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Antimicrobial resistance, genetic diversity and virulence associated factors of *Campylobacter* spp. isolated from poultry meat in Algeria

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Background: *Campylobacter* is the most common cause of bacterial food infections worldwide. In Algeria, data regarding the epidemiology, antimicrobial resistance and virulence of *Campylobacter* remain limited. This study aimed to investigate the antimicrobial resistance, genotyping and virulence associated mechanism of *Campylobacter* isolated from slaughterhouses in Algeria.
Methods: A total of 133 pooled poultry samples were collected from five slaughterhouses in Algiers. *Campylobacter* spp. was differentiated and the antimicrobial susceptibility testing against 12 antimicrobial agents was assessed using Kirby-Bauer-Method. The *tet*O gene and mutations in the *gyr*A gene associated with tetracyclines and fluoroquinolone resistance were investigated, respectively. The potential associated genes were screened. The *fla*A typing for isolated *Campylobacter* was performed using PCR-RFLP.
Results: *Campylobacter* spp. was detected in 28 (21.05%) poultry samples. The prevalence was significantly higher in caecal samples (61.11%) than in neck skin samples (14.78%). Among 28 isolates, 19 (67.86%), 8 (28.57%) and 1 (3.57%) were identified as *Campylobacter coli*, *Campylobacter jejuni* and *Campylobacter lari*, respectively. High levels of resistance to fluoroquinolones (92.86%), nalidixic acid (96.43%) and tetracycline (85.71%) were observed. Multidrug resistance to three or more classes of antimicrobial agents was ranged between 7.14 and 28.57%. The *tet*O gene was amplified in 20 (83.3%) phenotypic tetracycline resistant isolates while mutations in the *gyr*A gene associated with fluoroquinolone resistance were detected in 84.62% (22/26) of isolates. *Campylobacter* spp. was differentiated into 16 genotypes and two isolates from human were belonged to two different *fla*A genotypes. All 19 *Campylobacter coli* isolates carried *cad*F and *cdt*A, while *rac*R, *dna*J, *cdt*B and *cdt*C were detected in 36.84, 36.84, 84.21 and 52.63%, respectively. In turkeys, 22.22% of *Campylobacter coli* and 100% of *Campylobacter jejuni* harbored all virulence associated genes.
Conclusion: These findings emphasize the importance of integrated surveillance of *Campylobacter* across animal and human sectors to mitigate public health risks.

KEYWORDS
Algeria, antimicrobial resistance, *fla*A genotyping, poultry meat, thermotolerant *Campylobacter*, virulence genes

1 Introduction

Thermotolerant *Campylobacter* (*C.*), primarily *C. jejuni* and *C. coli*, are recognized as the leading bacterial cause of human gastroenteritis worldwide and represent a major public health concern (1). These pathogens are considered priority foodborne bacteria by international health authorities due to their high incidence, zoonotic nature and increasing antimicrobial resistance.

Poultry constitute the main reservoir of thermotolerant *Campylobacter* and plays a central role in the epidemiology of Campylobacteriosis (2). Human infection is predominantly associated with the handling and consumption of contaminated poultry products (3). Consequently, contamination at any stage of the poultry production and processing chain may directly impact human health.

The Algerian poultry industry produces annually on average 340.000 tons of white meat and more than 4.8 billion eggs (4). In Algeria, poultry meat, particularly broiler chicken and turkey, represents the most consumed animal protein source due to its affordability and nutritional value (5). Turkey meat is especially appreciated for its low-fat content, making it suitable for consumers with dietary or cholesterol-related constraints (6). Poultry meat consumption per capita reached 9.88 kg in 2023 in Algeria, according to Faostat (7).

The use of a direct isolation method inspired by modified ISO 10272-1 (2017) could represent a good alternative to the indirect isolation method in developing countries, as it would increase the detection rate of *Campylobacter* spp. due to the decrease in detection time, costs and contaminant rates (8). *Campylobacter* spp. presents a significant risk for transmission from infected animals or animal-derived products to humans, animals and the environment. Recent research highlighted poultry products as a primary reservoir, with domestic poultry implicated in various regions worldwide.

Africa has been identified as one of the endemic regions of campylobacteriosis. However, research attention is not geared toward *Campylobacter* source tracing like other enteropathogens, probably because of inadequate standard laboratory facilities and the fastidious nature of the organism. It was noted that the common and emerging species of *Campylobacter* have been documented in Africa and the major transmission pathways to humans are international travel and consumption of contaminated poultry (9).

The results of previous study in Algeria showed a high prevalence of thermotolerant *Campylobacter* with multidrug resistance profiles in poultry farms and slaughterhouses of Algiers (10). Potential sources of contamination of *Campylobacter* species were noticed in farms and slaughterhouses located in the middle area of Algeria. The most tested strains exhibited resistance to ciprofloxacin (75%) and erythromycin (25%) (11).

Despite the importance of poultry production, data on the prevalence, antimicrobial resistance and virulence of thermotolerant *Campylobacter* in Algeria remain scarce. In foods of animal origin, these bacteria may enter a viable but non-culturable (VBNC) state under environmental stress, enabling survival and persistence along the food chain (12). Although Campylobacteriosis is often self-limiting, severe infections require antimicrobial treatment, particularly in vulnerable populations (13).

Campylobacter is genetically highly diverse and undergoes frequent intraspecific recombination. Turkeys have been identified as an important reservoir for *C. jejuni* which is of public health significance (14). The emergence of resistance to critically important antimicrobials, notably fluoroquinolones and macrolides (15, 16), has further increased the public health relevance of *Campylobacter* (17, 18). This antimicrobial resistance threatens treatment efficacy and highlights the need for integrated surveillance at the human–animal–environment interface (19, 20).

Therefore, the present study aimed to investigate thermotolerant *Campylobacter* isolated from poultry in Algeria by assessing prevalence, antimicrobial resistance patterns, resistance mechanisms, and potential virulence gene profiles epidemiological links within their pathogenicity.

2 Materials and methods

2.1 Sampling strategy and sample collection

The sampling was conducted over a period extending in one year from January to November. Samples were collected across multiple seasons, with a higher representation in winter (13 samples), followed by autumn (6 samples) and spring (3 samples). Furthermore, a total of 7 different cities contributed to the sampled batches. The samples were collected at the slaughterhouses and not on farm site, that's why we cannot neither determine whether the farms located in the same city have the same origin nor provide the number of farms contributing to the batches. A total of 133 pooled samples resulting from 460 individual collections (115 neck skin samples and 18 caecal samples), including broiler chickens and turkeys, were aseptically and randomly collected from 23 poultry batches in five poultry slaughterhouses in the Algiers region, Algeria.

The caecal samples could not be collected from all 23 batches but only from 18 batches. In five batches (batches 1, 2, 3, 4 and 8), the slaughterhouse staff had discarded the ceca before my arrival. From the 23 visited poultry batches, 23 neck skins samples and 18 cecal samples were collected.

Fifteen carcasses were sampled per batch and approximately 10 g of neck skin were taken from each carcass. Neck skins from three carcasses of the same batch were pooled to obtain five composite samples per batch (21). Caecal samples were collected immediately after evisceration. Five caeca from five birds of the same batch were pooled to form a single sample. All samples were placed in sterile containers, transported in insulated boxes under refrigerated conditions, and processed within 2–4 h of collection (22).

2.2 Isolation and identification of *Campylobacter*

Caecal contents were aseptically removed and pooled. One gram of pooled caecal content was homogenized in 9 mL of sterile 0.9% physiological saline. For neck skin samples, 10 g of pooled skin were added to Bolton selective enrichment broth at a 1:10 ratio and homogenized in sterile stomacher bags. Isolation of thermotolerant *Campylobacter* was performed according to ISO 10272:2017 (21). Samples were streaked onto modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (Oxoid, Wesel, Germany) and incubated at 42 °C for 48 h under microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) (Anoxomat®, Advanced Instrument, USA). Presumptive colonies were sub-cultured on Columbia blood agar (Oxoid, Wesel, Germany) for purification.

Identification was based on colony morphology, Gram staining, oxidase and catalase tests, growth at different temperatures and biochemical characteristics, including susceptibility to nalidixic acid and cephalothin. Confirmation was achieved using latex agglutination tests, API Campy galleries (bioMérieux, France) and confirmed by PCR assays (23–25).

2.3 Antimicrobial susceptibility testing

Antimicrobial susceptibility test of isolated *Campylobacter* was performed against 12 antimicrobial agents using the Kirby–Bauer test according to EUCAST guidelines (26).

Zones diameters indicating resistance to erythromycin, tetracycline and ciprofloxacin were analyzed according to the EUCAST breakpoints for *Campylobacter* (26). While different reference interpretive standards according to CLSI breakpoints for *Enterobacteriaceae* were used for a tentative classification of the isolates as susceptible or resistant against Amoxicillin/Clavulanic acid (AMC), Ampicillin (AM), Cephotaxime (CTX) according to the EUCAST breakpoints (26). For Streptomycin (S), Gentamicin (GM), Kanamycin (K), Tobramycin (TM), Nalidixic Acid (NA) and Chloramphenicol (C) (27).

Campylobacter jejuni DSM 4688 (ATCC 33560) and *C. coli* DSM 4689 (ATCC 33559) (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) were used as reference strains for quality control in each batch of the antimicrobial susceptibility tests.

2.4 DNA extraction

Genomic DNA was extracted from pure cultures using a thermal lysis method. Briefly, *Campylobacter* spp. isolates were preserved on Columbia agar (Oxoid, Wesel, Germany). After incubation at 42 °C for 48 h under microaerophilic conditions, isolated *Campylobacter* colonies were harvested from pure cultures for DNA extraction. Colonies were transferred into sterile Eppendorf tubes containing 400 µL of sterile nuclease-free water (DNA- and RNA-free; Thermo Scientific™, HyClone® HyPure™). The suspensions were incubated at 100 °C for 10 min using a digital dry bath (Benchmark™, MyBlock™). Following lysis, samples were centrifuged at 10,000 rpm for 5 min using a microcentrifuge (Beckman Coulter®, Microfuge® 16). The resulting supernatants were transferred into sterile 1.5 mL microtubes and stored at −20 °C until PCR analysis.

2.5 Molecular identification and genotyping

Campylobacter spp. was identified and differentiated into *C. jejuni*, *C. coli* and *C. lari* using multiplex PCR (25) (Table 1).

TABLE 1 Primer sequences and expected amplicon sizes used in this study.

Target	Primer	Size (bp)	Sequences (5′-3′)	Reference
Campylobacter spp.	23SF	650	TATACCGGTAAGGAGTGCTGGAG	(25)
	23SR		ATCAATTAACCTTCGAGCACCG	
Campylobacter jejuni	CjF	323	ACTTCTTTATTGCTTGCTGC	
	CjR		GCCACAACAAGTAAAGAAGC	
Campylobacter coli	CcF	126	GTAAAACCAAAGCTTATCGTG	
	CcR		TCCAGCAATGTGTGCAATG	
Campylobacter lari	CL 1155 R	522	ATT TAG AGT GCT CAC CCG AAG	(24)
	CL 632-rv		GGG AAA CTG GTA ATC TAG AGT GG	
tetO	tet1	559	GGCGTTTTGTTTATGTGCG	(28)
	tet2		ATGGACAACCCGACAGAAGC	
gyrA	GzgyrA5	673	ATTTTTAGCAAAGATTCTGAT	(29)
	GzgyrA6		CCATAAATTATTCCACCTGT	
*cdt*A	GNW	165	GGAAATTGGATTTGGGGCTATACT	(30)
	IVH		ATCACAAGGATAATGGACAAT	
*cdt*B	VAT2	495	GTTAAAATCCCCTGCTATCAACCA	
	WMI-R		GTTGGCACTTGGAATTTGCAAGGC	
*cdt*C	WMI-F	555	TGGATGATAGCAGGGGATTTTAAC	
	LPF-X		TTGCACATAACCAAAAGGAAG	
*cad*F	F2B	400	TTGAGGGTAATTTAGATATG	(32)
	R1B		CTAATACCTAAAGTTGAAAC	
*rac*R	25	584	GATGATCCTGACTTTG	(31)
	593		TCTCCTATTTTTACCC	
dnaJ	299	720	AAGGCTTTGGCTCATC	
	1003		CTTTTTGTTCATCGTT	

The PCR was performed in a 50-µL reaction mixture containing 5.0 µL of 10 × Taq reaction buffer complete (Jena Bioscience GmbH, Jena, Germany), 2.0 µL of dNTP mix (2 mM each; Carl Roth GmbH, Karlsruhe, Germany), 2.0 µL of each primer (Jena Bioscience GmbH; Table 2) and 0.2 µL of Taq Pol thermostable DNA polymerase (Jena Bioscience GmbH). Amplification reactions were carried out in Eppendorf Thermoblock cycler (Eppendorf, Germany) using the following program: one cycle of 1 min at 96 °C followed by 35 cycles each consisting of 60 s at 95 °C, 90 s at 59 °C and 60 s at 72 °C. The PCR was terminated by a final extension step of 5 min at 72 °C. Amplification generated 650, 323, and 126 bp DNA fragments specific for the genus *Campylobacter*, *C. jejuni* and *C. coli*, respectively. The corresponding amplicon for *C. lari* was 522 bp. For analysis, 20 µL of PCR products were subjected to electrophoresis in a 1.5% agarose gel for 1 h, stained with ethidium bromide (0.5 µg/mL), and visualized under UV light. Results were documented using BioImage system GeneGenius (Syngene, Synoptics Ltd., Cambridge, UK). Reference strains *C. jejuni* DSM 4688 (ATCC 33560), *C. coli* DSM 4689 (ATCC 33559) and *C. lari* DSM 11375 (ATCC 352219) (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany) were used as positive controls.

2.6 Molecular detection of resistance and potential virulence associated mechanisms

The antimicrobial resistant genes (*tet*O) associated with phenotypic tetracycline resistance (28) and mutations in the *gyr*A gene were associated with fluoroquinolone resistance (29) were identified by PCR.

The potential virulence-associated genes (*cdt*A, *cdt*B, *cdt*C, *cad*F, *rac*R and *dna*J) were amplified using forward and reverse primers in Table 1 and PCR reaction was performed according to the previously published protocol (30–32).

2.7 *fla*A-RFLP assays

For *fla*A-RFLP analysis, extracted DNA was amplified as described previously (14, 33). Out of 28 *Campylobacter* isolated in this study, *fla*A gene was only amplified in 22 isolates. Two *C. jejuni* isolated from humans suffering from campylobacteriosis were included in the analysis. Amplification conditions were: initial denaturation for 60 s at 94 °C followed by 35 cycles each consisting of 15 s at 94 °C, 60 s at 45 °C, 120 s at 72 °C and a final extension step of 300 s at 72 °C. The *fla*A amplicon was digested for 18 h at 37 °C with *Sau3*AI (Jena Bioscience GmbH) and *Dde*I (Roche Diagnostics GmbH) enzymes using the incubation buffer as recommended by the manufacturers. The DNA segments were separated using 2.5% agarose gels (Starlab GmbH, Hamburg, Germany) in TBE buffer at 200 V for 1 h, stained with ethidium bromide and visualized under UV light. Documentation was done using a Bio Imaging System (Syngene, Cambridge, UK). TIF images of the restriction profiles for *fla*A-RFLP were incorporated for analysis into BioNumerics V. 7.1 (Applied Maths, Austin, TX, USA). Pair comparisons and cluster analysis were made using the Dice correlation coefficient and the unweighted pair group mathematical average (UPGMA) clustering algorithm. The optimization and position tolerance for band analysis were set at 4%, and a cut-off of 90% was used for the determination of the different restriction patterns for *fla*A-RFLP.

2.8 Statistical analysis

The results were analyzed using AnaStats software. The tests performed included the calculation of 95% confidence intervals (95% CI), as well as the Chi-square (χ^2) test, Fisher's exact test and G test (log-likelihood ratio test). Differences were considered statistically significant when the probability (p) was less than or equal to the significance level α ($p \leq 0.05$).

3 Results

3.1 Prevalence of thermotolerant *Campylobacter*

Out of 133 investigated poultry samples, thermotolerant *Campylobacter* was detected and confirmed by PCR in 28 (21.05%) samples. The prevalence was significantly higher in caecal samples (61.11%; 11/18) than in neck skin samples (14.78%; 17/115). Among 28 isolates, 19 (67.86%), 8 (28.57%) and 1 (3.57%) were identified as *C. coli*, *C. jejuni* and *C. lari*, respectively using PCR (Table 2).

The epidemiological distributions of the isolated *Campylobacter* according to the sample type and locations of slaughter houses were demonstrated in Table 3.

TABLE 2 Prevalence of thermotolerant *Campylobacter* isolated from 133 pooled samples.

Sample source	Samples number	Isolates *n* (%)	*Campylobacter coli n* (%)	*Campylobacter jejuni n* (%)	*Campylobacter lari n* (%)	95% confidence interval
Neck skin	115	17 (14.78)	12 (70.59)*	5 (29.41)*	0*	[8.3–21.3%]
• Broilers	70	10 (14.29)*	7 (70)	3 (30)	0	[7.07–24.71%]
• Turkeys	45	7 (15.56)*	5 (71.43)	2 (28.57)	0	[6.49–29.46%]
Caecal content	18	11 (61.11)	7 (63.64)*	3 (27.27)*	1 (9.09)*	[35.75–82.70%]
• Broilers	12	6 (50)*	3 (50)	2 (33.33)	1 (16.67)	[21.09%–78.91%]
• Turkeys	6	5 (83.33)*	4 (80)	1 (20)	0	[35.88%–99.58%]
Total	133	28 (21.05)	19 (67.86)	8 (28.57)	1 (3.57)	[14.1–28%]

*$p > 0.05$.

3.2 Antimicrobial resistance profiles

A high frequency of resistance to fluoroquinolones and tetracycline was observed among isolated *Campylobacter*. Resistance to macrolides was less frequent but was detected in some isolates (Table 4).

The highest resistance rates were observed for ampicillin (75%, 21/28; 95% CI: 55.13–89.31%), tetracycline (85.71%, 24/28; 95% CI: 67.33–95.97%), ciprofloxacin (92.86%, 26/28; 95% CI: 76.50–99.12%) and nalidixic acid (96.43%, 27/28; 95% CI: 81.64–99.91%) ($p > 0.05$). Approximately 50% of isolates were resistant to amoxicillin/clavulanic acid (42.86%, 12/28; 95% CI: 24.46–62.82%), cefotaxime (50%, 14/28; 95% CI: 30.65–89.35%), kanamycin (50%, 14/28; 95% CI: 30.65–89.35%), tobramycin (53.57%, 15/28; 95% CI: 33.87–72.49%) and erythromycin (53.57%, 15/28; 95% CI: 33.87–72.49%). Lower resistance rates were recorded for streptomycin (35.71%, 10/28; 95% CI: 18.64–55.93%) ($p > 0.05$). Chloramphenicol resistance was rare (3.57%, 1/28; 95% CI: 0.09–18.35%) and no resistance to gentamicin was observed (0%, 0/28; 95% CI: 0–12.34%). Additionally, 92.86% (26/28) of *Campylobacter* isolates exhibited cross-resistance between nalidixic acid and ciprofloxacin, while one isolate was resistant to nalidixic acid but sensitive to ciprofloxacin (3.57%, 1/28).

Multidrug resistance to three or more classes of antimicrobial agents was ranged between 7.14 and 28.57% of *Campylobacter* isolates, as shown in Table 5.

3.3 Molecular detection of resistance and virulence associated genes

Out of 24 phenotypically tetracycline resistant *Campylobacter*, *tet*O gene associated with tetracycline-resistant was amplified in 20 (83.3%) isolates while mutations in the *gyr*A gene associated with fluoroquinolone resistance were detected in 84.62% (22/26) of isolates.

Three colonization genes (*cad*F, *rac*R, *dna*J) and three pathogenicity genes (*cdt*A, *cdt*B, *cdt*C) were screened in all *Campylobacter* spp. isolates (Table 6). All 19 *C. coli* isolates carried *cad*F and *cdt*A, while *rac*R, *dna*J, *cdt*B and *cdt*C were detected in 36.84% (7/19), 36.84% (7/19), 84.21% (16/19) and 52.63% (10/19),

TABLE 3 Epidemiological distribution of isolated *Campylobacter* from slaughter houses in Algeria.

Source		*Campylobacter* species	Location	Number of birds	Age	Sex
Turkey	Neck skin	*Campylobacter coli*	Tizi Ouzou	20	5 months	♂
		Campylobacter coli	Issers	40	5 months	♂ + ♀
		Campylobacter coli				
		Campylobacter coli	Tizi Ouzou	30	5 months	♂
		Campylobacter coli				
		Campylobacter jejuni	Sétif	100	4 months	♂
		Campylobacter jejuni				
	Caecal content	*Campylobacter coli*	Tizi Ouzou	30	5 months	♂
		Campylobacter coli	Tizi Ouzou	30	5 months	♂
		Campylobacter jejuni	Sétif	100	4 months	♂
		Campylobacter coli	Tablat	30	5 months	♂
		Campylobacter coli	Sétif	100	4 months	♂
Broiler	Neck skin	*Campylobacter jejuni*	Médéa	600	60 days	♂
		Campylobacter coli				
		Campylobacter jejuni	Médéa	500	60 days	♂ + ♀
		Campylobacter coli	Sétif	1,400	44 days	♂ + ♀
		Campylobacter coli	Boumerdes	700	60 days	♂ + ♀
		Campylobacter coli				
		Campylobacter coli	Boumerdes	700	60 days	♂ + ♀
		Campylobacter coli				
		Campylobacter jejuni				
		Campylobacter coli				
	Caecal content	*Campylobacter coli*	Médéa	600	60 days	♂ + ♀
		Campylobacter lari	Boumerdes	250	55 days	♂ + ♀
		Campylobacter coli	Tablat	350	60 days	♂ + ♀
		Campylobacter jejuni	Sétif	1,300	60 days	♂ + ♀
		Campylobacter coli	Bejaia	700	58 days	♂ + ♀
		Campylobacter jejuni	Bejaia	700	60 days	♂ + ♀

TABLE 4 Breakpoints and Antimicrobial susceptibility patterns of *Campylobacter* spp. identified by the disk diffusion methods using disk diffusion methods (26, 27).

Antimicrobial agents	Disk content	Breakpoints (mm)		Resistance n (%)	95% confidence interval	References
		S*	R*			
Ampicillin (AM)	10 µg	≥ 14	< 14	21 (75%)	[55.13–89.31%]	(26)
Amoxicillin/Clavulanic acid (AMC)	20/10 µg	≥ 50	< 19	12 (42.86%)	[24.46–62.82%]	(26)
Cefotaxime (CTX)	5 µg	≥ 20	< 17	14 (50%)	[30.65–89.35%]	(26)
Streptomycin (S)	10 µg	≥ 15	< 11	10 (35.71%)	[18.64–55.93%]	(27)
Gentamicin (GM)	10 µg	≥ 15	< 12	0	[0–12.34%]	(27)
Kanamycin (K)	30 µg	≥ 18	< 13	14 (50%)	[30.65–89.35%]	(27)
Tobramycin (TM)	10 µg	≥ 15	< 12	15 (53.57%)	[33.87–72.49%]	(27)
Erythromycin (E)	15 µg	≥ 20 (18)	< 20 (18)	15 (53.57%)	[33.87–72.49%]	(26)
Nalidixic acid (NA)	30 µg	≥ 20	< 15	27 (96.43%)	[81.64–99.91%]	(27)
Ciprofloxacin (CIP)	5 µg	≥ 50	< 26	26 (92.86%)	[76.50–99.12%]	(26)
Tetracycline (TE)	30 µg	≥ 30	< 30	24 (85.71%)	[67.33–95.97%]	(26)
Chloramphenicol (C)	30 µg	≥ 18	< 12	1 (3.57%)	[0.09–18.35%]	(27)

*S, susceptible; R, resistant.

TABLE 5 Multi-drug-resistance (MDR) rates of thermotolerant *Campylobacter* against different antimicrobial classes.

MDR profiles	No. (%)
Resistance against 3 classes	**2 (7.14)**
Tetracyclines; Aminoglycosides; Fluoroquinolones	1 (3.57)
Fluoroquinolones; Penicillin; Macrolides	1 (3.57)
Resistance against 4 classes	3 (10.7)
β-Lactames; Tetracyclines; Aminoglycosides; Fluoroquinolones	1 (3.57)
β-Lactames; Tetracyclines; Fluoroquinolones; Penicillin	1 (3.57)
Tetracyclines; Aminoglycosides; Fluoroquinolones; Macrolides	1 (3.57)
Resistance against 5 classes	8 (28.57)
Tetracyclines; Aminoglycosides; Fluoroquinolones; Penicillin; Macrolides	1 (3.57)
Cephalosporins; Tetracyclines; Fluoroquinolones; Penicillin; Macrolides	1 (3.57)
Tetracyclines; Fluoroquinolones; Penicillin; Cephalosporins; Aminoglycosides	1 (3.57)
β-Lactames; Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides	1 (3.57)
β-Lactames; Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides	1 (3.57)
Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides; Cephalosporins	1 (3.57)
Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides; Macrolides	2 (7.14)
Resistance against 6 classes	8 (28.57)
β-Lactames; Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides; Cephalosporins	4 (14.3)
β-Lactames; Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides; Macrolides	1 (3.57)
Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides; Macrolides; Cephalosporins	1 (3.57)
β-Lactames; Tetracyclines; Fluoroquinolones; Aminoglycosides; Macrolides; Cephalosporins	1 (3.57)
Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides; Macrolides; Cephalosporins	1 (3.57)
Resistance against 7 classes	4 (14.29)
β-Lactames; Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides; Macrolides; Cephalosporins	3 (10.7)
Tetracyclines; Fluoroquinolones; Penicillin; Aminoglycosides; Macrolides; Cephalosporins; Phenicol	1 (3.57)

respectively. A three-gene (*cad*F-*rac*R-*dna*J) profile was present in *C. coli* from broilers (30%, 3/10; 95% CI: 6.67–65.25%) and turkeys (22.22%, 2/9; 95% CI: 2.81–60.01%).

Most *C. coli* isolated from poultry carried at least one pathogenicity gene (15.79%, 3/19; 95% CI: 3.38–39.58%). Most frequently observed genes in *C. jejuni* isolates were *cad*F, *dna*J, *cdt*A and *cdt*B

TABLE 6 Prevalence of colonization and pathogenicity associated genes for *Campylobacter* spp.

Gene	*Campylobacter coli* (*n* = 19)			95% confidence interval	*Campylobacter jejuni* (*n* = 8)			95% confidence interval	*Campylobacter lari* (*n* = 1)
	Broiler (*n* = 10)	Turkey (*n* = 9)	Total *n* (%)		Broiler (*n* = 5)	Turkey (*n* = 3)	Total *n* (%)		Broiler *n* (%)
*cad*F	10 (100)	9 (100)	19 (100)	82.3–100%	4 (80)	3 (100)	7 (87.5)	47.35–99.68%	0
*rac*R	4 (40)	3 (33.33)	7 (36.8)	16.29–61.64%	3 (60)	3 (100)	6 (75)	34.91–96.81%	1 (100)
dnaJ	3 (30)	4 (44.44)	7 (36.8)	16.29–61.64%	4 (80)	3 (100)	7 (87.5)	47.35–99.68%	0
*cad*F-*rac*R	1 (10.0)	1 (11.11)	2 (10.5)	0–24.3%	0	0	0	0–32.0%	0
*cad*F-*dnaJ*	0	2 (22.22)	2 (10.5)	0–24.3%	1 (20)	0	1 (12.5)	0.32–52.65%	0
*cad*F-*rac*R-*dnaJ*	3 (30)	2 (22.22)	5 (26.3)	6.5–46.1%	3 (60)	3 (100)	6 (75)	34.91–96.81%	0
*cdt*A	10 (100)	9 (100)	19 (100)	82.3–100%	4 (80)	3 (100)	7 (87.5)	47.35–99.68%	0
*cdt*B	9 (90)	7 (77.78)	16 (84.2)	60.42–96.62%	4 (80)	3 (100)	7 (87.5)	47.35–99.68%	0
*cdt*C	3 (30)	7 (77.78)	10 (52.6)	28.86–75.55%	2 (40)	3 (100)	5 (62.5)	24.49–91.48%	0
*cdt*A-*cdt*B	1 (10)	0	1 (5.3)	10.7–52.5%	0	0	0	0–55.0%	0
*cdt*A-*cdt*B-*cdt*C	3 (30)	7 (77.78)	10 (52.6)	28.86–75.55%	5 (62.5)	3 (100)	8 (100)	24.49–91.48%	0
*cad*F-*rac*R-*dnaJ*-*cdt*A-*cdt*B-*cdt*C	0	2 (22.22)	2 (10.5)	0–24.3%	2 (40)	3 (100)	5 (62.5)	24.49–91.48%	0

(87.5%, 7/8; 95% CI: 47.35–99.68%), while *rac*R and *cdt*C were present in 75% (6/8; 95% CI: 34.91–96.81%) and 62.5% (5/8), respectively.

The majority of *C. jejuni* (75%, 6/8; 95% CI: 34.91–96.81%) carried all three colonization genes *cad*F-*rac*R-*dnaJ* and represented as broilers (60%, 3/5; 95% CI: 14.66–94.73%) and turkeys (100%, 3/3; 95% CI: 29.24–100%). One *C. jejuni* isolate from broilers (12.5%, 1/8; 95% CI: 0.32–52.65%) lacked all colonization genes. Three-gene profiles (*cdt*A-*cdt*B-*cdt*C) were more frequent in isolates from turkeys than broilers for both *C. jejuni* (100%, 3/3; 95% CI: 29.24–100% vs. 40%, 2/5; 95% CI: 5.27–85.34%) and *C. coli* (77.78%, 7/9; 95% CI: 39.99–97.19% vs. 30%, 3/10; 95% CI: 6.67–65.25%). Overall, ten distinct virulence profiles were identified in poultry.

Among *C. jejuni* and *C. coli* 62.5% (5/8) and 10.5% (2/19) carried all virulence genes (*cad*F-*rac*R-*dna*J-*cdt*A-*cdt*B-*cdt*C), respectively. There is no *Campylobacter coli* isolated from broiler harboured all 6-virulence gene together, while 40% (2/5) of *C. jejuni* isolated from broiler carried all genes. While in turkeys, 22.22% (2/9) of *C. coli* and 100% (3/3) of *C. jejuni* harbored all virulence associated genes, indicating that turkey *C. jejuni* isolates may represent the most virulent strains.

Only *rac*R was detected in *C. lari*.

3.4 Molecular analysis of *fla*A-RFLP results

The PCR-RFLP analysis of *fla*A genes of the 22 *Campylobacter* spp. isolated from poultry revealed 16 genotypes when digested with *Sau3*AI and *Dde*I (Figure 1). All isolates gave identical results when experiments were repeated (data not shown). The two *C. jejuni* isolated from humans were not genotypically related to *Campylobacter* spp. isolated from poultry in this study.

4 Discussion

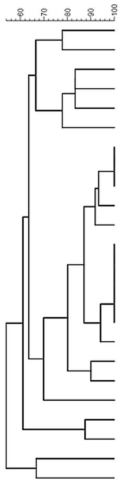
According to previous studies, variations in *Campylobacter* prevalence in poultry are influenced by multiple factors, including season, geographic location, detection methods, sample size and sample type (34, 35).

In this study, the overall prevalence of thermotolerant *Campylobacter* in the slaughterhouses was 21.05%. Despite a higher number of neck skin samples (*n* = 115) compared to cecal contents (*n* = 18), *Campylobacter* detection was higher in cecal contents (61.11%) than in neck skins (14.78%). This pattern is consistent with the fact that turkeys and broilers serve as primary reservoirs of thermotolerant *Campylobacter*, with higher bacterial loads in the intestinal tract than on neck skin in agreement with previous reports (36, 37).

The low detection rate may also be influenced by season, as most samples were collected during winter (12 batches) and autumn (8 batches), with only three batches in spring and none in summer. Peak contamination typically occurs in summer, decreasing thereafter (38). Additionally, *Campylobacter* are highly sensitive to desiccation; sampling from chilled carcasses may further reduce detection rates due to moisture loss and cold-induced inactivation during chilling and ventilation processes (39).

In poultry, *Campylobacter* prevalence in the intestinal tract is higher than that of *Salmonella* (40). Both broilers and turkeys are important hosts; colonization typically begins around 7 days of age, reaching near 100% within 14–28 days (41). Transmission within a flock is rapid, potentially infecting all birds within 72 h (24).

Three species were isolated in descending order of prevalence: *C. coli* (67.86%), *C. jejuni* (28.57%) and *C. lari* (3.57%). While some studies report *C. coli* as dominant in turkeys and broilers (42), most literature indicates *C. jejuni* predominance (61.9–73%), with *C. coli* ranging from 0–14.3% (43, 44).



Species	Sample origin	Type of sample	Flock No.	Slaughterhouse location	Farm location
C. jejuni	Turkey	Caecal content	12	Bab Ezzouar	Sétif
C. jejuni	Human	Feces -Hospital	-	-	-
C. coli	Turkey	Caecal content	5	Baba Ali	Tizi Ouzou
C. coli	Turkey	Caecal content	14	Rouiba	Tablat
C. coli	Broiler	Neck skin	20	Bordj El Kiffan	Sétif
C. coli	Broiler	Neck skin	22	Bordj El Kiffan	Boumerdes
C. jejuni	Broiler	Neck skin	23	Bordj El Kiffan	Boumerdes
C. coli	Broiler	Cecal content	9	Baba Ali	Médéa
C. coli	Broiler	Cecal content	13	Rouiba	Tablat
C. coli	Turkey	Neck skin	5	Baba Ali	Tizi Ouzou
C. coli	Turkey	Neck skin	5	Baba Ali	Tizi Ouzou
C. coli	Turkey	Caecal content	10	Baba Ali	Tizi Ouzou
C. coli	Turkey	Caecal content	15	Bab Ezzouar	Sétif
C. jejuni	Broiler	Neck skin	3	Baba Ali	Médéa
C. coli	Broiler	Neck skin	22	Bordj El Kiffan	Boumerdes
C. jejuni	Broiler	Cecal content	16	Bordj El Kiffan	Sétif
C. jejuni	Broiler	Caecal content	19	Bordj El Kiffan	Bejaia
C. jejuni	Human	Feces -Hospital	-	-	-
C. coli	Turkey	Neck skin	4	Baba Ali	Issers
C. jejuni	Turkey	Neck skin	12	Bab Ezzouar	Sétif
C. jejuni	Broiler	Neck skin	1	Baba Ali	Médéa
C. coli	Broiler	Neck skin	23	Bordj El Kiffan	Boumerdes
C. coli	Turkey	Neck skin	2	Baba Ali	Tizi Ouzou
C. Lari	Broiler	Cecal content	11	Sidi Moussa	Boumerdes

FIGURE 1
Dendrogram based on restriction profiles of 24 *Campylobacter* isolates were digested using *Sau3*AI and D*del* restriction enzymes. *fla*A-RFLP cluster analysis was performed with the Dice correlation coefficient and the unweighted pair group mathematical average clustering algorithm of BioNumerics V. 7.1.

Environmental stress responses differ between species. *Campylobacter jejuni* is generally more resilient to environmental stressors, whereas *C. coli* may survive better after transport and handling due to shifts in intestinal microflora (42, 45). *Campylobacter lari* is typically found in gulls but can cause severe gastrointestinal infections or bacteremia in humans (46).

Based on the available breakpoints reported for *Campylobacter* in both EUCAST and CLSI, the breakpoints were limited available only for fluoroquinolones, tetracyclines and macrolides. In this study, we tried to cover other clinically used antimicrobial agents used in the poultry farms in Algeria. For this reason, the resistance breakpoints for *Enterobacteriaceae* were used for Ampicillin (AM), Amoxicillin/Clavulanic Acid (AMC), Cefotaxime (CTX), Streptomycin (S), Gentamicin (GM), Kanamycin (K), Tobramycin (TM) and Chloramphenicol (C) as recommended by the Clinical and Laboratory Standards Institute and EUCAST (23, 47, 48).

The broth microdilution is considered the "gold standard" for determining minimum inhibitory concentrations (MICs). The disc diffusion is highly regarded for its simplicity, cost-effectiveness and flexibility. According to financial limit, disc diffusion (Kirby-Bauer) testing offers several practical advantages in routine clinical settings, small laboratories or when flexibility is required. Disc diffusion is significantly more cost-effective than broth microdilution, particularly for smaller laboratories or when testing a low number of samples (16, 49).

In this study, using the disc diffusion give flexibility for easily choose of antibiotic discs, allowing us to customize the panel to fit clinically relevant antimicrobial agents. Furthermore, the method is technically simple to perform, does not require specialized equipment, and is straightforward for laboratory technicians to interpret.

Nevertheless, several studies have demonstrated that the broth dilution method can be substituted by agar disk diffusion and the E-test, as the results obtained with these techniques show strong agreement with those of the dilution method (50–55). It should also be noted that the agar disk diffusion method was first standardized by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (16), and subsequently by the CLSI, which also recommends its use (56).

According to Yildirim *et al.* (2005), the agar disk diffusion test is a reliable, simple, and cost-effective method for determining the antibiotic susceptibility of *C. jejuni* and *C. coli* strains (54). The E-test, meanwhile, may also be reliable but requires a prior standardization step before it can be routinely used as a susceptibility testing method for *Campylobacter* strains (54, 55, 57).

High resistance rates were observed for nalidixic acid (96.43%), ciprofloxacin (92.86%) and tetracycline (85.71%), likely due to excessive use in poultry farms. Fluoroquinolone resistance is mainly chromosomal, associated with mutations in the *gyr*A gene QRDR region (Thr-86, Asp-90, Ala-70) and, to a lesser extent, by the CmeABC efflux pump (20, 23, 47, 58–60).

Campylobacter jejuni isolated from broilers, laying hens, and farm workers in Egypt demonstrated high rates of resistance to ampicillin (69.6%), ciprofloxacin (68.1%), erythromycin (66.7%), amoxicillin (65.2%), tetracycline (63.8%) and azithromycin (63.8%). In contrast, the lower rates of resistance to several other antibiotics ranged from 34.8 to 47.9% (61). While in Pakistan, the antimicrobial resistance rates of *C. jejuni* and *C. coli* in humans, animals and the environment were 89, 75, 70, 69, 54, 39 and 23% against tetracycline, ceftriaxone, cefotaxime, erythromycin, nalidixic acid, ciprofloxacin and gentamicin, respectively (62).

Tetracycline resistance is plasmid- or chromosome-mediated, primarily involving *tet*O and, rarely, *tet*M, which protect ribosomes from antibiotic binding (63, 64). The present study confirmed high prevalence of *tet*O among tetracycline-resistant isolates, particularly in *C. jejuni* (100%) and *C. coli* (88.24%). Some discrepancies between phenotypic and genotypic antimicrobial resistance may reflect primer sensitivity or the presence of additional resistance genes (65).

Resistance to β-lactams, including ampicillin (75%) and cefotaxime (50%), is mainly due to chromosomal β-lactamase production, with efflux pumps and low permeability contributing (66). Amoxicillin/clavulanic acid-maintained activity due to β-lactamase inhibition. Aminoglycoside resistance (kanamycin, streptomycin, tobramycin; 35.71–53.57%) is often plasmid-mediated, involving AAD, AAC, and APH enzymes (66). Macrolide resistance (erythromycin; 53.57%) is mainly chromosomal, involving 23S rRNA mutations, with efflux pumps and membrane alterations contributing (28, 67).

Chloramphenicol resistance was low (3.57%), reflecting restricted use, while the CmeABC efflux pump or plasmid-encoded CAT enzymes could explain the rare resistant isolates (66, 68). High-level resistance to ciprofloxacin, erythromycin and tetracycline may result from combined mechanisms including *gyr*A or 23S rRNA mutations, *tet*O and efflux pumps (58).

The ability of thermotolerant *Campylobacter* to colonize the intestine and cause infection in humans and animals is largely mediated by specific virulence genes (69, 70). In this study, the colonization genes *cad*F, *rac*R and *dnaJ* were the most frequently detected, confirming their major role in bacterial adhesion and survival in the host intestine (69, 71, 72). The *cad*F gene encodes a fibronectin-binding protein essential for bacterial adherence to intestinal epithelial cells (72). Similarly, *rac*R is a transcriptional regulator that modulates the expression of multiple colonization factors (71), while *dnaJ* is involved in stress response and contributes to bacterial survival in the gastrointestinal environment (69).

Regarding pathogenicity associated genes, *cdt*A, *cdt*B and *cdt*C, which encode the cytolethal distending toxin (CDT), were detected with variable frequencies depending on the species and the origin of the isolates (70). CDT is known to cause DNA damage in host cells, leading to cell cycle arrest and cell death (73, 74). The results of this study show that *C. jejuni* more frequently harbored the full set of *cdt* genes compared to *C. coli*, which may explain the higher potential virulence of this species in humans (70, 75).

The combination of colonization and pathogenicity associated genes suggests that certain strains may exhibit a potential complete virulence profile (*cad*F-*rac*R-*dnaJ*-*cdt*A-*cdt*B-*cdt*C), which might facilitate both intestinal colonization and the onset of severe gastrointestinal symptoms (70, 75). This observation is consistent with previous studies, which demonstrated that the presence of these genes together is associated with increased colonization and virulence potential (69, 70). Finally, our findings suggest that the prevalence of virulence genes may be influenced by the sample origin (broiler chicken *vs*. turkey), and that species such as *C. lari* possess a more limited set of virulence genes, which could explain their lower clinical incidence in humans (70, 76).

The genetic diversity amongst *Campylobacter* must be considered in epidemiological evaluations and microbial risk assessments of *Campylobacter* in poultry and poultry meat. Multiple genotypes can constitute the *Campylobacter* population within poultry flocks, suggesting different sources of exposure and/or genetic drifts within the *Campylobacter* population (77).

Molecular methods used for typing of *Campylobacter* spp., which are characterized by low complexity and high reproducibility, are needed to study the bacterial population structure. The use of *fla*A gene typing for epidemiological studies is controversial, due to the intra- and inter-genomic recombination within the flagellin genes that results in significant sequence heterogeneity (78). The discriminatory power of *fla*A-RFLP typing clearly depended upon the type of restriction enzyme used (14, 78, 79).

There are various methods used for typing *Campylobacter* as MLST, cgMLST and Whole Genome Sequencing which are considered the modern molecular tools for genotyping. The limitation in this study was based on the financial limit. According to previously published reports, it has been suggested that the sensitivity of the *fla*A gene locus to spontaneous genetic change is a limiting factor in its use in long-term epidemiological studies, but is suitable for the initial grouping of isolates in surveillance situations (80). The *fla*A gene of *Campylobacter* species serves as an epidemiological marker, as it shows extensive sequence heterogeneity (79). The *fla*A typing in *Campylobacter* is a commonly used, rapid and easy method for genotyping with an acceptable discriminatory power (81, 82). It has been shown that PCR-RFLP of *fla*A amplicons was suitable for discriminating *Campylobacter* isolates by generating DNA banding pattern. Different restriction enzymes can be used, and combining the enzyme patterns (composite analysis) has been shown to result in an increased degree of discrimination (14). Furthermore, the method is technically simple to perform, does not require specialized equipment, and is straightforward for laboratory technicians to interpret.

In this study, the genetic diversity among 22 *Campylobacter* spp. isolated from poultry and two *C. jejuni* isolated from human isolates was elucidated. Molecular biological typing was done using established *fla*A typing method. The present study demonstrated that poultry flocks can be simultaneously colonized with more than one *Campylobacter* genotype which in accordance with previous reports (14, 77). In contrast, other studies reported the detection of only one genotype per sampled flock (83, 84).

5 Conclusion

This study highlights the widespread presence of thermotolerant *Campylobacter* spp., particularly *C. jejuni* and *C. coli*, in poultry carcasses from slaughterhouses. The isolates exhibited high rates of antimicrobial resistance, including multidrug resistance and harbored multiple virulence genes associated with intestinal colonization and pathogenicity. These findings indicate that poultry products, even after refrigeration, may serve as reservoirs for pathogenic and antimicrobial-resistant *Campylobacter*, posing a significant public health risk.

The results also emphasize the need for improved hygiene practices, biosecurity measures, and targeted interventions along the poultry production and processing chain to limit bacterial contamination and the spread of resistance genes. Further research on the genetic mechanisms underlying resistance and potential virulence, as well as the development of standardized detection protocols, is essential to reduce the impact of *Campylobacter* contamination and protect human from infection.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

RB: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. TM-H: Conceptualization, Supervision, Validation, Writing – review & editing. CK: Data curation, Investigation, Methodology, Resources, Writing – review & editing. HT: Supervision, Writing – review & editing. HN: Resources, Supervision, Writing – review & editing. HE-A: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Sharma S, Kaur S, Naguib M, Bragg A, Schneider A, Kulkarni RR, et al. Major food-borne bacterial pathogens in poultry: implications for human health and the poultry industry and probiotic mitigation strategies. *Microorganisms*. (2025) 13:2363. doi: 10.3390/microorganisms13102363

2. Skarp CPA, Hänninen ML, Rautelin HIK. Campylobacteriosis: the role of poultry meat. *Clin Microbiol Infect*. (2016) 22:103–9. doi: 10.1016/j.cmi.2015.11.019

3. Igwaran A, Okoh AI. Human campylobacteriosis: a public health concern of global importance. *Heliyon*. (2019) 5:e02814. doi: 10.1016/j.heliyon.2019.e02814

4. Alloui N, Bennoune O. Poultry production in Algeria: current situation and future prospects. *Worlds Poult Sci J*. (2013) 69:613–20. doi: 10.1017/S0043933913000615

5. Ali F, Hacène I, Mohamed B. Structuring and development of poultry sectors in Algeria: limits of modernization policies: the case of the "Turkey" sector (2000-2020). *Agric Sci Dig*. (2024) 44:1147–53. doi: 10.18805/ag.DF-584

6. Brunel V, Jehl N, Drouet L, Portheau MC, editors. Viande de volailles: Sa valeur nutritionnelle présente bien des atouts. Viandes et produits carnés (Aubière). (2006) 25:18–28. Available online at: http://pascal-francis.inist.fr/vibad/index.php?action=getRecordDetail&idt=17688468

7. Faostat. Poultry Meat Consumption Per Capita in Algeria Czech Republic(2025). Available online at: https://www.helgilibrary.com/indicators/poultry-meat-consumption-per-capita/algeria/#:~:text=Data%20Source%20Settings&text=Poultry%20meat%20consumption%20per%20capita%20reached%205.84%20kg%20in%202021,of%201.51%20kg%20in%201963 (Accessed 25 September 2025).

8. Bouhamed R, Hamdi TM. *Campylobacter* spp. at different stages of the poultry slaughtering line in Algeria: evaluation of direct and indirect modified ISO 10272:2017 detection methods and characterization of the isolates. *Arch Razi Inst*. (2023) 78:1657–67. doi: 10.22092/ari.2023.78.5.1657

9. Ahmed AO, Ameh JA, Abubakar MB, Raufu IA. Campylobacteriosis in Africa: a neglected demon. *The Microbe*. (2025) 7:100409. doi: 10.1016/j.microb.2025.100409

10. Messad S, Hamdi TM, Bouhamed R, Ramdani-Bouguessa N, Tazir M. Frequency of contamination and antimicrobial resistance of thermotolerant *Campylobacter* isolated from some broiler farms and slaughterhouses in the region of Algiers. *Food Control*. (2014) 40:324–8. doi: 10.1016/j.foodcont.2013.12.016

11. Bouhamed R, Bouayad L, Messad S, Zenia S, Naïm M, Hamdi TM. Sources of contamination, prevalence, and antimicrobial resistance of thermophilic *Campylobacter* isolated from turkeys. *Vet World*. (2018) 11:1074–81. doi: 10.14202/vetworld.2018.1074-1081

12. Murphy C, Carroll C, Jordan KN. Environmental survival mechanisms of the food-borne pathogen *Campylobacter jejuni*. *J Appl Microbiol*. (2006) 100:623–32. doi: 10.1111/j.1365-2672.2006.02903.x

13. Derakhshan-Sefidi M, Bakhshi B, Hamidi F, Safaei S, Shams S, Kazemnejad A, et al. Prevalence and antimicrobial resistance patterns of *Campylobacter* spp. causing human gastroenteritis in Iran (2000-2025): a systematic review and meta-analysis. *BMC Infect Dis*. (2025) 25:1742. doi: 10.1186/s12879-025-12057-0

14. El-Adawy H, Hotzel H, Tomaso H, Neubauer H, Taboada EN, Ehricht R, et al. Detection of genetic diversity in *Campylobacter jejuni* isolated from a commercial Turkey flock using *fla*A typing, MLST analysis and microarray assay. *PLoS One*. (2013) 8:e51582. doi: 10.1371/journal.pone.0051582

15. Allos B. "Campylobacter infections". In: Bachman P and Abrutyn E, editors. *Bacterial Infections of Humans*, 4th Edn. USA: Springer Science, Business Media (2009). p. 189–212.

16. Ge B, Wang F, Sjölund-Karlsson M, McDermott PF. Antimicrobial resistance in *campylobacter*: susceptibility testing methods and resistance trends. *J Microbiol Methods*. (2013) 95:57–67. doi: 10.1016/j.mimet.2013.06.021

17. Salmani F, Mohamadi S, Tasara T, Ansarifar E, Sadighara P, Zeinali T. Antibiotic resistance of *Campylobacter* species isolated from foods, animals and humans in Iran through one health approach: a systematic review and Meta-analysis. *Vet Med Sci*. (2026) 12:e70746. doi: 10.1002/vms3.70746

18. Aghmiyuni ZF, Ahmadi MH, Javadi K. Global prevalence of fluoroquinolone resistance in common *Campylobacter* species associated with human infections. *New Microbes New Infect*. (2026) 69:101687. doi: 10.1016/j.nmni.2025.101687

19. Zhang Q, Beyi AF, Yin Y. Zoonotic and antibiotic-resistant *Campylobacter*: a view through the one health lens. *One Health Adv*. (2023) 1:4. doi: 10.1186/s44280-023-00003-1

20. Zhang Q, Lin J, Pereira S. Fluoroquinolone-resistant *Campylobacter* in animal reservoirs: dynamics of development, resistance mechanisms and ecological fitness. *Anim Health Res Rev*. (2003) 4:63–71. doi: 10.1079/ahr200356

21. ISO-10272-1. *Microbiology of the Food chain: Horizontal Method for Detection and Enumeration of Campylobacter Spp. Part 1: Detection Method*. Geneva, Switzerland: International Organization for Standardization (2017). p. 24.

22. Elvers KT, Morris VK, Newell DG, Allen VM. Molecular tracking, through processing, of *Campylobacter* strains colonizing broiler flocks. *Appl Environ Microbiol*. (2011) 77:5722–9. doi: 10.1128/aem.02419-10

23. El-Adawy H, Hotzel H, Düpre S, Tomaso H, Neubauer H, Hafez HM. Determination of antimicrobial sensitivities of *Campylobacter jejuni* isolated from commercial Turkey farms in Germany. *Avian Dis*. (2012) 56:685–92. doi: 10.1637/10135-031912-Reg.1

24. El-Adawy H, Hotzel H, Tomaso H, Neubauer H, Hafez HM. Elucidation of colonization time and prevalence of thermophilic *Campylobacter* species during Turkey rearing using multiplex polymerase chain reaction. *Poult Sci*. (2012) 91:454–9. doi: 10.3382/ps.2010-01810

25. Wang G, Clark CG, Taylor TM, Pucknell C, Barton C, Price L, et al. Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni*, *C. coli*, *C. lari*, *C. Upsaliensis*, and *C. fetus* subsp. *fetus*. *J Clin Microbiol*. (2002) 40:4744–7. doi: 10.1128/jcm.40.12.4744-4747.2002

26. EUCAST. *Breakpoint Tables for Interpretation of MICs and zone Diameters*.The European Committee on Antimicrobial Susceptibility Testing (2026). p. 87.

27. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing. M100*. 30th ed. Wayne, Pennsylvaniy, USA: Clinical and Laboratory Standards Institute (2020) 32–42.

28. Gibreel A, Kos VN, Keelan M, Trieber CA, Levesque S, Michaud S, et al. Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*: molecular mechanism and stability of the resistance phenotype. *Antimicrob Agents Chemother*. (2005) 49:2753–9. doi: 10.1128/aac.49.7.2753-2759.2005

29. Zirnstein G, Li Y, Swaminathan B, Angulo F. Ciprofloxacin resistance in *Campylobacter jejuni* isolates: detection of *gyr*A resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. *J Clin Microbiol*. (1999) 37:3276–80. doi: 10.1128/jcm.37.10.3276-3280.1999

30. Bang DD, Nielsen EM, Scheutz F, Pedersen K, Handberg K, Madsen M. PCR detection of seven virulence and toxin genes of *Campylobacter jejuni* and *Campylobacter coli*

isolates from Danish pigs and cattle and cytolethal distending toxin production of the isolates. *J Appl Microbiol*. (2003) 94:1003–14. doi: 10.1046/j.1365-2672.2003.01926.x

31. Datta S, Niwa H, Itoh K. Prevalence of 11 pathogenic genes of *Campylobacter jejuni* by PCR in strains isolated from humans, poultry meat and broiler and bovine faeces. *J Med Microbiol*. (2003) 52:345–8. doi: 10.1099/jmm.0.05056-0

32. Konkel ME, Gray SA, Kim BJ, Garvis SG, Yoon J. Identification of the enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the cadF virulence gene and its product. *J Clin Microbiol*. (1999) 37:510–7. doi: 10.1128/jcm.37.3.510-517.1999

33. Nachamkin I, Bohachick K, Patton CM. Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *J Clin Microbiol*. (1993) 31:1531–6. doi: 10.1128/jcm.31.6.1531-1536.1993

34. Berrang ME, Buhr RJ, Cason JA. *Campylobacter* recovery from external and internal organs of commercial broiler carcass prior to scalding. *Poult Sci*. (2000) 79:286–90. doi: 10.1093/ps/79.2.286

35. Jeffrey JS, Tonooka KH, Lozanot J. Prevalence of *Campylobacter* spp. from skin, crop, and intestine of commercial broiler chicken carcasses at processing. *Poult Sci*. (2001) 80:1390–2. doi: 10.1093/ps/80.9.1390

36. Corry JE, Atabay HI. Poultry as a source of *Campylobacter* and related organisms. *Symp Ser Soc Appl Microbiol*. (2001) 30:96s–114s. doi: 10.1046/j.1365-2672.2001.01358.x

37. Logue CM, Sherwood JS, Elijah LM, Olah PA, Dockter MR. The incidence of *Campylobacter* spp. on processed Turkey from processing plants in the midwestern United States. *J Appl Microbiol*. (2003) 95:234–41. doi: 10.1046/j.1365-2672.2003.01969.x

38. Reich F, Atanassova V, Haunhorst E, Klein G. The effects of Campylobacter numbers in caeca on the contamination of broiler carcasses with Campylobacter. *Int J Food Microbiol*. (2008) 127:116–20. doi: 10.1016/j.ijfoodmicro.2008.06.018

39. Wideman N, Bailey M, Bilgili SF, Thippareddi H, Wang L, Bratcher C, et al. Evaluating best practices for *Campylobacter* and Salmonella reduction in poultry processing plants. *Poult Sci*. (2016) 95:306–15. doi: 10.3382/ps/pev328

40. Guyard-Nicodème M, Anis N, Naguib D, Viscogliosi E, Chemaly M. Prevalence and association of *Campylobacter* spp., *Salmonella* spp., and *Blastocystis* sp. in poultry. *Microorganisms*. (2023) 11:1983. doi: 10.3390/microorganisms11081983

41. Wallace JS, Stanley KN, Jones K. The colonization of turkeys by thermophilic *campylobacters*. *J Appl Microbiol*. (1998) 85:224–30. doi: 10.1046/j.1365-2672.1998.00470.x

42. Wesley IV, Muraoka WT, Trampel DW, Hurd HS. Effect of preslaughter events on prevalence of *Campylobacter jejuni* and *Campylobacter coli* in market-weight turkeys. *Appl Environ Microbiol*. (2005) 71:2824–31. doi: 10.1128/aem.71.6.2824-2831.2005

43. Atanassova V, Reich F, Beckmann L, Klein G. Prevalence of *Campylobacter* spp. in Turkey meat from a slaughterhouse and in Turkey meat retail products. *FEMS Immunol Med Microbiol*. (2007) 49:141–5. doi: 10.1111/j.1574-695X.2006.00180.x

44. Perko-Mäkelä P, Isohanni P, Katzav M, Lund M, Hänninen ML, Lyhs U. A longitudinal study of *Campylobacter* distribution in a Turkey production chain. *Acta Vet Scand*. (2009) 51:18. doi: 10.1186/1751-0147-51-18

45. Alter T, Gaull F, Froeb A, Fehlhaber K. Distribution of *Campylobacter jejuni* strains at different stages of a Turkey slaughter line. *Food Microbiol*. (2005) 22:345–51.

46. Werno AM, Klena JD, Shaw GM, Murdoch DR. Fatal case of *Campylobacter lari* prosthetic joint infection and bacteremia in an immunocompetent patient. *J Clin Microbiol*. (2002) 40:1053–5. doi: 10.1128/jcm.40.3.1053-1055.2002

47. El-Adawy H, Ahmed MF, Hotzel H, Tomaso H, Tenhagen BA, Hartung J, et al. Antimicrobial susceptibilities of *Campylobacter jejuni* and *Campylobacter coli* recovered from organic Turkey farms in Germany. *Poult Sci*. (2015) 94:2831–7. doi: 10.3382/ps/pev259

48. El-Adawy H, Hotzel H, García-Soto S, Tomaso H, Hafez HM, Schwarz S, et al. Genomic insight into *Campylobacter jejuni* isolated from commercial Turkey flocks in Germany using whole-genome sequencing analysis. *Front Vet Sci*. (2023) 10:1092179. doi: 10.3389/fvets.2023.1092179

49. Ge MC, Kuo SF, Chang S-C, Chien CC, You HL, Lu JJ. Antimicrobial susceptibility and virulence surveillance of *Campylobacter* spp. isolated from patients in two tertiary medical centers in Taiwan. *Front Microbiol*. (2019, 2018) 9:3186. doi: 10.3389/fmicb.2018.03186

50. Al-Natour MQ, Alaboudi AR, Osaili TM, Obaidat MM. Resistance of *Campylobacter jejuni* isolated from layer farms in northern Jordan using microbroth dilution and disc diffusion techniques. *J Food Sci*. (2016) 81:M1749–53. doi: 10.1111/1750-3841.13363

51. Azrad M, Tkhawkho L, Isakovich N, Nitzan O, Peretz A. Antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli*: comparison between Etest and a broth dilution method. *Ann Clin Microbiol Antimicrob*. (2018) 17:23. doi: 10.1186/s12941-018-0275-8

52. Ge M, Kuo S, Chang S, Chien C, You H, Lu J. Antimicrobial susceptibility and virulence surveillance of *Campylobacter* spp. isolated from patients in two tertiary medical centers in Taiwan. *Front Microbiol*. (2019) 9:3186. doi: 10.3389/fmicb.2018.03186

53. Luangtongkum T, Morishita TY, El-Tayeb AB, Ison AJ, Zhang Q. Comparison of antimicrobial susceptibility testing of *Campylobacter* spp. by the agar dilution and the agar disk diffusion methods. *J Clin Microbiol*. (2007) 45:590–4. doi: 10.1128/jcm.00986-06

54. Yildirim M, Ersin I, Burcu A, Nilgun U. Comparison of disc diffusion and E test® for in vitro antimicrobial susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry. *Rev Med Vet*. (2005) 156:510–3.

55. Luber P, Bartelt E, Genschow E, Wagner J, Hahn H. Comparison of broth microdilution, E test, and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. *J Clin Microbiol*. (2003) 41:1062–8. doi: 10.1128/jcm.41.3.1062-1068.2003

56. CLSI. "Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious Bacteria". In: CaLSI e, editor, 3rd Edn. Wayne, Pennsylvania, USA: Clinical and Laboratory Standards Institute (2016)

57. Ge B, Bodeis S, Walker RD, White DG, Zhao S, McDermott PF, et al. Comparison of the etest and agar dilution for in vitro antimicrobial susceptibility testing of *Campylobacter*. *J Antimicrob Chemother*. (2002) 50:487–94. doi: 10.1093/jac/dkf162

58. Iovine NM. Resistance mechanisms in *Campylobacter jejuni*. *Virulence*. (2013) 4:230–40. doi: 10.4161/viru.23753

59. Lin J, Michel LO, Zhang Q. CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrob Agents Chemother*. (2002) 46:2124–31. doi: 10.1128/aac.46.7.2124-2131.2002

60. Payot S, Cloeckaert A, Chaslus-Dancla E. Selection and characterization of fluoroquinolone-resistant mutants of *Campylobacter jejuni* using enrofloxacin. *Microb Drug Resist*. (2002) 8:335–43. doi: 10.1089/10766290260469606

61. Shami A, Abdallah M, Alruways MW, Mostafa YS, Alamri SA, Ahmed AE, et al. Comparative prevalence of virulence genes and antibiotic resistance in *Campylobacter jejuni* isolated from broilers, laying hens and farmers. *Pak Vet J*. (2024) 44:200–4. doi: 10.29261/pakvetj/2024.133

62. Yaseen K, Ali S, Rahman SU, Sajid MS. Comparative molecular virulence typing and antibiotic resistance of *Campylobacter* species at the human-animal-environment interface. *Foodborne Pathog Dis*. (2025) 22:109–17. doi: 10.1089/fpd.2023.0104

63. Connell SR, Trieber CA, Dinos GP, Einfeldt E, Taylor DE, Nierhaus KH. Mechanism of *Tet*(O)-mediated tetracycline resistance. *EMBO J*. (2003) 22:945–53. doi: 10.1093/emboj/cdg093

64. Taylor DE, Tracz DM. "Campylobacter: molecular and cellular biology". In: Ketley JM and Konkel ME, editors. *Mechanisms of Antimicrobial Resistance in Campylobacter*. Wymondham, UK: Horizon Bioscience (2005). p. 193–204.

65. Guévremont E, Nadeau E, Sirois M, Quessy S. Antimicrobial susceptibilities of thermophilic *Campylobacter* from humans, swine, and chicken broilers. *Can J Vet Res*. (2006) 70:81–6.

66. Aarestrup FM, Engberg J. Antimicrobial resistance of thermophilic *Campylobacter*. *Vet Res*. (2001) 32:311–21. doi: 10.1051/vetres:2001127

67. Iovine NM, Blaser MJ. Antibiotics in animal feed and spread of resistant *Campylobacter* from poultry to humans. *Emerg Infect Dis*. (2004) 10:1158–89. doi: 10.3201/eid1006.040403

68. Payot S, Bolla JM, Corcoran D, Fanning S, Mégraud F, Zhang Q. Mechanisms of fluoroquinolone and macrolide resistance in *Campylobacter* spp. *Microbes Infect*. (2006) 8:1967–71. doi: 10.1016/j.micinf.2005.12.032

69. Elmi A, Nasher F, Dorrell N, Wren B, Gundogdu O. Revisiting *Campylobacter jejuni* virulence and fitness factors: role in sensing, adapting, and competing. *Front Cell Infect Microbiol*. (2020) 10:607704. doi: 10.3389/fcimb.2020.607704

70. Gharbi M, Kamoun S, Hkimi C, Ghedira K, Béjaoui A, Maaroufi A. Relationships between virulence genes and antibiotic resistance phenotypes/genotypes in *Campylobacter* spp. isolated from layer hens and eggs in the north of Tunisia: statistical and computational insights. *Foods*. (2022) 11:3554. doi: 10.3390/foods11223554

71. Flanagan RC, Neal-McKinney JM, Dhillon AS, Miller WG, Konkel ME. Examination of *Campylobacter jejuni* putative adhesins leads to the identification of a new protein, designated *Flp*A, required for chicken colonization. *Infect Immun*. (2009) 77:2399–407. doi: 10.1128/iai.01266-08

72. Konkel ME, Garvis SG, Tipton SL, Anderson DE, Cieplak W. Identification and molecular cloning of a gene encoding a fibronectin-binding protein (*Cad*F) from *Campylobacter jejuni*. *Mol Microbiol*. (1997) 24:953–63. doi: 10.1046/j.1365-2958.1997.4031771.x

73. Lara-Tejero M, Galán JE. A bacterial toxin that controls cell cycle progression as a deoxyribonuclease I-like protein. *Science*. (2000) 290:354–7. doi: 10.1126/science.290.5490.354

74. Lara-Tejero M, Galán JE. *Cdt*A, *Cdt*B, and *Cdt*C form a tripartite complex that is required for cytolethal distending toxin activity. *Infect Immun*. (2001) 69:4358–65. doi: 10.1128/iai.69.7.4358-4365.2001

75. Mohamed MI, Khalifa HO, Habib I. The role of virulence genes in Campylobacter pathogenicity: a perspective from the Gulf cooperation council countries. *Front Cell Infect Microbiol*. (2025) 15:1584835. doi: 10.3389/fcimb.2025.1584835

76. Takaku C, Sekizuka T, Tazumi A, Moore JE, Millar BC, Matsuda M. *Campylobacter lari*: molecular and comparative analyses of the virulence-associated chromosome locus J (vacJ) gene homologue, including the promoter region. *Br J Biomed Sci*. (2009) 66:85–92. doi: 10.1080/09674845.2009.11730250

77. Alter T, Weber RM, Hamedy A, Glünder G. Carry-over of thermophilic *Campylobacter* spp. between sequential and adjacent poultry flocks. *Vet Microbiol*. (2011) 147:90–5. doi: 10.1016/j.vetmic.2010.06.005

78. Eberle KN, Kiess AS. Phenotypic and genotypic methods for typing *Campylobacter jejuni* and *Campylobacter coli* in poultry. *Poult Sci*. (2012) 91:255–64. doi: 10.3382/ps.2011-01414

79. Wassenaar TM, Newell DG. Genotyping of *Campylobacter* spp. *Appl Environ Microbiol*. (2000) 66:1–9. doi: 10.1128/aem.66.1.1-9.2000

80. Petersen L, On SLW. Efficacy of flagellin gene typing for epidemiological studies of *Campylobacter jejuni* in poultry estimated by comparison with macrorestriction profiling. *Lett Appl Microbiol*. (2000) 31:14–9. doi: 10.1046/j.1472-765x.2000.00757.x

81. Behringer M, Miller WG, Oyarzabal OA. Typing of *Campylobacter jejuni* and *Campylobacter coli* isolated from live broilers and retail broiler meat by flaA-RFLP, MLST, PFGE and REP-PCR. *J Microbiol Methods*. (2011) 84:194–201. doi: 10.1016/j.mimet.2010.11.016

82. Meinersmann RJ, Helsel LO, Fields PI, Hiett KL. Discrimination of *Campylobacter jejuni* isolates by fla gene sequencing. *J Clin Microbiol*. (1997) 35:2810–4. doi: 10.1128/jcm.35.11.2810-2814.1997

83. Ring M, Zychowska MA, Stephan R. Dynamics of *Campylobacter* spp. spread investigated in 14 broiler flocks in Switzerland. *Avian Dis*. (2005) 49:390–6. doi: 10.1637/7319-010305r1.1

84. Chuma T, Makino K, Okamoto K, Yugi H. Analysis of distribution of *Campylobacter jejuni* and *Campylobacter coli* in broilers by using restriction fragment length polymorphism of flagellin gene. *J Vet Med Sci*. (1997) 59:1011–5. doi: 10.1292/jvms.59.1011