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Research Article

Investigating *Campylobacter* Spp. in Mekong Delta Duck Farms: Prevalence, Risk Factors, and Antibiotic Resistance

Nguyen Vu Thuy Hong Loan[1], Le Trung Kien[2], Do Ngoc Yen Phuong[1], Lam Thi Ngoc Trang[1], Le Minh Thanh[2] and Nguyen Trong Ngu[2]

[1]*Faculty of Veterinary Medicine and Animal Husbandry, HUTECH University, Ho Chi Minh City, Vietnam*
[2]*Faculty of Animal Sciences, Can Tho University, Can Tho City, Vietnam*

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Corresponding Author:
Nguyen Trong Ngu
Faculty of Animal Sciences,
Can Tho University, Can Tho
City, Vietnam
Email: ntngu@ctu.edu.vn

Abstract: *Campylobacter* spp. is a leading cause of gastroenteritis worldwide, contributing to significant health and economic burdens, especially in poor hygiene practices. Investigating this bacterium's prevalence and antibiotic resistance in ducks from the Mekong Delta is essential, as the region's extensive duck farming industry presents a potential risk for transmission to humans through the food chain. During August 2024 to February 2025, this study surveyed 15 households and collected 324 samples (78 wastewaters, 78 environmental/fecal, and 168 cloacal swabs) across three provinces (Soc Trang, Hau Giang, and Tien Giang) in the Mekong Delta to assess *Campylobacter* spp. prevalence. A questionnaire was collected to analyze risk factors, employing a combination of multiple correspondence analysis and logistic regression models to identify key determinants of *Campylobacter* spp. infection. The sample was cultured in nutrient broth medium for 24 hours to enhance proliferation before using the PCR method to detect Campylobacter spp. and identify associated antibiotic resistance genes. *Campylobacter* spp. was detected in 50.31% of 324 samples from Hau Giang, Soc Trang, and Tien Giang, with Tien Giang having the highest prevalence at 82.00% (n = 82), indicating significant regional variation ($p<0.001$). Sample type (environmental/fecal, cloacal swabs, or wastewater) showed no significant differences. Logistic regression identified Tien Giang as a significant risk factor for *Campylobacter* infection (OR = 16.3, $p<0.001$). Demographic and practice factors were not significantly associated. The *tetO* gene was the most prevalent (84.42%, n = 130), with Hau Giang showing the highest resistance rates. Environmental/fecal samples had the highest prevalence of resistance genes like *tetO* (93.18%, n = 41) and *cmeB* (54.55%, n = 24), highlighting the farm environment's role in spreading antibiotic resistance in the Mekong Delta. The results highlight the importance of focusing targeted biosecurity measures on high-risk areas. The detection of the highest rates of antibiotic-resistance genes in environmental fecal samples suggests that this could be a significant reservoir. This underscores the need for antibiotic stewardship and improved sanitation practices on farms.

Keywords: Antibiotic-Resistance, Campylobacter, Duck, Epidemiology, Mekong Delta

Introduction

Campylobacter spp. is a Gram-negative bacterium recognized as a leading cause of gastroenteritis and enteritis globally, exhibiting significant prevalence in low-, middle-, and high-income countries (Silva *et al.*, 2011; Igwaran and Okoh, 2019). This bacterium colonizes the intestinal tracts of livestock and poultry, particularly chickens and ducks, leading to the contamination of raw meat products (Fosse *et al.*, 2009; Hermans *et al.*, 2012).

Poultry meat, notably chicken, serves as a primary reservoir for *Campylobacter*, with *C. jejuni* identified as the dominant pathogenic species (Sheppard *et al*., 2009). Poor hygiene practices during slaughter and processing further elevate the risk of contamination, facilitating the entry of *Campylobacter* into the food supply chain (Pearce *et al*., 2003).

In developing nations such as Vietnam, inadequate food hygiene and unsatisfactory water quality contribute to heightened *Campylobacter* infections, resulting in elevated human infection rates in comparison to Salmonella and Shigella (Altekruse *et al*., 1999; World Health Organization, 2002). Although extensive research has been conducted on chickens (Hong *et al*., 2007; Huong *et al*., 2024; Rama *et al*., 2024), there is a lack of data regarding *Campylobacter* prevalence in ducks, attributed to the smaller scale of duck farming. Duck farming is a significant agricultural practice in Vietnam, providing essential sources of meat and eggs while supporting rural livelihoods (Nind and Tu, 1998). The consumption of duck meat and eggs is increasing, offering a crucial protein source for both urban and rural communities (Yitayih *et al*., 2021; Hossain *et al*., 2021). Recent studies in Vietnam reveal that the prevalence of *Campylobacter* in broiler chickens at slaughterhouses and markets ranges from 18.8 to 29.7% (Le Hong *et al*., 2023). In duck farms, the prevalence is reported at 23.9% (Carrique-Mas *et al*., 2014), in contrast to 31.9% in chicken farms. Ducks frequently harbor *C. jejuni* and *C. coli* asymptomatically, which can act as reservoirs for the pathogens, thereby posing a risk of transmission to other animals and potentially to humans (Adzitey *et al*., 2012). The significant prevalence of these bacteria in ducks and their role within Vietnam's food chain underscore their contribution to human *Campylobacter* infections, although precise transmission rates require further investigation.

The high prevalence of *Campylobacter* has led to an increased reliance on antibiotics in poultry farming, which in turn accelerates the emergence of antibiotic-resistant strains (Bukari *et al*., 2025). A study conducted by Han *et al.* (2019) identified resistance in duck-derived *Campylobacter* isolates to several antibiotics, including tetracycline, clindamycin, azithromycin, erythromycin, nalidixic acid, chloramphenicol, gentamicin, and ciprofloxacin, using PCR-based gene analysis. This resistance profile, which parallels that found in chickens, complicates treatment options and poses heightened public health risks (Tedersoo *et al*., 2022). It is imperative to implement continuous surveillance of antibiotic resistance genes through methodologies such as PCR, which is essential for monitoring resistance trends and guiding antibiotic stewardship. Additionally, taking measures to mitigate antimicrobial resistance in food-producing animals, including ducks, is crucial for the protection of public health (Dai *et al*., 2020).

Materials and Methods

Sample Collection

In each province of the study area, duck farming households were randomly selected from a list provided by the Sub-department of Animal Husbandry and Veterinary Medicine. The research team engaged directly with these selected households, and only those that consented to participate were incorporated into the sampling plan. Samples and information on duck farming households were collected from August 2024 to February 2025. Samples were collected from duck farming households, including cloacal swab samples obtained using sterile cotton swabs from individual ducks at each farm. Additionally, environmental fecal samples were gathered utilizing sterile cotton swabs to randomly collect feces from various locations within the pen, following a diagonal sampling pattern. These samples were pooled into a single sterile Falcon tube with a secure cap to avoid cross-contamination. Wastewater samples containing sediment were collected from different locations. Sediment was aspirated using a sterile 5 mL syringe from multiple sites and subsequently transferred into a sterile 50 mL Falcon tube, which was also securely capped. All samples were stored in an icebox at 2 - 8 degrees Celsius and transported to the laboratory for analysis within 24 hours to maintain quality. In total, 324 samples were collected across three provinces: Hau Giang, Soc Trang, and Tien Giang. This total comprised 168 cloacal swab samples, 78 environmental fecal swab samples, and 78 wastewater samples.

Campylobacter Spp. Detection

Cloacal swabs, environmental fecal swabs, and wastewater samples were transported to the laboratory and enriched for 24 hours. Samples were incubated at 37°C in NB medium for 24 hours to promote proliferation. DNA extraction followed the manufacturer’s protocol for the TopPURE® Genomic DNA Extraction Kit (ABT Biotechnology Solutions, Vietnam). Polymerase Chain Reaction (PCR) was conducted to detect the presence of *Campylobacter* spp. The PCR assay utilized the forward primer Campy_F: GGATGACACTTTTCGGAGC and reverse primer Campy_R: CATTGTAGCACGTGTGTC, targeting the 16S rRNA gene as recommended by Linton *et al.* (1996). The PCR reaction mixture for *Campylobacter* identification (16S rRNA gene) consisted of MyTaq mix (5.0 µl), forward primer (10 µM) (0.2 µl), reverse primer (10 µM) (0.2 µl), DNA template (2.0 µl), and PCR-grade water (2.6 µl). The reaction tubes were placed in a thermal cycler with the following program: 1 cycle at 95°C for 6 minutes, followed by 30 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 1 minute, concluding with 1 cycle at 72°C for 10 minutes.

Detection of Antibiotic Resistance Genes in Campylobacter Spp.

DNA samples testing positive for the *Campylobacter*-specific 16S rRNA gene primers were further analyzed to investigate the presence of selected antibiotic resistance genes. The specific primer pairs used for detecting these genes are listed in Table 1.

Multiple Correspondence Analysis

Multiple Correspondence Analysis (MCA), as described by (Snijders and Bosker, 1999), was used to visualize the connections between responses to two questionnaire sections: personal details and agricultural practices. MCA analyzes categorical variables by constructing a matrix that represents individual responses (I) and response categories (J). This matrix is treated as points in a high-dimensional Euclidean space, optimized to highlight the main sources of data variation. The results are shown in scatterplots of the first and second principal components, where similar response patterns are clustered together. Hierarchical clustering on these components (HCPC) employed Ward's method to group participants into comparable categories for each section. These clusters were utilized as independent variables in a multivariable logistic regression model to assess the likelihood of *Campylobacter* spp. infection in individual ducks. The MCA analysis was performed using the FactoMineR package (Husson *et al.*, 2008) in R version 4.4.1 (RCoreTeam, 2024).

Table 1: Nucleotide sequences of primer pairs used for detecting antibiotic-resistance genes in *Campylobacter* spp.

Antibiotic group	Gene	Nucleotide sequences	Tm	Product size	Reference
Beta-lactam	*blaOXA-61*	F- AGAGTATAATACAAGCG R- TAGTGAGTTGTCAAGCC	54	372	(Hadiyan *et al.*, 2022)
Tetracycline	*tetO*	F- GCGTTTTGTTTATGTGCG R- ATGGACAACCCGACAGAAG	54	559	(Pratt and Korolik, 2005)
Quinolone nalidixic acid	*gyrA*	F- GCTCTTGTTTTAGCTTGATGCA R- TTGTCGCCATCCTACAGCTA	50	620	(Abubakar *et al.*, 2019)
Amino-glycoside	*aph(2")-if*	F- AAGGAACTTTTTTAACACCAG R- CCWATTTCTTCTTCACTATCTTC	50	420	(Zhao *et al.*, 2015)
Multidrug efflux pump	*cmeA*	F- TAGCGGCGTAATAGTAAATAAAC R- ATAAAGAAATCTGCGTAAATAGGA	49.8	435	(Olah *et al.*, 2006)
	cmeC	F- CAAGTTGGCGCTGTAGGTGAA R- CCCCAATGAAAAATAGGCAGAGTA	52.3	431	
	cmeB	F- TCCTAGCAGCACAATATG R- AGCTTCGATAGCTGCATC	54	241	(Obeng *et al.*, 2012)

Mixed-Effects Logistic Regression

All variables grouped based on the MCA method were included in a fixed-effects logistic regression model to model the likelihood of *Campylobacter* spp. infection in ducks, considering variables with an unconditional association at $P \leq 0.2$. A backward stepwise method retained variables significant at $\alpha \leq 0.05$, and those that changed regression coefficients by over 20% were included. Although two-way interactions were examined, none were significant at $\alpha \leq 0.05$. The model was expanded to include farm-level effects regardless of significance. Normality and variance homogeneity were assessed through residual histograms and plots in the multilevel model. Variance estimates at the farm and bird levels relied on the logit scale's lowest variance, $\pi^2/3$ (π = 3.1416). The predicted *Campylobacter* spp. status was used to generate a Receiver Operating Characteristic (ROC) curve, with the Area Under the Curve (AUC) assessing the model's ability to distinguish *Campylobacter* spp. positive from *Campylobacter* spp. negative ducks. The AUC score of the ROC ranges from 0.5 to 1.0. Generally, the score categories are as follows: A score between 0.9 and 1.0 is considered excellent, 0.8 to 0.9 is good, 0.7 to 0.8 is fair, 0.6 to 0.7 is medium, and 0.5 to 0.6 is poor. Analyses were conducted using the epiR and lme4 packages in R version 4.4.1.

Results

Descriptive Statistics

This section summarizes the data on duck farm across various factors, including farmers' education level, age, gender, farming experience, income, farming purpose, livestock species, poultry age, flock size, farming system, feed type, water source, hygiene practices, and antibiotic use, as shown in Table 2. This study provides a descriptive analysis of duck farming practices among 15 households, emphasizing demographic characteristics, experience, economic conditions, and husbandry practices.

Table 2: Distribution of demographic variables and production practices at duck farms in the Mekong Delta

Variable	No. of farm	Percentage (%)	Variable	No. of farm	Percentage (%)
Age			Flock Size		
>50 year	7	46.67	30-299	8	53.33
41-50 year	8	53.33	≥300	7	46.67
Gender			Disinfection		
Male	12	80.00	Yes	2	13.33
Female	3	20.00	No	13	86.67
Education Level			Drinking Water		
Graduate	4	26.67	Tap	8	53.33
Elementary School	7	46.67	River	2	13.33
High School	4	26.67	Lake	5	33.33
Experience			Feed		
<1 year	1	6.67	Commercial	14	93.33
>10 year	14	93.33	Commercial and suplemetary	1	6.67
Income			Hand Washing with Soap		
31-50%	7	46.67	Always	12	80.00
>50%	8	53.33	Sometime	3	20.00
Purpose			Disinfection Frequency		
Meat	11	73.33	Yes	1	6.67
Egg	4	26.67	No	14	93.33
Species			Poultry Age		
Duck	2	13.33	≤14 day	1	6.67
Muscovy Duck	11	73.33	15-30 day	5	33.33
Sea Duck	2	13.33	>30 day	9	60.00
Farm Type			Antibiotic Use		
Close	1	6.67	Yes	11	73.33
Close and Grazing	14	93.33	No	4	26.67
Mixed Species			Probiotic Use		
Yes	10	66.67	Yes	12	80.00
No	5	33.33	No	3	20.00
Supplement Use					
Yes	4	26.67			
No	11	73.33			

In general, duck farming in the surveyed area is primarily managed by experienced, middle-aged male farmers who use mixed grazing systems. While personal hygiene practices are satisfactory, barn disinfection is lacking, and there is widespread use of antibiotics and probiotics, indicating opportunities for enhancing veterinary and husbandry practices.

Detection of Campylobacter Spp.

The results of the investigation into the presence of *Campylobacter* spp. in ducks from farming households in the Mekong Delta are presented in Table 3.

A total of 324 samples were collected and analyzed from three main provinces in the region: Hau Giang, Soc Trang, and Tien Giang. The overall prevalence of *Campylobacter* spp. was 50.31%, in which Tien Giang recorded the highest detection rate at 82.00%. Statistical analysis revealed significant differences in detection rates among the provinces, with an overall P-value of <0.001. Additionally, pairwise differences between provinces were noted. These findings indicate an uneven distribution of *Campylobacter* spp. across the provinces, with Tien Giang identified as the highest-risk area. This variation may be attributed to factors such as hygiene conditions, farming density, and management practices in each province. The results underscore the need for targeted control and surveillance measures for *Campylobacter* spp. in duck farming in the Mekong Delta, particularly in high-prevalence areas like Tien Giang.

There was a slight difference in the prevalence of *Campylobacter* spp. among the different sample types, as shown in Table 4. The highest prevalence was observed in environmental and fecal samples. However, the statistical analysis indicated that there were no statistically significant differences among the sample types (P = 0.2621). These findings suggest a relatively uniform distribution of *Campylobacter* spp. across the different sample types, with environmental fecal samples showing a slightly higher detection rate. This similarity may reflect the levels of bacterial contamination across various sources within the farming environment. The results emphasize the need for comprehensive control and surveillance measures for *Campylobacter* spp. throughout the duck farming system in the Mekong Delta, particularly focusing on the surrounding environment.

Table 3: Prevalence of *Campylobacter* spp. in duck farms by location

Province	No. of sample	Positive (+)	Prevalence (%)	*P*
Hau Giang	122	53	43.44^{a}	
Soc Trang	102	28	27.45^{b}	<0.001
Tien Giang	100	82	82.00^{c}	
Total	324	163	50.31	

^{a,b,c}: Different letters indicate significant differences with P-value <0.05

Table 4: Prevalence of *Campylobacter* spp. in duck farms by sample type

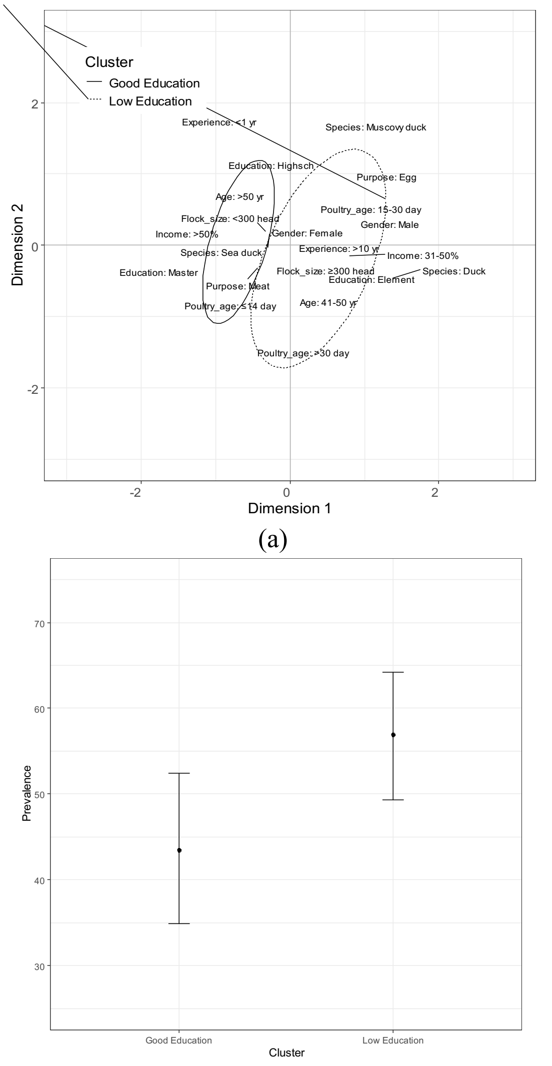
Sample type	No. of sample	Positive (+)	Prevalence (%)	*P*
Wastewater	78	35	44.87	
Environ/fecal	78	45	57.69	0.262
Cloacal swab	168	83	49.40	
Total	324	163	50.31	

Note: Environ: Environment

Multiple Correspondence Analysis (MCA) of Campylobacter Spp. Infection

Multiple Correspondence Analysis (MCA) was employed to elucidate the relationships between farmer characteristics, flock attributes, and the prevalence of *Campylobacter* spp. infection. Figure 1a clearly illustrates a significant difference in *Campylobacter* prevalence between two groups of farmers with differing education levels. The low-education group ("Low Education") exhibited a significantly higher prevalence (56.91%, 95% CI: 49.35–64.23) compared to the higher-education group ("Good Education", 43.51%, 95% CI: 34.88–52.45). This disparity likely reflects the influence of education on the ability to implement effective biosecurity and hygiene management practices in duck farming. Figure 1b displays the results of a Multiple Correspondence Analysis (MCA) examining the relationships between demographic factors, farming characteristics, and *Campylobacter* spp. Infections in ducks are present in households in the Mekong Delta. The analysis reveals two clusters based on education levels: Good Education and Low Education. The Good Education cluster (solid line) includes households with master's education, good farming experience (≥10 years), large flock sizes (≥300 head), middle income (31-50%), and a focus on meat production. These households are likely to adopt better farm management practices. Conversely, the Low Education cluster (dashed line) consists of households with secondary education, limited experience (<1 year), small flock sizes (<300 head), low income (<50%), and older farmers (>50 years) focusing on sea or Muscovy ducks for egg production. These conditions may heighten the risk of *Campylobacter* infections due to a lack of knowledge and control measures. Overall, the MCA highlights education as a significant factor influencing infection risk. Targeted educational programs on farm management are essential for low-education households to reduce *Campylobacter* spp. transmission in the Mekong Delta. This section explored the prevalence of *Campylobacter* spp. in ducks from farming households in the Mekong Delta, focusing on the impact of production practices. Figure 2a compares the prevalence between two groups: The Good Practice group, following standardized methods, and the Mix Practice group, using inconsistent practices. The Mix Practice group had a *Campylobacter* prevalence of 49.18% (95% CI: 36.14–62.30), while the Good Practice group showed a prevalence of 51.79% (95% CI: 45.42–58.12). Although the difference was not significant, the Mix Practice group exhibited greater variability, suggesting that inconsistent practices may increase the risk of *Campylobacter* infection. These findings suggest that adopting standardized production practices could help lower the risk of *Campylobacter* infections in duck farming in the Mekong Delta, highlighting the need for improved management in poultry farming.

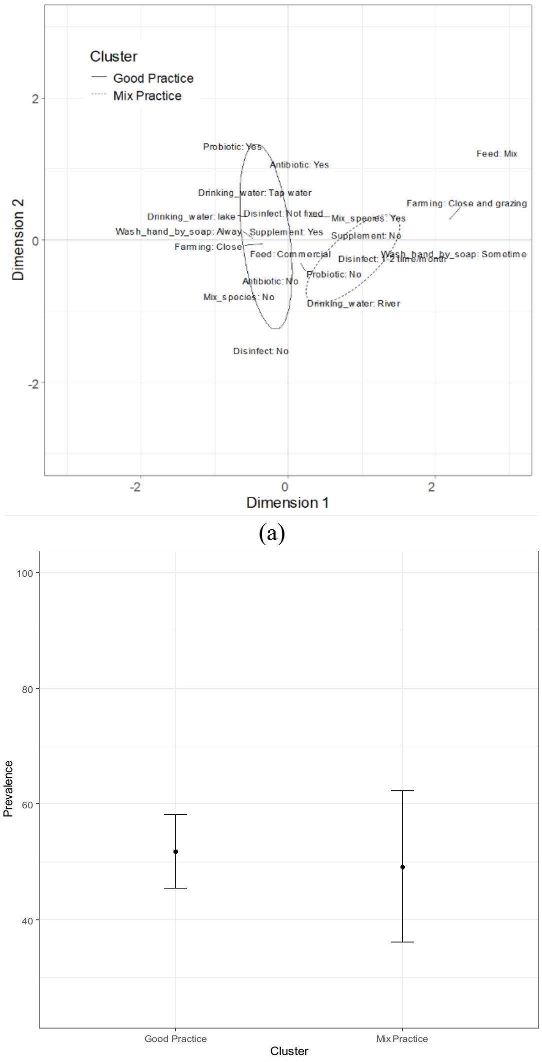
Figure 2b shows the results of the Multiple Correspondence Analysis (MCA), illustrating the relationship between production practices and *Campylobacter* spp. infection in duck farming households in the Mekong Delta. Two distinct clusters are identified: Good Practice and Mixed Practice. The Good Practice cluster (solid line), on the right side of the plot, is characterized by no antibiotic use, no probiotic use, use of commercial feed, regular disinfection (twice a month), occasional handwashing, and reliance on river water as a drinking source. This suggests that these households minimize antibiotic and probiotic use while maintaining basic hygiene, though river water usage poses a contamination risk. In contrast, the Mixed Practice cluster (dashed line), on the left side, is associated with: Antibiotic and probiotic use, mixed feed, multi-species farming, a combination of closed and free-range systems, lack of disinfection, infrequent handwashing, use of lake water for drinking, and inconsistent supplement use. This indicates that these households, with poor hygiene and diverse practices, are at a higher risk for *Campylobacter* infection. Overall, the MCA results highlight that production practices significantly influence the risk of *Campylobacter* infection. Emphasizing good hygiene and reducing unnecessary antibiotic use is essential to mitigate infection risks in duck farming in the Mekong Delta.



(a)

(b)

Fig. 1: (a) Multiple correspondence analysis biplot showing questionnaire responses related to respondent demographics. (b) Error bar plot showing Campylobacter spp. prevalence (and its 95% confidence interval) for the two clusters shown in (a) by demographic classification



(a)

(b)

Fig. 2: (a) Multiple correspondence analysis biplot showing questionnaire responses related to respondent practice. (b) Error bar plot showing Campylobacter spp. prevalence (and its 95% confidence interval) for the two clusters shown in (a) by practice classification

Table 5: Regression coefficients and their standard errors from a mixed-effects logistic regression model quantifying the association between location, demographic clusters, practice clusters, and *Campylobacter* spp. positivity

Explanatory variable	Samples	Positive samples	Coefficient (SE)	z	*P*-value	OR (95% CI)
Intercept	324	163	-0.6931(0.6628)			
Province						
Soc Trang	102	28	Reference	-	-	1.0
Hau Giang	122	53	0.5361(0.7225)	0.742	0.458	1.7 (0.4-7.0)
Tien Giang	100	82	2.7890(0.5264)	5.299	<.001	16.3 (5.8-45.6)
Demographic						
Good education	131	57	Reference	-	-	1.0
Low Education	181	103	-0.6492(0.8136)	-0.798	0.425	0.5 (0.1-2.6)
Practice						
Good Practice	251	130	Reference	-	-	1.0
Mix Practice	61	30	0.4262(0.5395)	0.790	0.430	1.5 (0.5-4.4)

Table 6: The proportion of antibiotic-resistance genes by location

Province	No. of sample	*blaOXA-61* (n/%)	*tetO* (n/%)	*gyrA* (n/%)	*aph(2")-if* (n/%)	*cmeA* (n/%)	*cmeB* (n/%)	*cmeC* (n/%)
Hau Giang	47	21 (44.68)	45 (95.74)	28 (59.57)	17 (36.17)	20 (42.55)	34 (72.34)	26 (55.32)
Soc Trang	27	1 (3.70)	21 (77.78)	11 (40.74)	6 (22.22)	9 (33.33)	18 (66.67)	11 (40.74)
Tien Giang	80	12 (15.00)	64 (80.00)	18 (22.50)	27 (33.75)	14 (17.50)	22 (27.50)	32 (40.00)
Total	154	34 (22.08)	130 (84.42)	57 (37.01)	50 (32.47)	43 (27.92)	74 (48.05)	69 (44.81)

Multivariable Logistic Regression

Table 5 presents the results of a multivariable logistic regression analysis examining the association between province, demographic factors, and production practices with *Campylobacter* spp. infections in ducks from farming households in the Mekong Delta. Out of 324 samples analyzed, 163 tested positive for *Campylobacter* spp. The model intercept was -0.6931 (SE = 0.6628), indicating the baseline probability of infection when all independent variables are set to their reference levels. Compared to Soc Trang (reference), Tien Giang exhibited a significantly higher risk of *Campylobacter* infection, with an Odds Ratio (OR) of 16.3 (95% CI: 5.8–45.6, P<0.001; coefficient = 2.7890, SE = 0.5264, z = 5.299). In contrast, Hau Giang showed no significant difference (OR = 1.7, 95% CI: 0.4–7.0, P = 0.458; coefficient = 0.5361, SE = 0.7225, z = 0.742), indicating that the risk in Tien Giang is markedly elevated, while Hau Giang does not show the same level of risk. Regarding education level, the low-education group (Low Education) exhibited a lower risk of infection compared to the high-education group (Good Education, reference), with an OR of 0.5 (95% CI: 0.1–2.6, P = 0.425; coefficient = -0.6492, SE = 0.8136, z = -0.798). However, this finding was not statistically significant. Similarly, the Mix Practice group, compared to the Good Practice group (reference), showed a higher risk (OR = 1.5, 95% CI: 0.5–4.4, P = 0.43; coefficient = 0.4262, SE = 0.5395, z = 0.79), but the association was also not statistically significant. The area under the ROC curve for the fixed-effects model was measured at 0.7 (data not shown), indicating a satisfactory to good ability to differentiate between ducks that tested positive for *Campylobacter* spp. and those that tested negative. In comparison, the area under the ROC curve for the mixed-effects model was recorded at 0.8, demonstrating a higher level of discrimination compared to the fixed-effects model. Overall, the analysis highlights the province, particularly Tien Giang, as the primary risk factor for *Campylobacter* spp. infection, presenting a 16.3-fold higher risk compared to Soc Trang. The demographic factors (such as education) and production practices did not show significant associations after controlling for other variables. These findings emphasize the importance of local factors in the transmission of *Campylobacter* and suggest that prioritizing control measures in high-risk areas like Tien Giang could help reduce the spread of this bacterium in duck farming throughout the Mekong Delta.

Antibiotic Resistance Genes

The investigation into the presence of antibiotic-resistance genes in *Campylobacter* spp. isolated from duck samples collected from farming households in the Mekong Delta is summarized in Table 6 Among the 163 *Campylobacter*-positive samples, 9 tested negative for all seven antibiotic-resistance genes. A total of 154 samples were analyzed from three provinces: Hau Giang, Soc Trang, and Tien Giang. The *tetO* gene exhibited the highest detection rate, with 130 out of 154 samples testing positive (84.42%). This was followed by the *cmeB* gene, found in 74 out of 154 samples (48.05%), and *cmeC*, detected in 69 out of 154 samples (44.81%). The *gyrA* and *aph(2")-if* genes were identified in 57 out of 154 samples (37.01%) and 50 out of 154 samples (32.47%), respectively. The lowest detection rates were noted for *cmeA* (27.92%, or 43 out of 154 samples) and *blaOXA-61* (22.08%, or 34 out of 154 samples). These findings indicate a high prevalence of the *tetO* gene among the *Campylobacter* population in the study area, while *blaOXA-61* was the least prevalent.

The distribution of antibiotic-resistance genes in *Campylobacter* isolates varied significantly across Hau Giang, Soc Trang, and Tien Giang. The *tetO* gene was most prevalent in Hau Giang (95.74%, or 45 out of 47 samples), followed by Tien Giang (80.00%, or 64 out of 80 samples) and Soc Trang (77.78%, or 21 out of 27 samples). The *cmeB* gene was more frequently found in Hau Giang (72.34%, or 34 out of 47 samples) and Soc Trang (66.67%, or 18 out of 27 samples), but was notably lower in Tien Giang (27.50%, or 22 out of 80 samples). Conversely, *blaOXA-61* had the lowest prevalence in Soc Trang (3.70%, or 1 out of 27 samples), compared to Hau Giang (44.68%, or 21 out of 47 samples) and Tien Giang (15.00%, or 12 out of 80 samples). The *cmeC* gene was more common in Hau Giang (55.32%, or 26 out of 47 samples) than in Soc Trang (40.74%, or 11 out of 27 samples) and Tien Giang (40.00%, or 32 out of 80 samples). Similarly, the genes *aph(2")-if* and *cmeA* were more prevalent in Hau Giang (36.17%, or 17 out of 47 samples and 42.55%, or 20 out of 47 samples, respectively) compared to Soc Trang (22.22%, or 6 out of 27 samples and 33.33%, or 9 out of 27 samples) and Tien Giang (33.75%, or 27 out of 80 samples and 17.50%, or 14 out of 80 samples). Finally, the *gyrA* gene showed the highest prevalence in Hau Giang (59.57%, or 28 out of 47 samples), decreasing in Soc Trang (40.74%, or 11 out of 27 samples) and Tien Giang (22.50%, or 18 out of 80 samples). These variations may reflect local factors such as patterns of antibiotic use and farming conditions.

Table 7: The proportion of antibiotic-resistance genes by sample type

Sample type	No. of sample	*blaOXA-61* (n/%)	*etO* (n/%)	*gyra* (n/%)	*aph(2")-if* (n/%)	*cmeA* (n/%)	*cmeB* (n/%)	*cmeC* (n/%)
Wastewater	35	6 (17.14)	30 (85.71)	14 (40.00)	11 (31.43)	9 (25.71)	15 (42.86)	17 (48.57)
Environmental/ fecal	44	14 (31.82)	41 (93.18)	21 (47.73)	16 (36.36)	19 (43.18)	24 (54.55)	24 (54.55)
Cloacal swab	75	14 (18.67)	59 (78.67)	22 (29.33)	23 (30.67)	15 (20.00)	35 (46.67)	28 (37.33)
Total	154	34 (22.08)	130 (84.42)	57 (37.01)	50 (32.47)	43 (27.92)	74 (48.05)	69 (44.81)

The investigation into antibiotic resistance genes in *Campylobacter* spp. Revealed significant variations across three sample types: Wastewater, environmental/feces, and cloacal swabs (Table 7). The *tetO* gene, associated with tetracycline resistance, exhibited the highest detection rate across all sample types, with environmental feces showing the highest prevalence (93.18%, 41/44), followed by wastewater (85.71%, 30/35), and cloacal swabs (78.67%, 59/75). The *cmeB* gene, linked to multidrug efflux pump, was most prevalent in environmental feces (54.55%, 24/44), compared to cloacal swabs (46.67%, 35/75) and wastewater (42.86%, 15/35). Similarly, the *blaOXA-61* gene, conferring beta-lactam resistance, had the highest prevalence in environmental feces (31.82%, 14/44), with lower rates in cloacal swabs (18.67%, 14/75) and wastewater (17.14%, 6/35). The *cmeC* gene, associated with multidrug efflux pump, showed higher prevalence in environmental feces (54.55%, 24/44) and wastewater (48.57%, 17/35) compared to cloacal swabs (37.33%, 28/75). The *aph(2")-if* gene, also conferring aminoglycoside resistance, was relatively evenly distributed, ranging from 30.67% (23/75) in cloacal swabs to 36.36% (16/44) in environmental feces. The *cmeA* gene, another multidrug efflux pump gene, was most prevalent in environmental feces (43.18%, 19/44), decreasing to 25.71% (9/35) in wastewater and 20.00% (15/75) in cloacal swabs. Finally, the *gyrA* gene, linked to quinolone resistance, showed the highest prevalence in environmental feces (47.73%, 21/44), followed by wastewater (40.00%, 14/35), and the lowest in cloacal swabs (29.33%, 22/75). In conclusion, the results indicate that the *tetO* gene is the most prevalent antibiotic resistance gene in *Campylobacter* isolates from ducks in the Mekong Delta, while *blaOXA-61* is the least common. The higher prevalence of most resistance genes in Hau Giang may be associated with greater antibiotic use, larger farming scales, or specific environmental conditions in the province. Additionally, environmental fecal samples consistently exhibited higher detection rates for most resistance genes, likely due to the accumulation of resistant *Campylobacter* strains in the farm environment. This variation underscores the critical role of the farm environment in disseminating antibiotic resistance genes, highlighting the need for stricter antibiotic control measures in the study region. These findings emphasize the importance of further research into the mechanisms of resistance gene dissemination and implementing targeted control measures in the area.

Discussion

This study reports a high prevalence of *Campylobacter* spp. in duck farming households across the Mekong Delta, with an overall rate of 50.31% (163 out of 324 samples). This is consistent with earlier reports from the Mekong Delta, which found Campylobacter spp. prevalence at 31.9% in chickens and 22.5% in ducks (Carrique-Mas *et al*., 2014). In broiler chickens, prevalence ranges from 18.8% in slaughterhouses to 29.7% in markets (Le Hong *et al*., 2023). Additionally, a study conducted in the northern region reported a Campylobacter spp. prevalence of 38.3% in chicken meat sold at retail markets (Huong *et al*., 2024). Notably, the prevalence varies by province, with Tien Giang showing the highest rate at 82.00%, compared to 43.44% in Hau Giang and 27.45% in Soc Trang. The high prevalence of Campylobacter spp. in Tien Giang may be closely tied to livestock density. According to the General Statistics Office of Vietnam, as of June 1, 2024, Tien Giang has the largest poultry population in the Mekong Delta region, totalling 16.57

million birds. Additionally, it has the highest pig population among the three provinces surveyed, with 293,000 pigs compared to 146,157 in Hau Giang and 191,685 in Soc Trang. The combination of high livestock density and proximity among different species increases the risk of *Campylobacter* spp. transmission between them (Huneau-Salaün *et al*., 2007; Carrique-Mas *et al*., 2014). Among sample types, environmental fecal samples had the highest prevalence (57.69%), followed by cloacal swabs (49.40%) and wastewater (44.87%). While differences among sample types were not statistically significant (P = 0.2621), the detection rate in environmental samples suggests they may act as a reservoir for *Campylobacter* spp. This aligns with findings that environmental contamination facilitates persistent presence (Newell *et al*., 2011). Ducks, as asymptomatic carriers, pose zoonotic risks through contaminated meat and water (Adzitey *et al*., 2012). The high prevalence in Tien Giang points to the need for targeted interventions, including enhanced biosecurity and environmental sanitation. The results underscore the urgency of addressing *Campylobacter* spp. in duck farming, which has received less attention than chicken farming. Future research should focus on assessing prevalence trends and identifying dominant strains and transmission pathways. The current study emphasizes the widespread presence of *Campylobacter* spp. in the Mekong Delta, revealing significant variations among different study areas. This is concerning, as *Campylobacter* is one of the four major causes of diarrhea worldwide and is recognized as the most common cause of gastroenteritis in humans. While *Campylobacter* infections are typically mild in adults, they can be severe and potentially fatal in young children, the elderly, and individuals with weakened immune systems. To prevent *Campylobacter* infections, it is essential to adhere to basic food safety and hygiene practices during food preparation, as *Campylobacter* can be easily eradicated through high temperatures. Therefore, in the Mekong Delta, particularly in duck farms, it is crucial to implement comprehensive surveillance, biosecurity measures, and strict hygiene protocols to minimize the risk of zoonotic transmission (World Health Organization, 2020).

This study utilized Multiple Correspondence Analysis (MCA) to examine the relationships among demographic factors, production practices, and the prevalence of *Campylobacter* spp. in duck farming households in the Mekong Delta. Compared to traditional analytical methods, such as univariate or logistic regression analyses, MCA presents several advantages in this context. First, MCA effectively handles categorical data, allowing for the simultaneous analysis of multiple qualitative variables (e.g., education, farming experience, and production practices) without assuming linear relationships, which is a limitation of traditional regression models (Le Roux and Rouanet, 2010). This capability is particularly valuable in veterinary epidemiology, where complex interactions between socio-demographic and management factors often occur. Second, the graphical output of MCA, including biplots, provides a visual representation of variable relationships and clusters, making it easier to identify patterns that may be obscured in traditional statistical outputs (Greenacre, 2017). For instance, the clear separation of the Good and Low Education clusters, as illustrated in Figure 1b, intuitively demonstrates the role of education in influencing infection risk. Additionally, MCA captures multidimensional associations, revealing how factors such as low education, small flock sizes, and egg production collectively impact *Campylobacter* spp. prevalence, which single-variable analyses might overlook (Husson *et al*., 2017). In practice, these variables may co-occur due to underlying trends or patterns, making MCA a more realistic approach for reflecting the complex interplay of factors in real-world settings. This comprehensive approach is essential for designing targeted interventions, such as educational programs for farmers with low education levels or standardized protocols for households employing mixed practices. However, it is critical to note that MCA is descriptive and does not establish causal relationships, necessitating complementary methods like logistic regression for hypothesis testing. Therefore, a logistic regression model based on MCA analysis was established in the present study, and the results showed that Tien Giang is a high-risk area for *Campylobacter* spp. infection in duck farming in the Mekong Delta, with a significantly elevated risk (OR = 16.3, 95% CI: 5.8–45.6, P<0.001) compared to Soc Trang. In contrast, Hau Giang showed no significant difference in risk (OR = 1.7, 95% CI: 0.4–7.0, P = 0.458). The analysis also revealed that neither education level (OR = 0.5, 95% CI: 0.1–2.6, P = 0.425) nor production practices (OR = 1.5, 95% CI: 0.5–4.4, P = 0.43) were significantly associated with infection risk after adjustments. The high risk observed in Tien Giang is likely due to regional factors, such as intensive farming practices or inadequate biosecurity measures that facilitate the transmission of *Campylobacter* (Huneau-Salaün *et al*., 2007). The lack of significant findings regarding education and farming practices suggests that regional effects might influence these factors or that larger sample sizes are needed to detect potential associations. Integrating Multiple Correspondence Analysis (MCA) with multivariable logistic regression improves the study's reliability by better reflecting real-world dynamics. MCA allows for the visualization of complex relationships between categorical variables (such as education and practices), while logistic regression quantifies risk associations while controlling for confounders (Le Roux and Rouanet, 2010). This combined approach offers a robust and comprehensive analysis, capturing complex interactions and ensuring that findings align with the epidemiological context of *Campylobacter* in duck farming (Husson *et al*., 2017). The results suggest the need for targeted biosecurity interventions in Tien Giang to help mitigate the spread of *Campylobacter*.

While conventional methods like minimum inhibitory concentration testing and disk diffusion are well-established and reliable for determining antibiotic resistance phenotypes, challenges in the cultivation process for isolating Campylobacter spp. can impact the accuracy of these methods. Therefore, using PCR to detect antibiotic resistance genes has the potential to identify antibiotic resistance phenotypes and address the limitations associated with isolating *Campylobacter* spp. through cultivation (Clinical and Laboratory Standards Institute, 2021). The presence of antibiotic resistance genes in Campylobacter spp. isolated from duck samples in the Mekong Delta is crucial for understanding the potential for bacterial resistance. The detection of genes such as tetO, cmeB, cmeC, gyrA, aph(2″)-if, cmeA, and blaOXA-61 indicates a potential that confer resistance to key classes of antibiotics, including tetracyclines, aminoglycosides, quinolones, and beta-lactams. Notably, the high prevalence of tetO suggests strong selective pressure from using tetracycline in veterinary practice. A high correlation between the tetO gene and tetracycline-resistant phenotypes in Campylobacter spp. was demonstrated in a recent study. In addition, Moderate agreement was found between the Kirby Bauer disk diffusion method and PCR for tetracycline in Campylobacter spp. (Wanja *et al*., 2023). In summary, the PCR results indicate a significant prevalence of tetracycline-resistant strains within the study area, thus allowing us to draw this conclusion without the need to employ the disk diffusion method for verification. This gene is often plasmid-mediated, facilitating horizontal gene transfer among Campylobacter populations and potentially to other pathogens (Gibreel *et al*., 2004). The presence of multidrug efflux pump genes (cmeB, cmeC, cmeA) enhances resistance by enabling Campylobacter to expel multiple antibiotics, complicating treatment and increasing the risk of therapeutic failure (Lin *et al*., 2002). Although the detection of gyrA and blaOXA-61 is less prevalent, it indicates emerging resistance to quinolones and beta-lactams, which are critical for treating Campylobacter infections in both veterinary and human medicine (Han *et al*., 2019). Together, these genes highlight the potential for Campylobacter to develop multidrug-resistant phenotypes, posing a significant challenge to public health, particularly in regions with high poultry consumption (Santos-Ferreira *et al*., 2022; Popa *et al*., 2025). The variation in the prevalence of resistance genes across provinces and sample types emphasizes the influence of local and environmental factors on resistance dissemination. Hau Giang exhibited the highest detection rates for most genes, likely due to intensive antibiotic use, larger farming scales, or environmental conditions that favor the persistence of resistant strains (Adzitey *et al*., 2012). In contrast, the lower prevalence in Soc Trang and Tien Giang may reflect differences in farming practices or antibiotic exposure. The consistently higher detection rates in environmental fecal samples compared to cloacal swabs and wastewater suggest that the farm environment serves as a primary reservoir for resistant Campylobacter strains. This aligns with evidence that fecal contamination in poultry environments perpetuates the dissemination of resistance genes (Newell *et al*., 2011). The elevated prevalence in environmental samples highlights the need for rigorous farm hygiene and waste management to disrupt resistance transmission pathways (Reichelt *et al*., 2022). These findings underscore the urgent need for antibiotic stewardship and environmental control measures in Mekong Delta duck farming. Targeted interventions in high-risk areas like Hau Giang and improved sanitation could help mitigate the spread of resistance. Future research should focus on molecular analyses to assess gene functionality and transferability and longitudinal studies to monitor resistance trends. In conclusion, the widespread presence of resistance genes in Campylobacter spp. emphasizes their role in driving bacterial resistance, necessitating comprehensive strategies to curb antibiotic misuse and environmental contamination.

Conclusion

This study highlights the high prevalence of Campylobacter spp. in duck farming households throughout the Mekong Delta, with an overall detection rate of 50.31%. Significant regional variation exists, particularly in Tien Giang, where the rate is notably higher at 82.00%. Identifying Tien Giang as an important risk factor (OR = 16.3, $P<0.001$) emphasizes this province's urgent need for targeted interventions. Additionally, the widespread presence of antibiotic resistance genes, particularly tetO (84.42%), especially in environmental and fecal samples, underscores the critical role of farm environments in the spread of antibiotic resistance. These findings indicate that poor hygiene practices and environmental contamination in duck farming systems pose substantial risks for transmitting Campylobacter to humans through the food chain. To mitigate these risks, it is essential to implement enhanced biosecurity measures, improve waste management, and promote prudent antibiotic use in duck farming. Furthermore, additional research is needed to monitor and control Campylobacter spp. and their resistance patterns in the Mekong Delta.

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Author’s Contributions

Nguyen Vu Thuy Hong Loan: Conceptualization, formal analysis, writing original draft, writing review and edited.

Le Trung Kien and Le Minh Thanh: Data curation, formal analysis, writing original draft, writing review and edited.

Do Ngoc Yen Phuong and Lam Thi Ngoc Trang: Data curation, formal analysis, writing review and edited.

Nguyen Trong Ngu: Conceptualization, formal analysis, writing original draft, writing review and edited, and supervision.

The manuscript was reviewed by the authors.

Ethics

All procedures involving animals in this study were conducted in strict accordance with the ethical standards outlined by Vietnamese legislation. The study protocol was reviewed and approved by the Animal Ethics Committee of Can Tho University under Approval Code: CTU-AEC24030.

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