Original Article

Prevalence, Phylogeny, and Virulence Genes of *Campylobacter jejuni* and *Campylobacter coli* in Slaughtered Broilers in Iraq

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ABSTRACT

Background: *Campylobacter* is a leading cause of bacterial foodborne illness worldwide, affecting developed and developing countries.

Objectives: This study aimed to identify *Campylobacter jejuni* and *Campylobacter coli* species in broilers at slaughter in four governorates in Iraq and analyze phylogeny and occurrence of virulence genes in both species.

Methods: A total of 200 broilers were randomly selected from 20 farms. Cecal samples were collected and subjected to bacteriological identification. Multiplex PCR was used to identify *C. jejuni* and *C. coli* using specific primers for the *hipO* and *glyA* genes. *16S rRNA* sequencing and PCR-based detection of virulence genes (*cadF* and *cdtB*) were also performed.

Results: The overall prevalence of *Campylobacter* spp. was 36.5%, with *C. jejuni* (75.3%) being more prevalent than *C. coli* (24.7%). Phylogenetic analysis of *16S rRNA* sequences of *C. jejuni* isolates are grouped into a cluster with four clades. The local strains showed close genetic relationships with two *C. jejuni* strains from Thailand (MZ948926.1 and MZ948908.1) and India (ON920206.1), while the greatest divergence was observed between the local isolates, Polish (MN708183.1), and Philippine (MZ028017.1) strains. The local *C. coli* isolates (OP263114, OP263119, OP263120) exhibit a high degree of genetic relatedness to *C. coli* isolates from Spain (MT453963.1; MT453947.1; MT453968.1; MT453951.1), but greater genetic distance from a *Campylobacter* sp. isolate from Germany (PP989493.1). The virulence genes *cadF* and *cdtB* were detected in 76.7% and 82.2% of the isolates, respectively. The *cadF* gene was more prevalent in *C. coli* (77.7%) than in *C. jejuni* (76.36%) isolates, while *cdtB* was more prevalent in *C. jejuni* (83.6%) than in *C. coli* (77.7%) isolates.

Conclusion: This study highlights the high prevalence, genetic diversity, and virulence potential of *C. jejuni* and *C. coli* in Iraqi broilers at slaughterhouses, emphasizing the need for enhanced surveillance, biosecurity measures, and genomic studies to mitigate public health risks.

Keywords: *Campylobacter jejuni*, *Campylobacter coli*, *16S rRNA*, *cadF*, *cdtB*, Prevalence

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Introduction

Campylobacter species (spp.) are widespread and fastidious microorganisms (Waje et al., 2024). These thermophilic, gram-negative bacteria possess a single polar flagellum at one or both ends, exhibiting corkscrew motility and not forming spores. *Campylobacter* spp thrive at 37-42 ˚C under microaerophilic conditions. They typically appear as S-shaped spirally curved rods or seagull shapes (WOAH, 2024).

Although *Campylobacter* is rarely detected in broilers younger than 2-3 weeks of age, its prevalence increases, peaking at the time of slaughter (Perez-Arnedo & Gonzalez-Fandos, 2019). The slaughtering process significantly contributes to carcass contamination with *Campylobacter*, primarily during the evisceration step (Shange et al., 2019). European countries employ several strategies to reduce infection rates, including early screening of broiler flocks before slaughter, using bacteriophages and probiotics, and eradication programs for rodents and flies to control infection in chicken houses and slaughterhouses (EFSA, 2016).

The genus *Campylobacter* comprises at least 39 recognized species widely distributed across various hosts and environments (Ammar, 2021). Zoonotic *Campylobacter* spp. are naturally occurring organisms in the intestines of various mammals, birds, and reptiles and can also be found in associated environments such as water and soil (Huang & Mariano Garcia, 2022). Poultry is considered the major reservoir of zoonotic *Campylobacter* spp. and serve as a significant source of human campylobacteriosis (Thames et al., 2022). A meta-analysis and systematic review study conducted by Mia et al. (2024) found the global prevalence of campylobacteriosis in poultry and poultry products to be 44% and 43% in Asian countries.

Campylobacteriosis is currently considered the most commonly reported bacterial foodborne illness worldwide, affecting both developed and developing countries (Igwaran & Okoh, 2019; European Food Safety Authority [EFSA] and European Centre for Disease Prevention and Control [ECDC], 2019). *Campylobacter* spp. is responsible for about 500 million cases of gastroenteritis infections worldwide each year (Igwaran & Okoh, 2019). Campylobacteriosis usually is characterized by fever, abdominal cramps, and bloody diarrhea, which may result in hospitalization, especially in immunocompromised patients. *Campylobacter jejuni* and *Campylobacter coli* are the two main species associated with human campylobacteriosis globally (EFSA, 2020). The infection transmits primarily through undercooked chicken meat or meat products. Consequently, *Campylobacter* represents a global public health concern (Dogan et al., 2019).

To ensure consumer safety, it is crucial to identify and characterize pathogenicity markers in foodborne strains of *Campylobacter*. Many virulence factors of *Campylobacter* spp. found to be essential in pathogenesis, disease severity, and post-infection sequelae (Wieczorek et al., 2018). Campylobacter adherence to the fibronectin (*cadF*) gene encodes a protein that mediates bacterial cell adhesion by attaching to fibronectin. Additionally, the invasive ability of *Campylobacter* is associated with the secretion of toxins such as the cytolethal distending toxin (CDT), which is encoded by a three-gene operon (*cdtABC*). The *cdtB* gene encodes for the CdtB toxic subunit, whose main action is nucleus catalytic action, which is responsible for cell cycle arrest and apoptosis of immune cells and intestinal epithelial cells (Kemper & Hensel, 2023).

Phenotypic methods for species identification and differentiation of the genus *Campylobacter* are often difficult and unreliable due to the close similarity of the isolates in biochemical reactions. However, molecular techniques have been developed to study the genetic diversity of *Campylobacter* species and the relationships among these isolates to address human and animal health issues (Natsos et al., 2019; García-Sánchez et al., 2018). *Campylobacter* spp. are classified specifically by the *hipO* gene and *glyA* to *C. jejuni* and *C. coli*, respectively (Syarifah et al., 2020). PCR-based *16S rRNA* sequencing can identify the genus *Campylobacter*, differentiate isolates at the species level, and determine phylogenetic relationships among *Campylobacter* isolates (Hansson et al., 2008).

In Iraq, data on the prevalence and genetic relatedness of *Campylobacter* isolated from broilers at slaughtering are very limited. Therefore, the objectives of this study were to estimate the prevalence, identify and characterize potential sources of *C. jejuni* and *C. coli* contamination in broilers collected from different farms at the slaughterhouse, analyze the presence of two virulence genes, and study the relationship between some the isolates.

Materials and Methods

Study design and sample size calculation

This cross-sectional study used the formula $n=Z^2 p (1-p)/d^2$ (Thrusfield, 2018) to calculate the minimum sample size, where n=calculated sample size, Z=1.96 (95% confidence level), p=0.50 (expected 50% prevalence to maximize sample size due to variability in *Campylobacter* prevalence) (Hue et al., 2010), and d=0.07 (desired precision) (Phosa et al., 2022). It yielded a minimum sample size of 196. A sample size of 200 broilers was chosen to ensure adequate statistical power and precision for estimating the prevalence of *Campylobacter*.

Broiler samples

This study was conducted between mid-January and June 2022 at a local chicken abattoir in Babylon Governorate. Sampling was performed during the evisceration phase to ensure standardized sampling conditions and minimize variability (FAO, 2019).

A total of 200 broilers were randomly selected from 20 farms distributed across four governorates (10 farms from Najaf, 4 from Karbala, 3 from Babylon, and 3 from Muthanna). These governorates were selected based on two criteria: their status as key poultry suppliers to the slaughterhouse and their geographic location within the Middle Euphrates region, which reflects central-south Iraq's poultry production landscape. To ensure randomization, farms were selected from slaughterhouse records using a simple random sampling approach. Within each farm, 10 birds were sampled proportionally from different slaughter batches (15000–20000 birds per batch) without prior knowledge of their health status or origin. This selection minimized bias and ensured representativeness across batches. Sampling across different farms and batches also helped mitigate potential clustering effects associated with farm-level management practices. To further reduce variability, five cecal swabs were pooled per carcass to create a composite sample, following FAO (2019) guidelines. This measure allowed us to maintain analytical consistency and improve statistical robustness while capturing the broader microbial profile.

Isolation and bacterial identification

As mentioned previously, ten broiler carcasses were selected randomly. Cecal swabs were collected in transport media (Cary Blair C & S collection vial, Hardy diagnostic, USA) and transported within 2-3 hours in a coolbox to the laboratory. A loopful inoculum from each transport broth was plated on the *Campylobacter* selective medium (Criterion, Hardy diagnostic). Medium enriched with 5%-10% (vol/ vol) sheep blood and *Campylobacter* selective supplement (Karmali, Oxoid). All plates were maintained at 42 ˚C for 48 hours in microaerophilic conditions created by CampyloGen TM (CN0035, Oxoid) inside an anaerobic jar. The colonies that showed mucoid, grayish, nonhemolytic, flat, and spreading colonies were selected. A presumptive diagnosis was made for each suspected colony by gram staining, biochemical testing for catalase and oxidase production, and indoxyl hydrolysis (WOAH, 2024).

Multiplex PCR for identifying *C. jejuni* and *C. coli*

Genomic DNA was extracted from two to three colonies of preliminary identified *Campylobacter* isolates using Bioneer extraction DNA kit®. Multiplex PCR assay was used to identify *C. jejuni* and *C. coli* by specific primers (Table 1) for the *hipO* gene and *glyA* gene, respectively, for all presumptively identified *Campylobacter* spp. isolates according to previous studies (Wang et al., 2002; Khalil et al., 2020). The PCR was carried out in 25 uL volume. The reaction mixture consisted of 2 uL of bacterial DNA, 4 uL of each primer, 4 uL Taq DNA polymerase, 0.24 mM deoxy nucleoside phosphate, and 2 Mm $MgCl_2$. The PCR cycling conditions were performed as described previously (Wang et al., 2002), as follows: Initial denaturation step of 94 ˚C for 6 min, followed by 35 cycles, each consisting of 30 s at 95 ˚C, 30 s at 59 ˚C, 30 s at 72 ˚C, and a final extension at 72 ˚C for 7 min.

PCR amplification, sequencing, and bioinformatics analysis of *16S rRNA* gene

All *C. jejuni* and *C. coli* isolates confirmed by species-specific PCR were subjected to PCR reaction targeting the *16S rRNA* gene using universal primer pairs (Table 1).

A stratified random sampling approach was used to ensure representative selection of *Campylobacter* isolates for sequencing. Isolates were stratified into four groups based on geographic origin (Najaf, Karbala, Babylon, Muthanna) and further subdivided by colony morphology of *C. jejuni* and *C. coli* and PCR confirmation. Simple random sampling was applied to select isolates from different farms within each subgroup. Nine isolates (6 *C. jejuni*, 3 *C. coli*) were chosen for *16S rRNA* sequencing, proportional to their prevalence in the overall dataset (Sahu, 2016; Temesgen et al., 2025). This number was selected to balance sequencing cost with the need to reflect the diversity of *Campylobacter* populations in the

study. The *16S rRNA* PCR products and primer pairs were then shipped to Bioneer Company (Korea) for sequencing. Genetic identities of the received sequences for *C. jejuni* and *C. coli* strains were confirmed using BLAST analysis through the National Center for Biotechnology Information (NCBI) BLAST tool (BLAST, 2025).

The Neighbor-Joining (NJ) method inferred the evolutionary history (Saitou & Nei, 1987). The bootstrap consensus tree inferred from 500 replicates represents the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% of bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Tamura-Nei method (Tamura & Nei, 1993) and are in the units of the number of base substitutions per site. This analysis involved 9 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 1287 positions in the final dataset. Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021).

Characterizing *Campylobacter* virulence genes

The confirmed *C. jejuni* and *C. coli* were assessed for the presence of each *cadF* and *cdtB* virulence gene. The primer details are presented in Table 1, and cycling program conditions were as mentioned above, with annealing at 45 ˚C for *cadF* and 57 ˚C for *cdtB* genes (Pacholewicz et al., 2020).

Statistical analysis

Chi-square and Fisher's exact tests were used to compare significance between two percentages, which were performed using Social Science Statistics online software. A significant difference was considered when the p-value was less than 0.05 (Social Science Statistics, 2025).

Results

Prevalence of *Campylobacter* in broilers at slaughter

Bacteriological identification and traditional biochemical tests (oxidase, catalase, and indoxyl hydrolysis) were initially identified 89 *Campylobacter* spp. isolates. Multiplex PCR was the definitive confirmation method for identifying *C. jejuni* and *C. coli*. Totally 73 strains (36.5%) were characterized by multiplex PCR, 55 isolates (75.3%) were *C. jejuni*, and 18(24.7%) were *C. coli* (Figures 1 and 2), while 16 amplified no products. The chi-squared analysis comparing the prevalence of two predominant species revealed a significant difference (χ^2=18.75, P<0.0001), suggesting that *C. jejuni* is more prevalent in broilers at slaughter.

Table 2 lists a higher prevalence of *Campylobacter* in broilers collected from Babylon farms (56.7%), followed by those from Muthanna (50%), both with a statistical difference (χ^2=11.88, P<0.05) than the other two studied provinces.

16S rRNA sequencing and phylogenetic analysis

As shown in Figure 3, all PCR-confirmed isolates of *C. jejuni* and *C. coli* were amplified 1500 bp bands corresponding to the *16S rRNA* gene. After DNA sequencing, the sequences were analyzed using the NCBI BLAST tool to confirm the identity of the isolates. The sequences were deposited in NCBI's GeneBank database, and accession numbers were obtained for nine *Campylobacter* isolates (Table 3).

The phylogenetic tree (Figure 4), constructed using the NJ method in CLC Sequence Viewer 8.1, elucidated the genetic relationships among local *C. jejuni* isolates (OP263112, OP263113, OP263115, OP263116, OP263117, OP263118) and reference strains from Poland (MN708183.1), the Philippines (MZ028017.1), Thailand (MZ948908.1, MZ948926.1), and India (ON920206.1) based on *16S rRNA* gene sequences.

Although the *C. jejuni* isolates formed four distinct clades, they are grouped into a distinct cluster. The genetic distance within the local isolates is smaller than their genetic distance from reference strains. The local *C. jejuni* strains have genetic relatedness (99.56%-99.91%) with the Indian (ON920206.1) and Thai (MZ948926.1 and MZ948908.1) reference strains that clustered together and related to the local cluster, indicating a close evolutionary relationship. In contrast, the greatest divergence was observed between the local isolates and Polish (MN708183.1) and Philippine (MZ028017.1) strains.

Figure 5 displays a phylogenetic tree illustrating the genetic relatedness among several *Campylobacter* isolates, specifically focusing on the *C. coli* isolates from this study (identified by accession numbers OP263114, OP263119, and OP263120) and reference strains from other locations. The tree was constructed using the NJ method in CLC Sequence Viewer 8.1. The three *C. coli* isolates from this study appear to cluster together. The local *C. coli* isolates (OP263114, OP263119, OP263120) exhibit

Table 1. List of primer sequences utilized in the study with the expected size

Target Gene	Primer	Primer Sequence (5′-3′)	Size	Ref.
C. Jejuni hipO	CJF	ACTTCTTTATTGCTTGCTGC	323 bp	Wang et al. (2002)
	CJR	GCCACAACAAGTAAAGAAGC		
C. coli glyA	CCF	GTAAAACCAAAGCTTATCGTG	126 bp	Wang et al. (2002)
	CCR	TCCAGCAATGTGTGCAATG		
16S rRNA universal primer	F8–27	GGTTACCTTGTTACGACTT	1500 bp	Eden et al. (1991)
	R1510–1492	AGAGTTTGATCCTGGCTCAG		
CadF	cad F	TTGAAGGTAATTTAGATATG	400 Bp	Konkel et al. (1999)
	cad R	CTAATACCTAAAGTTGAAAC		
CdtB	cdtB-F	GTTGGCACTTGGAATTTGCAAGGC	495 bp	Bang et al. (2003)
	cdtB-R	GTTAAAATCCCCTGCTATCAACCA		

a high degree of genetic relatedness (99.37%- 99.91%) to *C. coli* isolates from Spain (MT453963.1; MT453947.1; MT453968.1; MT453951.1), while a *Campylobacter* sp. isolate from Germany (PP989493.1) was showed a higher genetic distance from local *C. coli* strains.

Molecular detection of virulence factors

It can be seen from the data in Table 4 that out of 73 *C. jejuni* and *C. coli* isolates, 56 isolates (76.7%) were positive for the *cadF* gene, while 60(82.2%) were positive for the *cdtB* gene as detected by PCR (Figures 6 and 7). Statistical analysis showed no significant difference in the prevalence of these genes among the isolates (χ^2=0.38, P=0.54). Regarding the distribution of these genes among two species, the *cadF* gene was identified in 42(76.36%) *C. jejuni* and 14(77.7%) *C. coli* isolates. The *cdtB* gene was found in 46(83.6%) *C. jejuni* and 14(77.7%) *C. coli* isolates. There was no statistically significant difference in the prevalence of the *cadF* gene between *C. jejuni* and *C. coli* (χ^2=0.015, P>0.05).

Discussion

Concerning the first research question, it was found that the prevalence rate of molecularly confirmed *Campylobacter* spp. in the present study was 36.5%, which is comparable to findings from many countries such as Jordan (31.6%; Alaboudi et al., 2020), Iran 25.09% (Mousavinafchi et al., 2023), Italy (34%; Stella et al., 2017), and Poland (32.5%; Popa et al., 2025). However, a higher prevalence (66%) was reported in Uganda (Okello et al., 2025). This elevated prevalence may be attributed to *Campylobacter* contamination of broilers during transport to the slaughterhouse, potentially due to stress and feed withdrawal (Hakeem & Lu, 2021). Globally, the control of *Campylobacter* in broiler production remains challenging, and a multi-factorial approach should be applied on farms and in slaughterhouses, including transportation, biosecurity, and prevention programs (Olsen et al., 2024). Beyond immediate control measures, a principled and systematic approach is needed, including uninterrupted surveillance programs, source attribution, risk assessment, antimicrobial resistance monitoring, and

Table 2. Isolation rate of *Campylobacter* among samples collected from four provinces during the period of study

Province	Number of Farms	No. of Samples Collected	Isolation Rate of Confirmed Isolates (No.)	95% CI
Karbala	4	40	22.5% (9)	12.32-37.50
Najaf	10	100	32% (32)	23.67-41.66
Babylon	3	30	56.7% (17)	39.23-72.65
Muthanna	3	30	50% (15)	33.15-66.85

Table 3. List of Gene Bank 1 numbers of nine locally isolated *C. jejuni* and *C. coli*

Strain	Accession Number
C. jejuni	Op263112
C. jejuni	Op263113
C. coli	Op263114
C. jejuni	Op263115
C. jejuni	Op263116
C. jejuni	Op263117
C. jejuni	Op263118
C. coli	Op263119
C. coli	Op263120

evaluation of intervention strategies, particularly in developing countries (Nakhaee & Hafez, 2023).

Among 73 *Campylobacter* isolates, *C. jejuni* had the highest prevalence rate (75.3%), followed by *C. coli* (24.7%). Following the present results, previous studies have demonstrated that *C. jejuni* is the most dominant species isolated from broiler carcasses globally (Ansarifar et al., 2023; Bioumy et al., 2024; Okello et al., 2025; Phu et al., 2023). Detecting these species in the present study is of great importance related to foodborne infections and human health.

Another important finding was the significantly higher isolation rates of *Campylobacter* in broilers in Babylon and Muthanna provinces compared to Karbala and Najaf. A possible explanation for this variation might be the high poultry production density in Babylon and Muthanna, which may facilitate gut colonization with *Campylobacter* due to increased environmental contamination and bird-to-bird transmission (Hakeem & Lu, 2021). Additionally, differences in poultry farming practices, including management systems, biosecurity measures, and antimicrobial usage may have influenced the prevalence of *Campylobacter* (Gržinić et al., 2023; Wang et al., 2023). Specific biosecurity practices that could contribute to these differences include fly and rodent control, water sanitization protocols, broiler flock thinning schedules, and litter management programs. Previous studies have shown inadequate biosecurity and hygiene measures in broiler farms and slaughterhouses contribute to higher bacterial loads (Gržinić et al., 2023). Furthermore, the use of antibiotics at the slaughter stage might select resistant *Campylobacter* strains, influencing isolation rates (Sodagari et al., 2024). Lastly, the uneven distribution of sampled farms, only three farms each from Babylon and Muthanna compared to four from Karbala and ten from Najaf, may have also contributed to the observed variation in prevalence across governorates.

C. jejuni and *C. coli* are closely related to the bacterial species that cause many clinical cases of gastroenteritis worldwide (Mohan et al., 2025). Accurate and reliable molecular typing methods are therefore crucial for epidemiological investigations, source tracking, and understanding the transmission dynamics of these pathogens. The *16S rRNA* gene sequence is used as a molecular tool to study phylogenetic relatedness and evolutionary history of bacterial isolates due to its conserved nature and sufficient variability for species differentiation (Nayak et al., 2014; Weinroth et al., 2021).

Table 4. The number and percentage of *cadF* and *cdt* genes among *C. jejuni* and *C. coli* isolated from broilers at slaughter

Gene	No. (%)	
	***C. jejuni* (55 Isolate)**	***C. coli* (18 Isolates)**
CadF	42(76.36)	14(77.7)
CdtB	46(83.6)	14(77.7)

Figure 1. PCR amplification products of the *hipO* gene for detection of *C. jejuni*

Note: Lane M: Safe-green™ 100bp Opti-DNA marker (ABM, Canada); Lanes: 1-6, and 8-11 shows positive results with *C. jejuni*, *hipO* gene (323 bp).

Phylogenetic analyses based on *16S rRNA* sequence data showed that the local *C. jejuni* isolates (OP263112, OP263113–OP263118) form a distinct cluster, indicating a close genetic relationship among them, suggesting they share a common lineage separate from most reference strains. This cluster's relatively short branch lengths imply minimal genetic variation among these isolates, consistent with localized transmission or a recent common ancestor (Clarridge, 2004). The local strains showed close genetic relationships with two *C. jejuni* strains from Thailand (MZ948926.1 and MZ948908.1) and a strain from India (ON920206.1), suggesting a common ancestry or similar evolutionary pathway, potentially influenced by global trade or environmental factors (Sheppard & Maiden, 2015). Despite this, the Polish and Philippine strains (MN708183.1 and MZ028071.1) are more genetically distant from the local isolates, highlighting potential geographical variation in *C. jejuni* populations, as previously noted in studies of *Campylobacter* diversity (Colles et al., 2003). These results highlight the genetic homogeneity of local

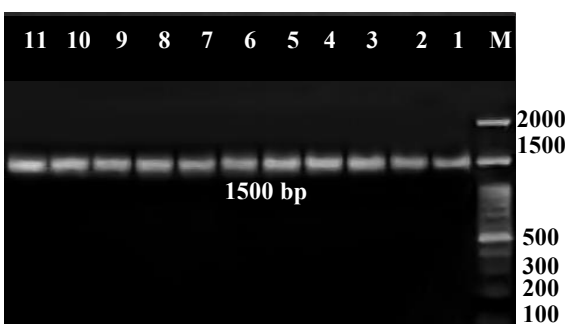

Figure 3. Agarose gel electrophoresis results of PCR amplification of *16S rRNA* gene in *C. jejuni* and *C. coli* isolates

Note: Lane M: Safe-green™ 100bp Opti-DNA Marker (ABM, Canada); Lanes: 1-11 shows positive results with *16S rRNA* gene (1500 bp).

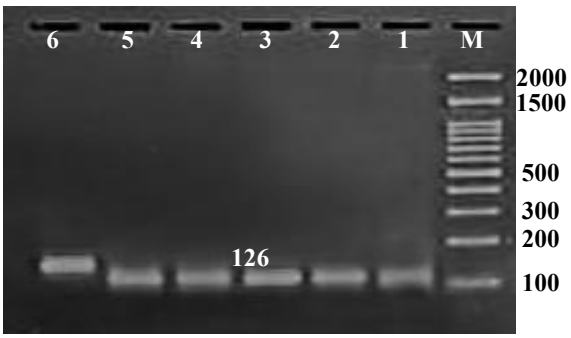

Figure 2. PCR amplification products of the *glyA* gene for detection of *C. coli*

Note: Lane M: Safe-green™ 100bp OptiDNA marker (ABM, Canada); Lanes: 1-6, show positive results with *C. coli glyA* gene (126 bp).

C. jejuni strains and their closer evolutionary relationship with strains from Southeast Asia.

The phylogenetic tree analysis also elucidated the close relationship among local *C. coli* strains. The clustering of the OP263114, OP263119, and OP263120 isolates suggests they are genetically closely related, potentially indicating a common source or recent diversification within the study area (Mbindyo, 2023). On the other hand, several *C. coli* isolates from Spain and *Campylobacter* isolates from Germany showed relatively close relationships to the local *C. coli* isolates. It suggests potential links or shared ancestry with *C. coli* strains circulating in these European countries. These relationships may partly be explained by importing chicks and domesticated animals from these countries or through migratory wild birds (Kovač et al., 2015; Cody et al., 2015).

These findings underscore the need for further genomic analysis, such as whole-genome sequencing (WGS), to explore virulence, antimicrobial resistance, and host adaptation mechanisms in these local isolates with higher resolution and accuracy. It is well known that WGS provides greater discriminatory power than *16S rRNA* sequencing alone, enabling detailed insights into pathogen evolution and epidemiology (Llarena et al., 2017). Such approaches could enhance our understanding of the local *Campylobacter* population structure and inform targeted public health interventions.

This study aimed to assess the presence of key virulence genes associated with adherence, invasion, and cytotoxicity using PCR. The *cadF* gene, which encodes a fibronectin-binding protein essential for bacterial attachment to host epithelial cells, is a crucial determinant of *Campylobacter* pathogenicity (Lu et al., 2025).

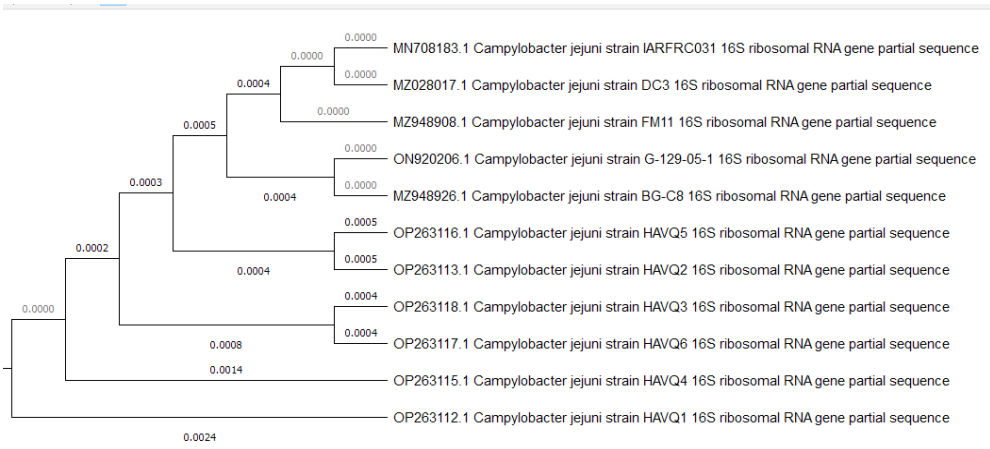

Figure 4. Phylogenetic tree constructed using the NJ method in CLC Sequence Viewer 8.1, depicting the genetic relationships among *C. jejuni* isolates, focusing on local isolates OP263112, OP263113, OP263115, OP263116, OP263117, and OP263118, and reference strains from Poland (MN708183.1), Philippines (MZ028017.1), Thailand (MZ948908.1 and MZ948926.1), and one isolate from India (ON920206.1)

Note: Bootstrap values are shown at the nodes. The branch lengths are proportional to the genetic distance.

The cdt operon, responsible for encoding cytolethal distending toxin, plays a significant role in host cell cycle arrest and apoptosis, with *cdtB* acting as the catalytic subunit responsible for DNase activity (Chen, 2024).

In the present study, the prevalence of *cadF* was 83.3% in *C. jejuni* and 76.3% in *C. coli* isolates, indicating a widespread presence of this adhesion factor among the tested isolates. Similar observations have exhibited a higher prevalence of the *cadF* gene in *Campylobacter* recovered from broilers at slaughter in Egypt, Pakistan, and China (Yaseen et al., 2025; Bai et al., 2024; Mahmoud et al., 2024). Moreover, the *cdtB* gene was identified in most strains of *C. jejuni* and *C. coli* (77.7% and 83.6%, respectively). The presence of *cdtB* suggests that these isolates can cause cellular damage, contributing to the diarrheagenic potential of *Campylobacter* human infections (Reddy, 2016). Several studies have reported a higher prevalence of this gene in both species (Andrzejewska et al., 2015; Bioumy et al., 2024; Khoshbakht et al., 2013; Ripabelli et al., 2010). In Brazil, a very low prevalence (11.5%) of the *cdtB* gene was found in *C. jejuni* recovered from broiler carcasses (Clemente et al., 2024). Previous studies have demonstrated such genes in *Campylobacter* isolated from humans (Abraham et al., 2020; Kim et al., 2019). As a result, the presence of these genes in current

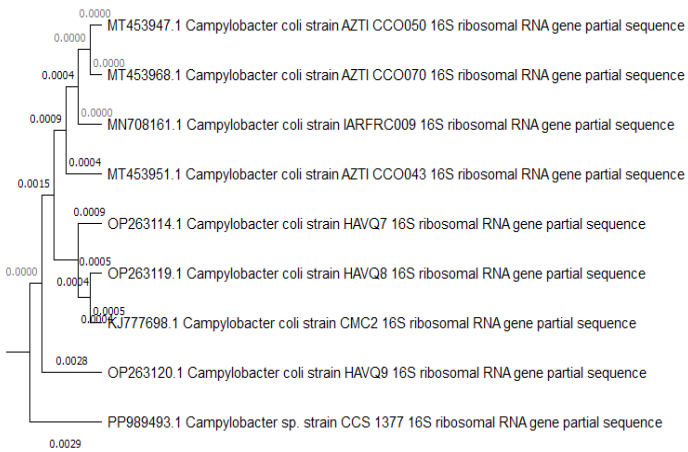

Figure 5. Phylogenetic tree constructed using the NJ method in CLC Sequence Viewer 8.1, depicting the genetic relatedness among the three *C. coli* isolates under study (OP263114, OP263119, OP263120), reference isolates from Spain (MT453963.1; MT453947.1; MT453968.1; MT453951.1) and Germany (PP989493.1)

Note: Bootstrap values are shown at the nodes. The branch lengths are proportional to the genetic distance.

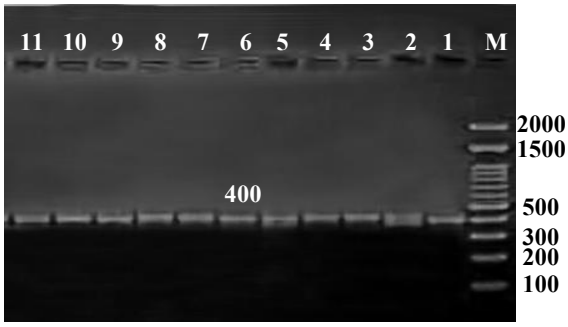

Figure 6. Red-safe stained 1% agarose gel (90 volts per 42 minutes) of amplified PCR products for *cadF* gene (400 bp)

Note: Lane M: Safe-green™ 100bp Opti-DNA marker (ABM, Canada); Lanes: 1-11, positive results with *cadF* gene.

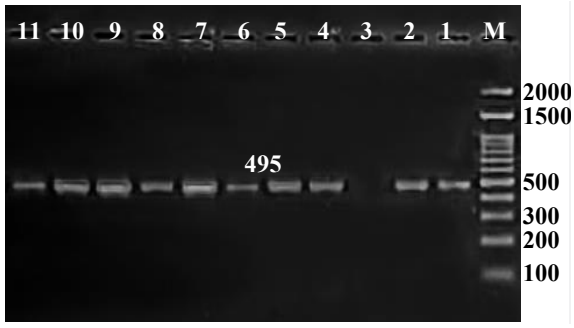

Figure 7. Red-safe stained 1% agarose gel (90 volts per 42 minutes) of PCR products for detection of *cdtB* gene

Note: Lane M: Safe-green™ 100bp Opti-DNA marker (ABM, Canada); Lanes: C1-2 and 4-10 shows positive results with *cdtB* (495 bp).

Campylobacter isolates may be related to their pathogenicity and ability to cause human infections (Mousavinafchi et al., 2023; Wieczorek et al., 2018).

These results may highlight the pathogenic potential of Campylobacter isolates from broilers, necessitating rigorous control measures in poultry production. The widespread presence of *cadF* and *cdtB* in isolates from different regions suggests that virulence-associated genes are conserved in *Campylobacter* populations. The co-occurrence of these virulence genes in most isolates amplifies the risk of severe gastrointestinal infections in humans exposed to contaminated poultry products, particularly in areas with suboptimal food safety practices or limited surveillance systems. Consequently, these results underline the urgent need for targeted interventions at multiple poultry production stages and public health awareness to limit the spread of pathogenic and potentially zoonotic *Campylobacter* strains.

Conclusion

This study provides critical insights into the prevalence, genetic diversity, and virulence-associated factors of *C. jejuni* and *C. coli* in broilers from four governorates in Iraq. The findings reveal a significant prevalence of *Campylobacter* spp., with *C. jejuni* being more prevalent than *C. coli*. Farm management practices, biosecurity measures, and environmental factors may influence regional variations in *Campylobacter* prevalence. Phylogenetic analysis revealed close genetic relationships among local isolates, with some clustering alongside international reference strains, suggesting possible transmission routes or shared evolutionary origins. Additionally, detecting key virulence genes, including *cadF* and *cdtB*, underscores the pathogenic potential of these isolates, posing a notable public health risk. These findings advocate for targeted interventions, including enhanced farm biosecurity, optimized slaughterhouse protocols, regional surveillance, innovative approaches such as probiotics, bacteriophages, and vaccine development, and robust public health education. Implementing these strategies can curtail *Campylobacter* contamination and mitigate associated zoonotic risks. Future studies using whole-genome sequencing are recommended to explore antimicrobial resistance and transmission dynamics further, supporting evidence-based policies to protect public health.

Ethical Considerations

Compliance with ethical guidelines

This study collected cecal fecal samples from slaughtered chickens without further intervention. Farm owners provided verbal consent for their farms' inclusion in the study. According to the guidelines of the Biosafety Committee at the University of Kufa, Kufa, Iraq, ethical approval was not required.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest.

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