step at 95°C for 6 min, followed by 30 cycles of 95°C for 30 s, 58°C for 30 s, 72°C for 1 min, and a final extension at 72°C for 10 min, then holding at 10°C. The resulting PCR products were visualized on 1.5% tris-acetate-EDTA agarose gels stained with ethidium

bromide alongside a 1,000 bp ladder (Bioline, UK) and photographed under UV transillumination. DNA extract of *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559 were used as positive controls, as well as sterile water was for control contamination.

Table 1 Primers for identification *Campylobacter* spp. by mPCR

Target gene	Primer	Sequence (5′→3′)	Product (bp)	Reference
16S rRNA	Camp_CH412_F	GGATGACACTTTTCGGAGC	816	(Platts-Mills et al., 2014)
	Camp_CH412_R	CATTGTAGCACGTGTGTC		
hipO	*C. jejuni_hipO*_F	ACTTCTTTATTGCTTGCTGC	323	(Wang et al., 2002)
	*C. jejuni_hipO*_R	GCCACAACAAGTAAAGAAGC		
glyA	*C. coli_glyA*_F	GTAAAACCAAAGCTTATCGTG	126	(Wang et al., 2002)
	*C. coli_glyA*_R	TCCAGCAATGTGTGCAATG		

Antimicrobial susceptibility testing

All *Campylobacter* isolates were evaluated the antimicrobial susceptibility with the Kirby-Bauer method for eleven antibiotics including ampicillin 10 µg (AMP10), imipenem 10 µg (IPM10), meropenem 30 µg (MEM), gentamicin 10 µg (GEN10), nalidixic acid 30 µg (NAL30), ciprofloxacin 5 µg (CIP5), sulfamethoxazole–trimethoprim 1.25/23.75 µg (SXT25), clindamycin 2 µg (CLI2), erythromycin 15 µg (ERY15), chloramphenicol 30 µg (CHL30), tetracycline 30 µg (TCY30). *C. jejuni* ATCC 33560 was used as a reference strain. Interpret the inhibition zone diameters according to the standards of the Clinical and Laboratory Standards Institute (CLSI, 2018; CLSI, 2023). The *Campylobacter* and *Enterobacteriaceae* breakpoints published by CLSI were used to interpret the inhibition zone diameters around antibiotic disks. If an isolate was resistant to three or more antimicrobial classes, it was considered multi-resistant bacteria.

Statistical analysis

Using Excel (version 2021) software to summarize data and descriptive statistics of primary data. Comparison of the difference of the ratio data was statistically analyzed by the Chi-Square Test method (χ^2) on Minitab (version 16.0) software. Use WHONET software to input antibiotic sensitivity test results, interpret results, and analyze susceptible (S) and resistant (R) rates. A comparison of the results between resistant and susceptible strains was performed by Fisher's exact test. *P*-values of less than 0.05 were considered to be significant.

RESULTS

Identification and differentiation of thermotolerant *Campylobacter*

Thirty-nine isolates were recovered from 177 samples, including 112 fecal samples, 28 cecal samples, and 37 rectal swab samples. All these were further confirmed by mPCR. Of those, 21 isolates were from fecal dropping (18.8%), 7 were from cecal (25%) and 11 were from rectal swabs (29.7%) (Table 2, Table 3).

Table 2 Prevalence of *Campylobacter* in slaughterhouses

Location	Fecal dropping (%) (n positive/n total)	Cecum (%) (n positive/n total)	Rectal swab (%) (n positive/n total)
An Giang	0 (0/16)	20.0 (1/5)	16.7 (1/6)
Can Tho	45.0 (9/20)	40.0 (2/5)	28.6 (2/7)
Soc Trang	15.0 (3/20)	25.0 (1/4)	33.3 (2/6)

Table 3 Prevalence of *Campylobacter* in live chicken markets

Location	Fecal dropping (%) (n positive/n total)	Cecum (%) (n positive/n total)	Rectal swab (%) (n positive/n total)
An Giang	6.3 (1/16)	16.7 (1/6)	16.7 (1/6)
Can Tho	25.0 (5/20)	16.7 (1/6)	33.3 (2/6)
Soc Trang	15.0 (3/20)	16.7 (1/6)	50.0 (3/6)

By species, identification through mPCR showed that *C. coli* had a higher infection rate than *C. jejuni* with 12.4% (22 isolates) compared to 3.4% (6 isolates) respectively. Among three types of samples, *C. coli* was found most frequently in the rectal swab with 8/37 samples (21.6%), followed by cecal with 5/28 samples (17.9%), and the fecal dropping with isolates accounted for 8% (9/112 samples). By contrast, the cecal has the highest infection rate of *C. jejuni* with 7.1% (2/28 samples), followed by rectal swabs and droppings with 2.7% (1/37 samples) and 1.8% (2/112 samples) respectively (Table 4). In general, *Campylobacter* is usually confirmed by identifying the organism in cultures of samples from stool or cecal (Vidal et al., 2013; Urdaneta et al., 2015).

Table 4 *Campylobacter* identification by using mPCR

Sample	No. of samples	No. of positive samples (%)	mPCR identification of *Campylobacter* (n=39)		
			C. jejuni	*C. coli*	Undetermined
Fecal dropping	112	21 (18.8)	3	9	9
Cecum	28	7 (25.0)	2	5	0
Rectal swab	37	11 (29.7)	1	8	2
Total	**177**	**39 (22.0)**	**6**	**22**	**11**

P>0.05

Antimicrobial susceptibility testing

Only 28 of *Campylobacter* isolates identified by the conventional culture methods could be re-cultivated after storage in brain heart infusion broth (BHI) with 20% glycerol. Twenty-eight *Campylobacter* isolates were investigated using CLSI as a reference for the antibiotic susceptibility testing and cut-off values. The results of antimicrobial susceptibility testing for isolated *Campylobacter* spp. against 11 chosen antimicrobial agents are presented in Table 5.

Table 5 The antimicrobial susceptibilities profile of *Campylobacter* spp. (n=28)

Antimicrobial agent	Disk content	Resistant (R)		Susceptible (S)	
		No. of isolates (%)	95% CI	No. of isolates (%)	95% CI
AMP	10 µg	19 (67.9)	47.6-83.4	7 (25.0)	11.4-45.2
IPM	10 µg	0 (0.0)	0.0-15.0	28 (100)	85.0-100
MEM	30 µg	0 (0.0)	0.0-15.0	28 (100)	85.0-100
GEN	10 µg	16 (57.1)	37.4-75.0	12 (42.9)	25.0-62.6
NAL	30 µg	25 (89.3)	70.6-97.2	1 (3.6)	0.2-20.2
CIP	5 µg	26 (92.9)	75.0-98.8	2 (7.1)	1.2-25.0
SXT	25 µg	14 (50.0)	31.1-68.9	14 (50.0)	31.1-68.9
CLI	2 µg	15 (53.6)	34.2-72.0	13 (46.4)	28.0-65.8
ERY	15 µg	14 (50.0)	31.1-68.9	14 (50.0)	31.1-68.9
CHL	30 µg	10 (35.7)	19.3-55.9	18 (64.3)	44.1-80.7
TCY	30 µg	27 (96.4)	79.8-99.8	0 (0.0)	0.0-15.0

Abbreviations of antimicrobial agents: AMP: ampicillin, IPM: imipenem, MEM: meropenem, GEN: gentamicin, NAL: nalidixic acid, CIP: ciprofloxacin, SXT: trimethoprim/sulfamethoxazole, CLI: clindamycin, ERY: erythromycin, CHL: chloramphenicol, TCY: tetracycline.

The results show that 100% of *Campylobacter* isolates were susceptible to imipenem and meropenem, followed by chloramphenicol (64.3%). *Campylobacter* was moderately susceptible to erythromycin and trimethoprim/sulfamethoxazole with 50% each; meanwhile, its susceptibility rate for clindamycin and gentamicin were 53.6% and 57.1%, respectively. The majority of isolates had high levels (above 85%) of resistance to tetracycline (96.4%), ciprofloxacin, and nalidixic acid (92.9% and 89.3% respectively). Ampicillin was also resistant to most *Campylobacter* with 67.9%. Multidrug-resistant profiles of isolated *Campylobacter* spp. are shown in Table 6. Almost *Campylobacter* spp. isolates were cross-resistant to two or more classes of antibiotics. Half of the isolates generally exhibited MDR with 14 of the 28 tested strains. The MDR rates of *C. coli* isolates were slightly higher than that of *C. jejuni* (16.7% compared to 59.1%, with no statistically significant difference (P=0.17). Quinolones, aminoglycosides, and tetracyclines seem the most common antimicrobial resistance patterns in *Campylobacter*. There were also five *C. coli* isolates (22.7%) resistant to greater than five antimicrobial classes tested.

Table 6 Antimicrobial susceptibility patterns of *Campylobacter*

Resistant to	Number of isolates		Total	*P* value
	C. jejuni (n=6)	*C. coli* (n=22)		
CIP/NAL	1 (16.7)	2 (9.1)	3 (10.7)	0.83
CIP/NAL-GEN	1 (16.7)	2 (9.1)	3 (10.7)	0.83
CIP/NAL-TCY	2 (33.3)	4 (18.2)	6 (21.4)	0.81
CIP/NAL-ERY-TCY	0	1 (9.1)	1 (3.6)	Not applicable
CIP/NAL-CHL-GEN	0	1 (9.1)	1 (3.6)	Not applicable
CIP/NAL-CHL-TCY	0	2 (18.2)	2 (7.1)	Not applicable
CIP/NAL-TCY-GEN	1 (16.7)	1 (9.1)	2 (7.1)	0.90
CIP/NAL-CLI-CHL-TCY	0	1 (9.1)	1 (3.6)	Not applicable
CIP/NAL-CLI-TCY-GEN	0	1 (9.1)	1 (3.6)	Not applicable
CIP/NAL-ERY-CLI-GEN	0	1 (9.1)	1 (3.6)	Not applicable
CIP/NAL-ERY-CHL-TCY	0	1 (9.1)	1 (3.6)	Not applicable
CIP/NAL-ERY-TCY-GEN	0	2 (18.2)	2 (7.1)	Not applicable
CIP/NAL-CHL-TCY-GEN	0	1 (9.1)	2 (7.1)	Not applicable
Greater than five agents	0	5 (22.7)	5 (17.9)	Not applicable
MDR (%)	**1 (16.7)**	**13 (59.1)**	**14 (50.0)**	**0.17**

Abbreviations of antimicrobial agents: AMP: ampicillin, IPM: imipenem, MEM: meropenem, GEN: gentamicin, NAL: nalidixic acid, CIP: ciprofloxacin, SXT: trimethoprim/sulfamethoxazole, CLI: clindamycin, ERY: erythromycin, CHL: chloramphenicol, TCY: tetracycline, MDR: multidrug-resistant Campylobacter.

DISCUSSION

Poultry is considered to be a natural reservoir for thermophilic *Campylobacter* spp. Commercial chickens are frequently colonized in the intestinal tract with high levels, primarily *C. jejuni* and *C. coli* (Sahin, 2015). The present study evaluated the presence of *Campylobacter* in both slaughterhouses and live chicken markets along with antimicrobial resistance. The prevalence of *Campylobacter* was 22%. The number of *Campylobacter* isolates from rectal swabs was the highest at 29.7%, followed by cecal samples at 25% and the lowest fecal-dropping samples at 18.8%. However, there were no statistically significant differences (P>0.05). The findings of this study are similar to the previous study by (Urdaneta et al., 2015), which reported the proportion of positive samples obtained with cloacal swabs was equal to or higher than that with cecal samples (P=0.04). Another has found that the growth kinetics of bacteria in the cecal was higher than that from the fecal (Woldemariam et al., 2008). Indeed, *Campylobacter* is associated with the cecum which could reach the titer of up to 10^9 CFU/1 g cecal contents (Allen et al., 2007). This phenomenon might explain the higher prevalence of *Campylobacter* detected from cecal samples compared to that from rectal swabs or fecal droppings.

By species, *C. coli* had a higher infection rate than *C. jejuni* with 12.4% (22 isolates) compared to 3.4% (6 isolates) respectively. In this study, the mPCR has helped to identify the presence of bacteria and accurately distinguish bacterial species at the same time. Indeed, more than one target sequence was used, specifically, 16S rRNA, *hipO*, and *glyA* for identifying *Campylobacter* spp., *C. jejuni*, and *C. coli*. This method could amplify the target DNA templates by using various pairs of primers in a single reaction. It has the potential to produce a considerable saving of time and effort in the laboratory without compromising test utility. These findings differed from the previous studies (Carrique-Mas et al., 2014; Nguyen et al., 2014) which the percentage of *Campylobacter* infections in poultry ranged from 22% to 35%. The result of the prevalence of *Campylobacter* among three types of samples was similar to a previous study by Truong et al. (2020) with the proportion of *C. coli* infection higher than that of *C. jejuni* with 60.5% and 35.1%, respectively. However, its result differed from those of Carrique-Mas et al. (2014) which more *C. jejuni* (28.4%) was isolated than *C. coli* (3.5%).

Twenty-eight *Campylobacter* isolates recovered from BHI with 20% glycerol-stored stocks were antimicrobial susceptibility testing. The number of strains recovered was smaller than that of stored strains. It is due to *Campylobacter*s being very sensitive to changes in environmental factors and can eventually lose viability during storage (Madden and Madden, 2019). There were high level resistances against antimicrobial groups such as quinolones and tetracyclines, which greater than 90% isolates observed and above 65% resistant to ampicillin. The results are similar to the study of Truong et al. (2020), which reported more than half of *Campylobacter* in chickens were resistant to the tested antibiotics. Specifically, 100% of bacteria were resistant to ampicillin, followed by ciprofloxacin (76.7%), nalidixic acid (75.3%), and tetracycline (63.3%). According to Carrique-Mas et al. (2014), 92% of *Campylobacter* was resistant to quinolones, while 100% and 99% were resistant to erythromycin and trimethoprim/sulfamethoxazole, respectively.

This is different from our results that isolates resisted moderately including erythromycin (50%), clindamycin (45.8%), gentamicin and trimethoprim/sulfamethoxazole (41.7% each). All *Campylobacter* isolates were susceptible to imipenem and meropenem, consistent with previous studies showing a high susceptibility of bacteria to carbapenems, ranging from 80% to 100%.

The high resistance rates may be due to the indiscriminate use of common antibiotics leading to the formation of resistance. In Vietnam, the annual estimate of antimicrobial usage in feeds aimed at animal production is approximately 1,023 tons representing 1.6% of the global (Van Boeckel et al., 2015). The study by Nguyen et al. (2016a) based on data from a survey in the Mekong Delta between March and June 2015 indicated that about 15-20% of total antimicrobial was used in chicken production and an estimated 690 mg of antibiotic used per chicken raised.

In the Mekong Delta, the most common antimicrobials reported were amoxicillin (10/10 studies); followed by enrofloxacin, norfloxacin, and doxycycline (9/10 studies); ampicillin and colistin (8/10 studies); neomycin, gentamicin, tylosin, trimethoprim, and florfenicol (7/10 studies); lastly erythromycin, sulfamethoxazole, and chlortetracycline (6/10 studies) (Nguyen et al., 2016b). Whilst penicillins, lincosamides, quinolones, and combinations of sulphonamides with trimethoprim were the most quantitatively used compounds in the Mekong Delta chicken farms, and in 84% of cases, antimicrobials were administered for prophylactic purposes (Nguyen et al., 2016b). Besides the main purpose of treating and preventing animal diseases in veterinary medicine, antimicrobials are often added to feedstuffs as antimicrobial growth promoters (AGP) in order to increase productivity. Over the years, there has been mounting evidence that the use of antimicrobials in agriculture (including AGP) contributes to the development of antimicrobial resistance (Nguyen et al., 2016b). Although the magnitude of this contribution is unknown, it is likely to be variable depending on the types and quantities of antimicrobials used previously (Nguyen et al., 2016b).

This study announced that *Campylobacter* showed a multidrug resistance phenotype. The obtained bacterial strains showed cross-resistance within the same antibiotic group as well as multi-resistance between different ones. Almost *C. jejuni* displayed MDR to fluoroquinolones, aminoglycosides, and tetracyclines, while *C. coli* likely to be multi-resistant to various classes of agents consisting of fluoroquinolones, aminoglycosides, tetracyclines, macrolides as well as phenicols. A high MDR prevalence was also observed in the previous surveillance studies (Chen et al., 2010; Li et al., 2017; Thomrongsuwannakij et al., 2017). It is worth noting that the MDR rate tends to be higher in *C. coli* compared to *C. jejuni* (Li et al., 2017; Ma et al., 2017; Szczepanska et al., 2017). The extremely high rates of antimicrobial resistance in *Campylobacter* spp. isolates in this study suggested that some of the antimicrobial classes, such as quinolones and tetracyclines, may no longer be suitable for the treatment of *Campylobacter* infection in the Mekong Delta. Antibiotic resistance may not simply diminish with the removal of antibiotic selection pressure once it becomes prevalent. Thus, prudent measures for antimicrobial usage and active surveillance should be established to reduce the prevalence and spread of antimicrobial-resistant *Campylobacter*.

CONCLUSIONS

This study indicated the prevalence of *Campylobacter* as well as the antibiotic resistance risk of the bacteria in the Mekong Delta. This study demonstrated a high contamination of *Campylobacter* in all types of samples, which the prevalence accounting for 22%. Besides, *C. coli* was observed to have a higher prevalence than *C. jejuni* with 12.4% compared to 3.4%. Moreover, most *Campylobacter* showed high resistance to (96.4%), ciprofloxacin (92.9%), nalidixic acid (89.3%), and ampicillin (67.9%).

CONFLICT OF INTEREST

We have no conflict of interest.

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How to cite this article;

Le Hong Phong, Nguyen Phuc Bao Tran, Nguyen Thi Kim Cuc, Nguyen Thanh Lam, Nguyen Duc Hien. Prevalence of Campylobacter and antibiotic susceptibility in chickens at slaughterhouses and retail markets in the Mekong Delta. Veterinary Integrative Sciences. 2024; 22(1): 501 - 511