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Original article

Profiles of *Campylobacter jejuni* from raw retail chicken meat: genetic diversity, pathogenic features, and antibiotic resistance

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Summary The study aimed to assess *Campylobacter jejuni* prevalence in chicken meat, biofilm formation, virulence factors, antibiotic resistance, and molecular typing. In the study, 200 chicken meat samples were collected from local outlets and 51 (25.5%) isolates were identified as *C. jejuni*. Resistance rates to ampicillin, tetracycline, sulfamethoxazole/trimethoprim, and ciprofloxacin were 59%, 60%, 64%, and 64% respectively. Many of the isolates (49%) exhibited multidrug resistance. Beta-lactamase and tetracycline resistance genes were found in 82.3% and 86.2% of isolates, respectively. Virulence genes were detected in various proportions. Biofilm formation was weak to moderate. ERIC-PCR showed varied band profiles. Whole genome sequencing confirmed findings. The study highlights *C. jejuni* presence with antibiotic resistance, virulence and biofilm features in chicken meat, posing public health risks.

Keywords Antibiotic resistance, *Campylobacter jejuni*, chicken meats, ERIC-PCR, whole genome sequencing.

Introduction

Campylobacter species are Gram-negative, non-sporeforming, microaerophilic bacteria, primarily motile microorganisms, causing foodborne gastroenteritis (Kayman *et al.*, 2019). *Campylobacter jejuni* is one of the main responsible for campylobacteriosis, with other species like *C. coli, C. upsaliensis*, and *C. lari* also implicated (Ammar *et al.*, 2021; Hizlisoy *et al.*, 2023; Alobaidy *et al.*, 2024). *C. jejuni*, commonly found in chicken intestines, contaminates poultry carcasses during processing, leading to human transmission through contaminated foods (Kaakoush *et al.*, 2015; Thames & Theradiyil Sukumaran, 2020). Studies worldwide have linked undercooked or raw poultry consumption with *C. jejuni* infection (Cokal *et al.*, 2009; Ahmed *et al.*, 2015; Guyard-Nicodème *et al.*, 2015; Büyükünal, 2017; Dramé *et al.*, 2020). *C. jejuni*, which can cause many diseases and economic burdens, is found at high rates worldwide, including the USA, the European Union, China, and Asian countries. However, further research is needed to fully understand *Campylobacter* spp. prevalence (Wang *et al.*, 2021).

Consumption of contaminated food can cause approximately 500 bacterial cells (5×10^2 CFU mL^{-1}) to colonise the small intestine and cause various diseases (Elhadidy *et al.*, 2020). Symptoms of *C. jejuni*-induced gastroenteritis typically appear after an incubation period of several days to a week (Hizlisoy *et al.*, 2023) and include abdominal cramps, fever and acute inflammatory gastroenteritis. Stool analysis may show leukocytes, erythrocytes and epithelial cells presence (Mousavi *et al.*, 2020). Several mechanisms contributed to the pathogenicity of *C. jejuni*, including motility, adhesion, host cells invasion, induction of cell death, and evasion from host defence mechanisms. Genetic factors, such as *flaA, cadF, virB11, ciaB, cdtABC* and *cgtB* have been implicated in virulence (Hizlisoy *et al.*, 2020). Despite extensive research into virulence genes, data remain insufficient (Lapierre *et al.*, 2016; Lim *et al.*, 2016; Lluque *et al.*, 2017). Although sensitive to environmental factors, *C. jejuni* can adopt protective measures such as entering a viable

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but non-culturable (VBNC) state, aerotolerance and biofilm formation (Zhong *et al*., 2020). This persistence therefore poses a challenge to the food industry. Biofilm formation (extracellular polymeric (EPS) matrix), which facilitates survival and organisation through dense populations (Klančnik *et al*., 2021), also contributes to antibiotic resistance transmission (Tram *et al*., 2020). Numerous antibiotics, notably quinolones, macrolides and tetracyclines are used to treat *C. jejuni* infections (Kayman *et al*., 2019), while aminoglycosides are preferred for systemic infections, such as bacteraemia (Zhang *et al*., 2021). The persistence of quinolone resistance in *Campylobacter* strains may be related to the use of quinolones, especially in treating humans and livestock, as well as the circulation of isolates resistant to many antibiotics among different reservoirs (Gahamanyi *et al*., 2021). However, multidrug-resistant strains are on the rise in both animals and humans, posing significant treatment challenges (Abay *et al*., 2014). This trend towards antibiotic resistance in food of animal origin has significant public health implications (Elhadidy *et al*., 2020). Bacterial resistance to antibiotics is particularly dangerous for immunocompromised individuals, as it severely limits the therapeutic effect of antibiotics. Therefore, understanding the drug resistance status of *C. jejuni* can inform the clinical use of antibiotics (Wang *et al*., 2021).

Campylobacter spp. infections are diagnosed using culture, or molecular-based methods (PCR, MLST, PFGE, etc.) (Gerace *et al*., 2022; Aydin *et al*., 2023). Moreover, whole genome sequencing (WGS) is preferred for its high discriminatory power in identifying genetic relationships between isolates and understanding molecular aspects of virulence and antimicrobial resistance in foodborne pathogens (Quino *et al*., 2022).

During literature review, we noticed the lack of a comprehensive study on *C. jejuni* in chicken meat. This inspired us to investigate and determine the biofilm characteristics, antibiotic resistance patterns, virulence factors, and clonal diversity of *C. jejuni*, one of the most significant sources of foodborne bacterial infections in recent times.

Materials and methods

Samples

Totally 200 chicken meat specimens, including dressed-whole chicken (n = 50), thigh (n = 50), wing (n = 50), and breast meat (n = 50), were analysed for the isolation and identification of *C. jejuni*. Between 2018 and 2020, eight samples were collected per month from different local supermarkets and chicken meat sales points in Kayseri, Turkey and the samples included two dressed-whole carcasses, thighs, wings, and breasts and were randomly selected.

Each sample was individually placed in sterile plastic bags and packaged to prevent leakage and moisture loss. Samples were transported to the laboratory within 12 h under cold storage (+4 °C) and were processed immediately. For dressed-whole chickens, samples were taken from the surface of the meat with skin, for others, a representative test portion (a piece of poultry meat with the skin, approximately 25 g, cut neatly) was taken from the sample.

Isolation and identification

The ISO (ISO 10272, 1995) method was used to isolate *Campylobacter* species from a total of 200 fresh chicken meats. Briefly, 25 g of the meat samples were enriched in 225 mL Bolton broth medium (Merck, M100068.0500, Germany) and were incubated at 37 °C for 4–6 h and then at 41.5 °C for 48–72 h in a microaerobic atmosphere. The samples were then seeded on modified charcoal-cefoperazone deoxycholate (mCCD) agar (Merck, M100070.0500, Germany) including charcoal-cefoperazone deoxycholate (CCDA) selective supplement (Oxoid SR0155E, UK) and were kept microaerobically at 41.5 °C for 48–72 h. When the incubation period ended, suspected *Campylobacter* colonies were selected. Gram staining and microscopic examination, motility, catalase, oxidase, hippurate hydrolysis, and H_2S formation tests were applied for phenotypic identification.

Molecular identification

The DNA of the isolates was extracted using an extraction kit (Ultraclean DNA extraction kit, 50 test Mobio 12 224–50, USA) in accordance with the manufacturer's instructions. Qubit 3.0 fluorometer (Thermo Fisher, USA) was used to determine DNA concentrations ($\mu g\ \mu L^{-1}$) of the samples and kept at −20 °C until analysis. PCR assay conditions using only *C. jejuni* (for species-level) and genus primers (for genus-level) were carried out as reported by Wang *et al*. (2002). The primers (Sentromer, Istanbul) used for PCR were given in Table 1. Briefly, in a reaction mixture with a total volume of 25 µL for each sample; 2.5 µL DNA sample, 1x PCR buffer (Thermo Scientific, USA), 20 mM $MgCl_2$ (Vivantis, Malaysia), 0.2 mM dNTP mix (Thermo Scientific, USA), 0.5 µM *C. jejuni* and 0.2 µM *Campylobacter* 23S rRNA primers and 1.25 U, was prepared as Taq polymerase (Thermo Scientific, USA). DNA amplification was performed with an initial denaturation of 6 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, primer annealing at 59 °C for 30 s, extension at 72 °C for 30 s and finally 72 °C. This was carried out in a thermal cycler (Arktic™ Thermal Cycler; Thermo Fisher, USA) with a final extension of 7 min. The

Table 1 Primers and expected band sizes

Primers	Sequence 5′-3′s	Band sizes (bp)
Primer-I (CJF)	5′-ACTTCTTTATTGCTTGCTGC-3′	323
Primer-II (CJR)	5′-GCCACAACAAGTAAAGAAGC-3′	
23SrRNA-F (Genus)	5′-TATACCGGTAAGGAGTGCTGGAG-3′	650
23SrRNA-R (Genus)	5′-ATCAATTAACCTTCGAGCACCG-3′	
flaAF	5′-ATGGGATTTCGTATTAACAC-3′	1700
flaAR	5′-CTGTAGTAAATCTTAAAACATTTTG-3′	
cdtAF	5′-GGAAATTGGATTTGGGGCTATACT-3′	165
cdtAR	5′-ATCACAAGGATAATGGACAAT-3′	
cdtBF	5′-GTTAAAATCCCTGCTATCAACCA-3′	495
cdtBR	5′-GTTGGCACTTGGAATTTGCAAGGC-3′	
cdtCF	5′-TGGATGATAGCAGGGGATTTTAAC-3′	555
cdtCR	5′-TTGCACATAACCAAAAGGAAG-3′	
virB11F	5′-TCTTGTGAGTTGCCTTACCCCTTTT-3′	494
virB11R	5′-CCTGCGTGTCCTGTGTTATTTACCC-3′	
ceuEF	5′-CCTGCTCGGTGAAAGTTTTG-3′	794
ceuER	5′-GATCTTTTTGTTTTGTGCTGC-3′	
cadFR1BF	5′-TTGAAGGTAATTTAGATATG-3′	400
cadFR1BR	5′-CTAATACCTAAAGTTGAAAC-3′	
iamF	5′-GCGCAAAATATTATCACCC-3	518
iamR	5′-TTCACGACTACTATGCGG-3′	
ERIC-I	5′-ATGTA AGCTCCTGGGGATTCAC-3′	-
ERIC-II	5′-AAGTAAGTGACTGGGGTGAGCG-3′	-
DMT1 (TetOF)	5′-GGCGTTTTGTTTATGTGCG-3′	559 bp
DMT2 (TetOR)	5′-ATGGACAACCCGACAGAAGC-3′	
23SRNA-F	5′-TTAGCTAATGTTGCCCGTACCG-3′	697 bp
23SRNA-R	5′-AGCCAACCTTTGTAAGCCTCCG-3′	
ERY2074-R	5′-AGTAAAGGTCCACGGGGTCTGG-3′	485 bp
ERY2075-R	5′-TAGTAAAGGTCCACGGGGTCGC-3′	
GZgyrA5	5′-ATTTTTAGCAAAGATTCTGAT-3′	673 bp
GZgyrA6	5′-CCATAAATTATTCCACCTGT-3′	
CampyMAMAgyrA1-F	5′-TTT TTAGCAAAGATTCTG AT-3′	265 bp
CampyMAMAgyrA5 R	5′-CAAAGC ATCATAAACTGC AA-3′	
cmeB	5′-TCCTAGCAGCACAATATG-3′	241 bp
cmeB	5′-AGCTTCGATAGCTGCATC-3′	
BlaOXA-61	5′-AGAGTATAATACAAGCG-3′	372 bp
BlaOXA-61	5′-TAGTGAGTTGTCAAGCC-3′	
aphA-3-1	5′-TGCGTAAAAGATACGGAAG-3′	701 bp
aphA-3-1	5′-CAATCAGGCTTGATCCCC-3′	
aadE1	5′-GAACAGGATGAACGTATTCG-3′	837 bp
aadE1	5′-GCATATGTGCTATCCAGG-3′	
GZgyrA7	5′-TTATTATAGGTCGTGCTTTG-3′	FS
GZgyrA8	5′-TAGAAGGTAAAACATCAGGTT-3′	
23SRNA-F	5′-TTAGCTAATGTTGCCCGTACCG-3′	FS
23SRNA-R	5′-AGCCAACCTTTGTAAGCCTCCG-3′	

FS, for sequencing.

PCR products obtained because of amplification were visualised using the UVP gel documentation system (Vilber Lourmat, France) after running on a 1.5% agarose gel at 90 V for 90 min in an electrophoresis device (Thermo EC 330, USA).

Antibiotic susceptibility testing

Disc diffusion test was performed on 51 *C. jejuni* isolates, according to the procedures suggested by Clinical and Laboratory Standards Institute (CLSI, 2022). Antibiotics were as follows: erythromycin (E, 15 μg), azithromycin (AZM, 15 μg), enrofloxacin (ENR, 5 μg), tetracycline (TE, 30 μg), trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μg), amoxicillin-clavulanic acid (AMC, 20/10 μg), gentamicin (CN, 10 μg), amikacin (A, 30 μg), ciprofloxacin (CIP, 5 μg), nalidixic acid (NA, 30 μg), clindamycin (DA, 2 μg), piperacillin–tazobactam (TZP, 100/10 μg), doxycycline (DO, 30 μg), cefotaxime (CTX, 30 μg) and

ampicillin (AMP, 10 μg, Oxoid, UK). In the test, isolates were incubated on 5% defibrinated sheep blood agar in a microaerobic environment at 37 °C for 24–48 h, and the growing colonies were suspended in 0.075% NaCl solution. After adjusting bacterial density according to the 0.5 McFarland turbidity standard, it was inoculated into blood agar in a volume of 0.1 mL and antibiotic susceptibility test discs were placed on the medium. Petri dishes were incubated in a microaerobic environment at 37 °C for 24–48 h. The diameters of the inhibition zones formed at the end of the test were measured and evaluated according to the "Clinical and Laboratory Standards Institute (CLSI)" recommendations.

Reference strains

As positive control for biofilm formation, *Campylobacter jejuni* NCTC 11168 was utilised. *Campylobacter jejuni* ATCC 700819 and *Campylobacter jejuni* ATCC 33560 were also employed as quality control strains throughout the study.

Virulence genes

A total of 51 *C. jejuni* isolates were evaluated for virulence genes using molecular methods (Hizlisoy *et al*., 2020). PCR was conducted by combining 1× PCR buffer (Thermo Scientific, USA), 1.5 mM $MgCl_2$ (Vivantis, Malaysia), 250 mM dNTP mix (Thermo Scientific, USA), 1 μM of each primer, 0.5 U Taq polymerase (Thermo Scientific, USA), and adjusting the final volume to 40 μL with sterile distilled water. PCR conditions in a thermocycler (Techne TC512, UK) were as follows: 30 cycles of 94 °C for 1 min, 56 °C for 30 s and 72 °C for 30 s: followed by a final extension at 72 °C for 10 min. The PCR products were electrophoresed on a 1.5% (w/v) agarose gel at 100 V for 1 h and visualised using a UV transilluminator (Biorad Gel Doc XR, USA).

Molecular detection of antibiotic resistance

The method described by Gibreel *et al*. (2004) was used to determine the presence of *tetO* gene of 51 *C. jejuni* isolates for tetracycline resistance. In the test, the reaction mixture contains 0.5 μM each of primers (DMT1 and DMT2 primers), 200 μM dNTPs, 1× reaction buffer (50 μM KCl, 10 mM Tris–HCl pH 8.3, 1.5 mM $MgCl_2$), 1 U Taq polymerase and consists of template DNA. The reaction was carried out in a total volume of 25 μL by adding 2 μL of target DNA to 23 μL of the mixture. It was performed in 30 cycles (95 °C 1 min, 50 °C 1 min, 72 °C 1 min) following preliminary denaturation at 95 °C for 1 min (Techne TC512, UK). PCR products obtained of amplification were subjected to electrophoresis for 1.5 h at 120 Volts in 1.5% agarose gel and then visualised using a gel documentation system (Biorad Gel Doc XR, USA).

PCR reactions were performed according to method described by Alonso *et al*. (2005) for the detection of ERY2074 and ERY2075 mutations in macrolide resistance. In the PCR reaction mixture; 1× PCR buffer (10 mM Tris–HCl, 1.5 mM $MgCl_2$, 50 mM KCl, pH 8.3), 80 ng genomic DNA, 0.2 μM of each primer, 0.2 mM DNTPs and 1 U Taq polymerase. It was carried out in a total volume of 25 μL by adding 5 μL of target DNA to the 20 μL mixture containing in the two-stage PCR reactions, first with ERY2074 primer and then with ERY2075 primer, 5 min of predenaturation at 94 °C, 30 s at 94 °C, 30 s at 59 °C and 30 cycles of 45 s at 72 °C, and a final extension step of 5 min at 72 °C was applied (Techne TC512, UK). The PCR products obtained from amplification were subjected to electrophoresis in 2% agarose gel at 120 volts for 1.5 h and then visualised using the gel documentation system.

For quinolone resistance, initially, quinolone resistance determining region (QRDR) was examined to detect quinolone resistance. In the PCR process, the reaction mixture contains 20 pmol primers (GZgyrA5 and GZgyrA6 primers), 200 μM dNTPs, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U Taq polymerase and template DNA. The reaction was performed in a total volume of 100 μL by adding 5 μL target DNA to 95 μL of the mixture. PCR reaction; after pre-denaturation at 94 °C for 3 min, it was performed in 30 cycles (94 °C 1 min, 50 °C 1 min, 72 °C 1 min) with a final extension step at 72 °C for 5 min (Techne TC512, UK).

Secondly, for the detection of mutations in *gyrA* gene of *C. jejuni*, the MAMA-PCR reaction described by Zirnstein *et al*. (1999) was used. In the PCR, the reaction mixture contains 20 pmol primers (CampyMAMAgyrA1 and CampyMAMAgyrA5 primers), 200 μM dNTPs, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2.5 U Taq polymerase (Thermo Scientific, USA) and consists of template DNAs. The reaction was performed in a total volume of 50 μL by adding 5 μL target DNA to the 45 μL mixture. PCR reaction: after pre-denaturation at 94 °C for 3 min, it was performed in 30 cycles (94 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s) with a final extension step at 72 °C for 3 min (Techne TC512, UK). The PCR products obtained from amplification were subjected to electrophoresis in 2% agarose gel at 120 volts for 1.5 h and then visualised using the gel documentation system.

Furthermore, aph-3-1, bla_{OXA-61}, and *cmeB* genes were also screened for aminoglycoside, ampicillin, and multidrug efflux pumps by multiplex PCR as explained by Obeng *et al*. (2012). In mPCR amplification; initial

denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, coupling at 54°C for 30 s and extension at 72°C for 1 min (Techne TC512, UK). Each reaction consisted of 4 mmol L^{-1}, $MgCl_2$, 1 μL of 25 pmol of each primer, 2 μL of 2 mmol L^{-1} dNTP and 4 μL of 5× PCR reaction buffer in a total reaction volume of 25 μL with 1 U of Taq polymerase and 2 μL of DNA template. The PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide in 1X TAE buffer and then visualised using the gel documentation system.

Biofilm formation

Biofilm formation of 51 *C. jejuni* isolates was evaluated by the bacterial potential cellulose production (microtiter plate test) and carried out on Congo red agar (Merck, Germany) plates according to the method suggested by Brown *et al.* (2014). However, Zhong *et al.* (2020) declared the quantitative measurement conducted with the microtiter plate test. In the test, firstly, isolates were inoculated onto Congo red agar and kept under microaerobic conditions at 37 °C for 24–48 h and evaluated according to colony morphology; white colonies were referred as non-cellulose producers and red colonies as cellulose producers (Brown *et al.*, 2014). The quantification of biofilm formation was then performed using the classification system outlined by Stepanović *et al.* (2000).

Enterobacterial repetitive intergenic consensus (ERIC)-PCR

ERIC-PCR method was reported by Houf *et al.* (2002). PCR protocol includes an initial denaturation at 94 °C for 5 min, followed by 40 cycles comprising 1 min at 94 °C, 1 min at 25 °C, and 2 min at 72 °C, concluding with a final extension step of 10 min at 72 °C. Subsequently, amplified products of 51 *C. jejuni* isolates were analysed through 2% agarose gel electrophoresis and visualised using a UV transilluminator (Bio-Rad, USA). Band patterns were captured using gel documentation and band sizes were manually converted into a binary code indicating the presence (1) or absence (0) of bands. Band profile similarity indices were determined using open-access online software iTOL version 5 (UPGMA, Letunic & Bork, 2019) to build a dendrogram that was constructed based on the Jaccard similarity coefficient and the arithmetic mean unweighted pair-group method.

Whole genome sequencing

Campylobacter jejuni HH22 isolate has been chosen for whole genome sequencing (WGS) according to its abundant virulence gene and antibiotic resistance profiles from sample HH22 obtained from chicken whole-dressed carcass. Genomic DNA obtained was used for library construction according to the manufacturer's instructions for Nextera XT library preparation kit (Illumina, USA). Next-generation sequencing was carried out as 2× 151 bp pair-end chemistry (500× depth coverage) with Novaseq6000 (Illumina, USA), and raw reads were gained in 'fastq.gz' format. Raw reads were subjected to TrimGalore v0.6.5 open-source software for barcode trimming (Krueger, 2019). SPAdes v3.13.0 (Bankevich *et al.*, 2012) was used to assemble truncated barcodes. QUAST v5.0.2 (Gurevich *et al.*, 2013) was used to assess the quality of the assemblies. Annotation of the assembled genomes was performed using Prokka (Seemann, 2014). Assembled contigs were used for some WGS-based predicted testing. For antibiotic-resistance genes, the CARD programme was used. VFanalyzer (Liu *et al.*, 2019) was used for virulence genes. PathogenFinder 1.1 was used to predict pathogenicity towards human hosts of isolate. MLST 2.0 (Larsen *et al.*, 2012) was used for multilocus sequence typing (MLST). Also, a phylogenetic dendrogram was constructed for understanding the genetic relation of HH22 with some *C. jejuni* isolated from chicken samples published in GenBank. For that aim, BV-BRC 3.28.21 was used for analysis using the programme RAxML (Stamatakis *et al.*, 2008; Stamatakis, 2014).

Statistical analysis

The association between *C. jejuni* positivity and chicken meat samples was assessed using Chi-square analysis, conducted with SPSS 28.0.1.0 (IBM Inc., Chicago, IL, USA). The relationship between the profiles of antibiotic resistance and the resistance genes was also investigated. The statistical significance level in the study was set at $P < 0.05$.

Results

At the end of the isolation and phenotypic identification tests, 51 (25.5%) of 200 samples were positive for *Campylobacter* spp. In molecular identification tests (mPCR), isolates were confirmed as *C. jejuni*.

Among 51 *C. jejuni* isolates, 59% exhibited resistance to ampicillin, 60% to tetracycline, 64% to sulphamethoxazole/trimethoprim, 50% to enrofloxacin, and 64% to ciprofloxacin (Table 2). Moreover, 49% of the isolates exhibited multidrug resistance. While 23.5% of the isolates were resistant to three antibiotic types, 17.6% were resistant to four classes of antibiotics (Table 3).

The virulence genes including *cdtC, cadF, cdtB, cdtA, ceuE*, and *flaA* were detected in 92.1%, 80.3%, 43.1%, 43.1%, 15.6% and 11.8% of 51 *C. jejuni*

Table 2 Antimicrobial resistance of *C. jejuni* isolates

Name of antibiotics[†]	*S* (%)	*I* (%)	*R* (%)
Amoxicillin/clavulanic acid (AMC)	31 (60%)	5 (9%)	15 (29%)
Piperacillin/tazobactam (TZP)	33 (64%)	1 (1%)	17 (35%)
Erythromycin (E)	43 (84%)	-	8 (15%)
Ampicillin (AMP)	21 (41%)	-	30 (59%)
Tetracycline (TE)	20 (39%)	-	31 (60%)
Trimethoprim/sulphamethoxazole (SXT)	18 (33%)	-	33 (64%)
Cefotaxime (CTX)	37 (72%)	3 (5%)	11 (21%)
Azithromycin (AZM)	43 (84%)	-	8 (15%)
Enrofloxacin (ENR)	25 (49%)	-	26 (50%)
Gentamycin (CN)	43 (84%)	-	8 (15%)
Ciprofloxacin (CIP)	18 (33%)	-	33 (64%)
Doxycycline (DO)	31 (60%)	-	20 (39%)
Amikacin (AK)	43 (84%)	-	8 (15%)
Nalidixic acid (NA)	27 (52%)	3 (5%)	21 (41%)

S; susceptible, I; intermedier, R; resistance.
[†]Since it does not provide a breakpoint for *Campylobacter* species, the limits given for Enterobacterales were used as a reference.

Table 3 Multidrug resistance profiles of *C. jejuni* isolates

Antibiotics	Number of isolates
AMP, TE, SXT	12
AMP, TE, SXT, CTX	3
E, AMP, TE, SXT	2
DA, E, AMP, TE	1
ENR, CN, CIP, DO	1
SXT, CTX, AZM, ENR	1
CIP, DO, AK, NA	1
TE, SXT, AZM, CTX, ENR	1
SXT, CTX, AZM, ENR, CN, CIP	1
AMP, TE, SXT, CTX, AZM, ENR, CN, CIP	1
AMP, TE, SXT, CTX, AZM, ENR, CN, CIP, NA, AK, DO	1

isolates, respectively. However, in any isolates, *iam* and *virB11* could not be found (Table 4) in 51 *C. jejuni* isolates.

Quinolone and macrolide resistance genes were not found in the isolates, whereas tetracycline resistance genes (*tetO*) were found in 44 (86.2%) isolates, and beta-lactamase resistance (*blaOXA*) genes were detected in 42 (82.3%) of 51 *C. jejuni* isolates.

In Congo red agar method, biofilm positivity was observed in five isolates, from which two isolates had weak, and three isolates had moderate biofilm ability. In the microtiter plate method, seven isolates had moderate, 42 isolates showed weak biofilm production ability, while two of them could not have biofilm ability. Biofilm formation and antibiotic sensitivity tests indicated that the biofilm-forming strains were multidrug resistant.

In the study, we typed 51 *C. jejuni* isolates by ERIC-PCR. Band patterns showed 1–14 fragments of DNA ranging in size from 100 to 1800 bp. Cluster analysis showed that ERIC-PCR patterns, classified with a Jaccard coefficient of 0.72, were separated into 14 (A–N) main clusters and two singletons. ERIC-PCR patterns of *C. jejuni* strains demonstrated that isolates were genetically diverse and had heterogeneous populations in spite of their common ancestry (Fig. 1).

Assembled contigs (*Campylobacter jejuni* HH22) were submitted to GenBank and the accession number was assigned as JARGYB000000000. Results of the predicted genomic characterisation of isolate were given in Table 5 and the phylogenetic relationships of *Campylobacter jejuni* HH22 with references isolated from other chicken meat samples and randomly chosen from NCBI GenBank were presented in Fig. 2. In Fig. 3, the schematic representation of the draft genome sequence of an isolate was given. Moreover, In Fig. 4, the general distribution of antibiotic resistance genes of isolate according to the CARD-RGI programme was demonstrated.

In this study, *C. jejuni* presence in chicken meat samples and the correlation between antimicrobial resistance of isolates and resistance genes were examined using Chi-square analysis in SPSS 28.0.1.0 (IBM Inc., Chicago, IL, USA), with differences considered insignificant when $P > 0.05$.

Discussion

Poultry meat and products are favoured by consumers for their affordability, availability and nutritional value (Barbut & Leishman, 2022). However, *C. jejuni*, which is naturally found in the poultry microbiota, can cause diseases such as gastroenteritis (Thames & Theradiyil Sukumaran, 2020). Foodborne gastroenteritis cases caused by *Campylobacter* species are the second most common bacterial infections worldwide, following infections caused by *Salmonella* spp. (Kaakoush *et al.*, 2015). However, foods contaminated with

Table 4 The distribution of virulence genes in *C. jejuni* isolates

Number of positive isolates	Virulence genes *CdtC*	*CadF*	*cdtB*	*cdtA*	*ceuE*	*flaA*
51	47 (92.1%)	41 (90.3%)	22 (43.1%)	22 (43.1%)	8 (15.6%)	6 (11.8%)

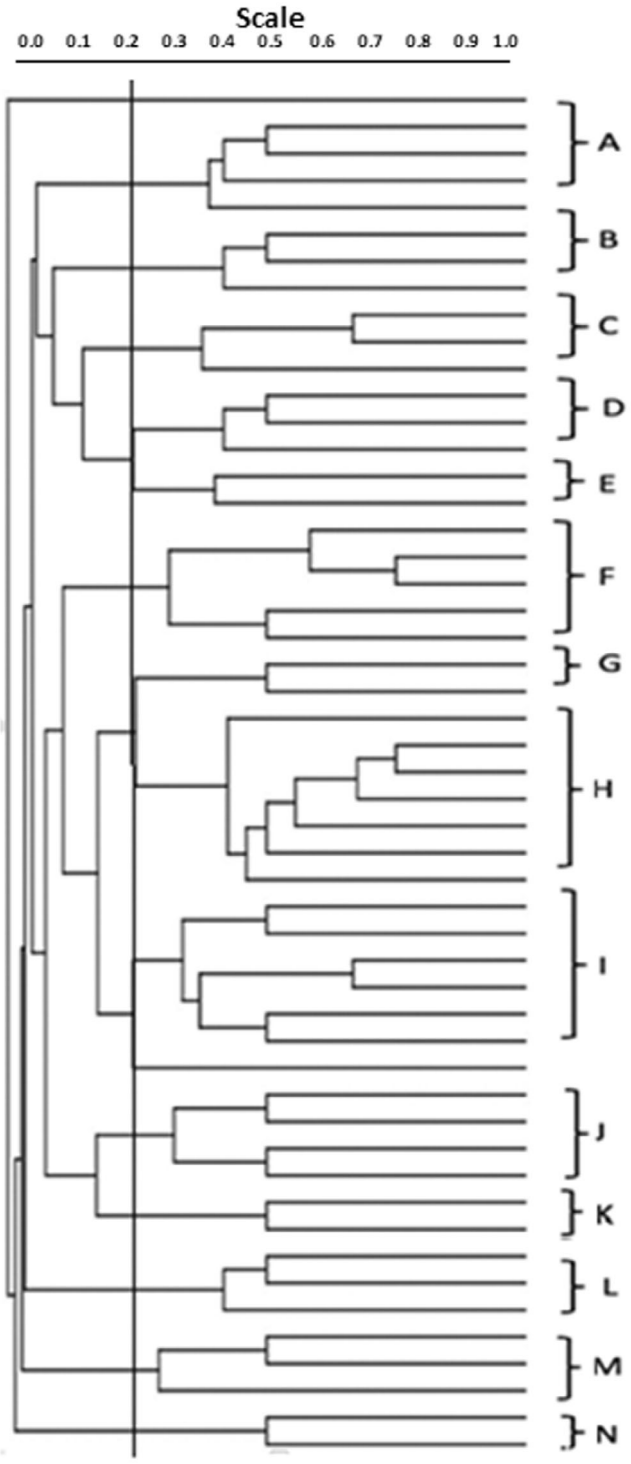

Figure 1 ERIC-PCR Dendogram of *Campylobacter jejuni* isolates.

Campylobacter species can be made safe through effective cooking and processing (Santos-Ferreira *et al.*, 2021).

Table 5 Predicted genomic characterisation of *Campylobacter jejuni* HH22 isolate based on WGS

Characteristics	Method	Profile
Antibiotic resistance genes[†]	CARD	blaOXA-61; tet(O/32/O); ant(6)-Ia
Virulence genes[†]	VFanalyzer	Adherence: CadF; JlpA; MOMP; PEB1/CBF1 Invasion: ciaB Immun evasion: LOS Secretion system: virB4; virB8-10; virD4 Toxin: cdtA; cdtB; cdtC
Probability of being a human pathogen	PathogenFinder 1.1	0.919
MLST type	MLST 2.0	*C. jejuni* 11 846

[†]Detailed virulence profiles of H22 isolate were given as Appendix S1.

In the study, the general prevalence of *C. jejuni* was 25.5%. Similarly, the occurrence of *C. jejuni* in the samples was found to be 33.1% by Salem *et al.* (2019). However, Mikulić *et al.* (2016) documented a higher prevalence compared with the findings in our study. In line with our investigation, Nowaczek *et al.* (2019) declared that in 140 chicken samples, *C. jejuni* was found at the rate of 22.1%. However, in some studies, researchers found a relatively low prevalence. Büyükünal (2017) found the prevalence of *C. jejuni* to be 8.52% in a study on the presence of thermophilic *Campylobacter* species from chicken meat. Differences in study rates may be due to seasonal fluctuations, climate variations and chicken meat sampling quality, regional disparities and hygiene standards in processing plants (Cokal *et al.*, 2009).

Administering antibiotics in small amounts over extended periods to promote livestock growth leads to the development of resistant bacteria, posing a threat to public health. The utilisation of enrofloxacin and quinolones in veterinary practices has resulted in the emergence of ciprofloxacin resistance in *Campylobacter* spp. found in both poultry and humans (Hizlisoy *et al.*, 2023). Recent reports indicate elevated rates of antibiotic resistance among *Campylobacter* species isolated globally (Casagrande Proietti *et al.*, 2020; Dramé *et al.*, 2020; Wang *et al.*, 2021). As a result, multidrug-resistant (MDR) campylobacteria isolated from both humans, animals and foods have become widespread (Abay *et al.*, 2014; Abebe *et al.*, 2020). In the antibiotic susceptibility testing of 51 *C. jejuni* isolates revealed that 59%, 60%, 64%, 50%, and 64% of the strains have shown resistance to ampicillin, tetracycline, sulphamethoxazole/trimethoprim, enrofloxacin and ciprofloxacin, respectively. Cokal *et al.* (2009)

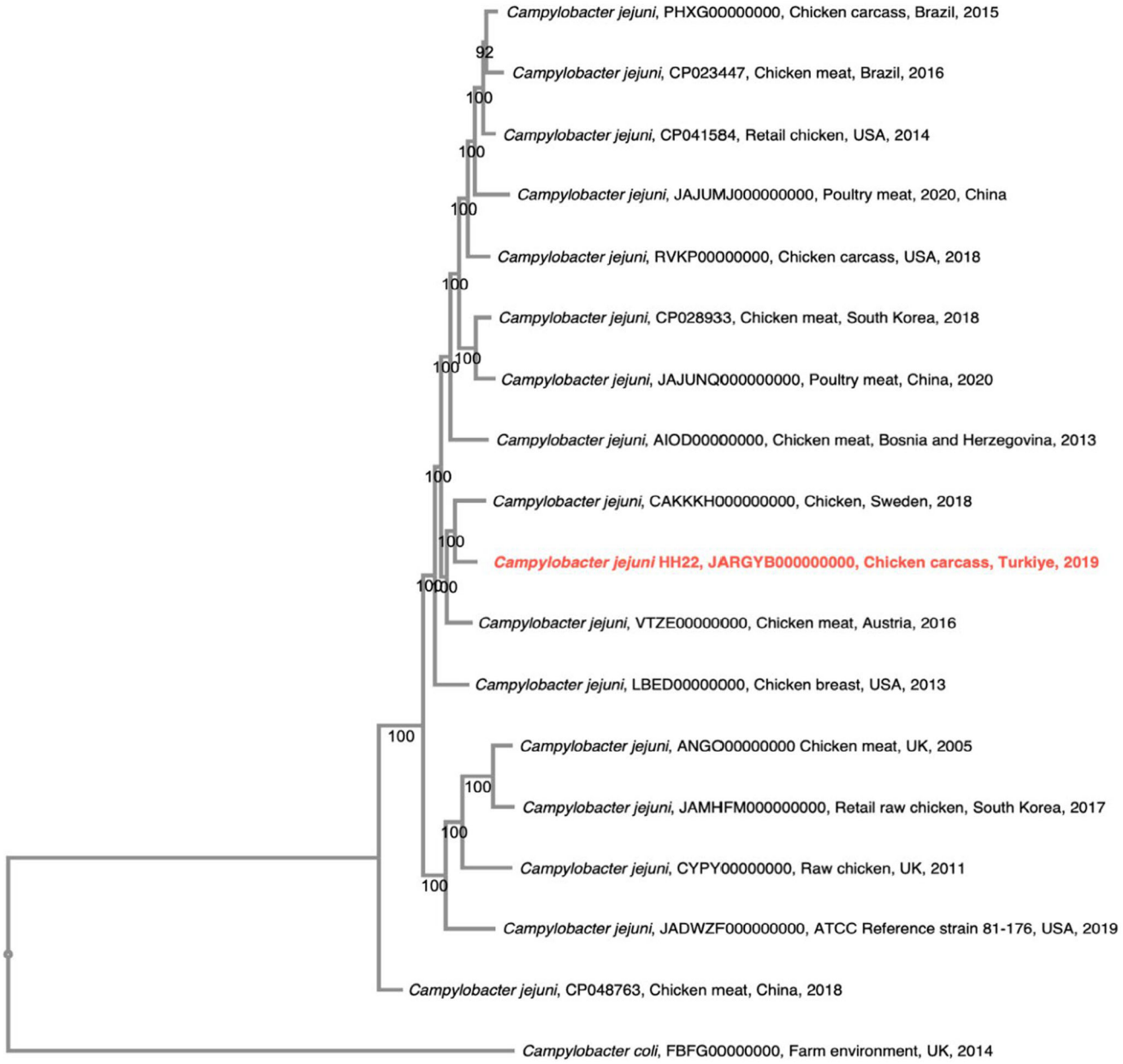

Figure 2 Phylogenetic relationships of *Campylobacter jejuni* HH22 (identified in this study are coloured with red) and other chicken meat isolates deposited in NCBI GenBank based on whole genome sequences (Number of genes: 1000). The sequences are given as GenBank accession number, isolation source country, and isolation years (*Campylobacter coli* FBFG00000000 from farm environment is used as outgroup).

found resistance to 79.4% nalidixic acid, 76.3% tetracycline and 74.2% ciprofloxacin in *C. jejuni* isolates. Yildirim *et al.* (2005) reported that 31.3% of *C. jejuni* strains have exhibited resistance to quinolones. In the study of Abay *et al.* (2014), 93% ciprofloxacin, 88% enrofloxacin and 7% erythromycin resistance were reported. In Switzerland, Wirz *et al.* (2010) reported that quinolone resistance was determined in 19.8% of isolates. In France, resistance to ciprofloxacin (32.9%), nalidixic acid (32%), and tetracycline (53.6%) were found in isolates (Guyard-Nicodème *et al.*, 2015). In Canada, Dramé *et al.* (2020) stated that among *C. jejuni* isolates, tetracycline had the highest resistance at a rate of 39%, and quinolone–tetracycline was the second with 6.6%. In Italy, Casagrande Proietti *et al.* (2020) reported 20 *C. jejuni* isolates were

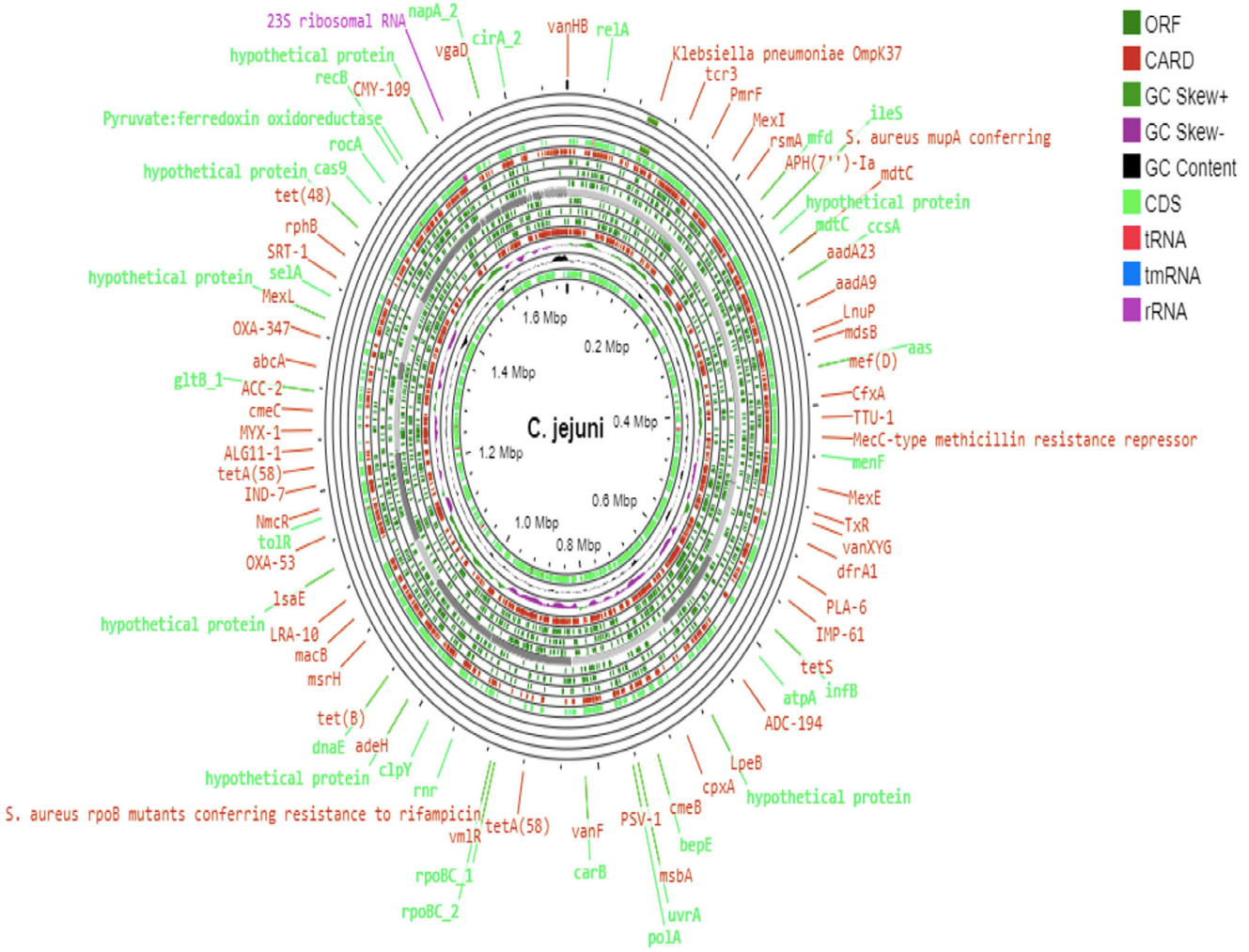

Figure 3 Schematic representation of the draft genome sequence of a *Campylobacter jejuni* isolate.

found to have beta-lactam resistance. In addition, *C. jejuni* isolates were reported to have 10% resistance to ticarcillin/clavulanic, 60% to ampicillin/sulbactam and 90% to amoxicillin/clavulanic acid.

As mentioned above, some studies (Yildirim *et al.*, 2005; Abay *et al.*, 2014) are consistent with the results of the presented study, while others have different rates (Cokal *et al.*, 2009; Wirz *et al.*, 2010; Guyard-Nicodème *et al.*, 2015; Casagrande Proietti *et al.*, 2020; Klančnik *et al.*, 2021). The variations in the results might have resulted from the antibiotics, antibiotic susceptibility method or the different mechanisms that caused antibiotic resistance in microorganisms (Jeon *et al.*, 2008).

Beyond the host response to *C. jejuni*, the pathogenic characteristics of bacteria play a crucial role in the diversity of clinical symptoms (Hizlisoy *et al.*, 2020). Campylobacters synthesise toxins with *cdt*A, B and C. *Fla*A is responsible for motility, *cadF* is for adhesion and invasion to the intestinal epithelial cells and *ceuE* gene encoding a lipoprotein in *C. jejuni* (Gharbi *et al.*, 2022). In the study, *cdtC, cadF, cdtB, cdtA, ceuE* and *flaA* were detected in 92.1%, 80.3%, 43.1%, 43.1%, 15.6% and 11.8% of isolates, respectively. The presence of these virulence genes poses a significant risk to human health in terms of the pathogenic potential of the bacterium and Campylobacter-related diseases. Of note, *iam* and *virB11* genes could not be found. In Campylobacters, the *iam* and *virB11* genes have been associated with invasion (Gharbi *et al.*, 2022). Although many genes play a role in the invasion of campylobacters into epithelial cells, the absence of *iam* and *virB11* genes is an important deficiency in terms of invasion. A study by Lluque *et al.* (2017) determined the presence of *cdt*ABC, *cadF* and *iam*, in *C. jejuni* isolates. In Chile, the most common genes were reported to be *flaA* and

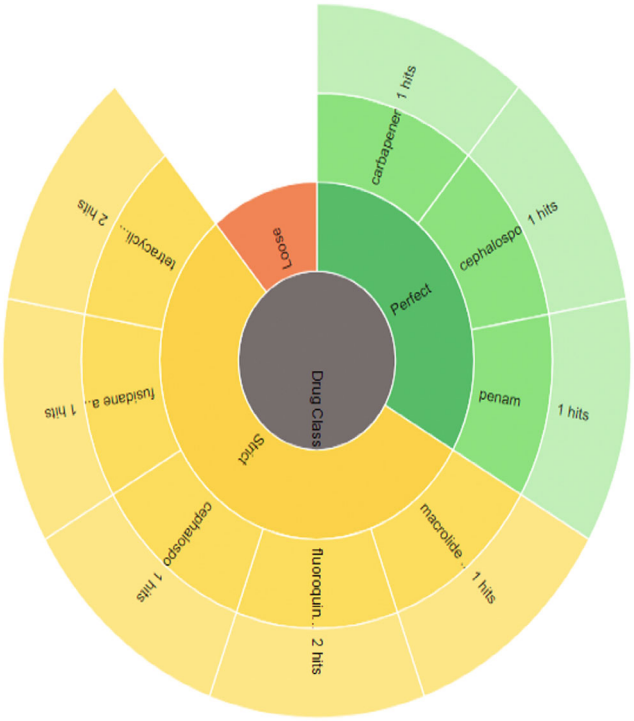

Figure 4 General distribution of antibiotic resistance genes of a *Campylobacter jejuni* isolate according to the CARD-RGI program in whole genome sequence analysis.

cdtA (63%), followed by *cdtC* (60%) (Lapierre *et al*., 2016). In a study by Lim *et al*. (2016), the presence of 12 virulence factors in campylobacters was examined and *flaA, cdtA, cdtB* and *cdtC* were found in all isolates. In Campylobacter isolates obtained from different slaughterhouses, *cdtC, flaA* and *iam* were found in 7, 6 and 1 of the *C. jejuni* isolates, respectively (Hizlisoy *et al*., 2020).

According to the PCR analysis, the tetracycline resistance gene, *tetO*, was detected in 86.2% of isolates, while beta-lactamase resistance genes were detected in 82.3% of isolates. Macrolide and quinolone resistance genes were not found in *C. jejuni* isolates. The mechanism of resistance has not been revealed genotypically. Other resistance mechanisms (efflux pumps) in quinolone (Engberg *et al*., 2001) and in macrolide resistances (reduced permeability, enzymatic pathways or efflux pumps) may be involved (Iovine, 2013). In contrast to our study, the studies of Aydin *et al*., 2023, Hizlisoy *et al*., 2023, and Alobaidy *et al*., 2024 have reported the presence of quinolone resistance genes in *C. jejuni* isolates. A group of tet determinants, typically found on plasmids, is responsible for tetracycline resistance, potentially transferred between bacteria (Kayman *et al*., 2019). The mechanism involves membrane proteins like *tetO* actively expelling tetracyclines (Taylor & Tracz, 2005). All tetracycline-resistant isolates carried the *tetO* gene. Similar to Kayman *et al*., 2019, the study did not specify *tetO*'s location. Additional information about *tetO* genes' plasmid origin is detailed in studies by Gibreel *et al*. (2004) and Dasti *et al*. (2007). However, Oyarzabal *et al*. (2008), nine isolates were found to carry the *tetO* gene, but it was stated that they were of genomic origin and not plasmid origin.

Ampicillin resistance in *C. jejuni* isolates may stem from the synthesis of beta-lactamases, low-affinity binding of beta-lactams to penicillin-binding proteins (PBP), or decreased permeability of outer membrane porins (Aslantaş, 2019). In the study, the resistance was related to enzymatic inactivation by $bla_{\text{OXA-61}}$. In 42 (82.3%) of isolates, $bla_{\text{OXA-61}}$ genes were detected. However, in *C. jejuni* isolates only 30 (59%) of isolates have ampicillin resistance. In the studies conducted around the world, the authors reported that the ampicillin resistance in *C. jejuni* isolates from poultry was between 33.3% and 65% (Obeng *et al*., 2012; Sierra-Arguello *et al*., 2015; Casagrande Proietti *et al*., 2020). The antibiotic resistance gene rate was higher than the present study's phenotypic resistance rate. Other factors involved in antibiotic susceptibility and resistance may explain this situation (Hedayatianfard *et al*., 2014). As it is known, microorganisms can transfer genes between each other in several ways (transformation, transduction, conjugation, etc.). During the transfer of genes, losses may occur in the chromosome. This may be the reason why some genes could not be identified (Jeon *et al*., 2008). Although *C. jejuni* is microaerophilic, it can survive for extended periods in biofilms under normal or harsh conditions (Zhong *et al*., 2020), becoming highly antibiotic-resistant (Kim *et al*., 2015). Antibiotics act slowly on bacteria within biofilm layers, providing protection to *Campylobacter* species. Sublethal antibiotic concentrations encountered by biofilm bacteria not only slow growth but also induce resistance genes (Klančnik *et al*., 2021). In the study, when examining the biofilm features, isolates have weak or moderate biofilm abilities. In earlier studies examining the biofilm ability of *C. jejuni*, isolates with biofilm production capacity at different levels have been noted (Zhong *et al*., 2020; Wang *et al*., 2021). In the study, the isolates capable of forming biofilms exhibited multiple resistance to antibiotics tested. Hence, this sheds light on the issue of biofilm-forming isolates and their resistance to multiple antibiotics (Tram *et al*., 2020). Similarly, in the study of Bae & Jeon (2013), increased fluoroquinolone resistance was reported from the biofilms of isolates. These results provide an opportunity for further research to explore new approaches and develop innovative solutions to address the interplay between the formation of biofilms and resistance to antibiotics. The flagella of *Campylobacter* spp., play a key role in adhering to various surfaces and forming biofilms. Activation

of flagellin A (*flaA*) expression has been reported to be associated with biofilm formation (Ohadi *et al*., 2022). In this study, it was understood that six *flaA*-positive *C. jejuni* isolates had weak or moderate biofilm ability. Although our study only covers chicken samples offered for retail sale, the presence of *C. jejuni* isolates with biofilm characteristics in chicken slaughter units and equipment, and thus the possibility of cross-contamination poses a risk to public health.

Although various typing methods (PFGE, Rep-PCR, MLST, RFLP) have been used in different studies to reveal *Campylobacter* spp. genetic relatedness (Abay *et al*., 2014; Aydin *et al*., 2023), ERIC-PCR was preferred in this study due to its ease of use, economic cost, and the fact that it can be performed even by simple microbiology laboratories without the need for additional software and/or hardware (Ranjbar *et al*., 2017). Concerning cluster analysis based on ERIC-PCR results, in spite of their common ancestry, raw chicken *C. jejuni* isolates exhibited clonal diversity and heterogeneous populations. Similar to the study conducted, some authors (Wieczorek, 2009; Ahmed *et al*., 2015; Staji *et al*., 2018) found genetic heterogeneity among isolates in their studies investigating genetic similarity using ERIC-PCR.

In the analysis of antibiotic resistance in WGS, *blaOXA*-61, *tetO*/32/O and *ant*(6)-la resistance genes were found. Similarly, these genes were found in a study conducted by Chen *et al*. (2020). Investigations showed that the isolate has a 91.9% probability of being a human pathogen, indicating that the organism has a very high potential to cause human diseases.

Conclusion

C. jejuni, forming biofilms on chicken meat surfaces, has virulence properties and is resistant to antibiotics. It has been detected in raw chicken meat as an important cause of gastroenteritis, strict hygiene practices should be followed during processing, and awareness should be raised among consumers and food processors. Besides, effective cooking and processing can render chicken meat contaminated with *Campylobacter* species safe. Therefore, we should make sure that the poultry meat consumed is sufficiently cooked.

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Author contributions

Harun Hizlisoy: Methodology; project administration; writing – original draft. **Mukaddes Barel:** Methodology; project administration; writing – original draft. **Adalet Dishan:** Writing – original draft. **Serhat Al:** Writing – original draft. **Candan Candemir:** Writing – original draft. **Kursat Koskeroglu:** Writing – original draft. **H. Burak Disli:** Writing – original draft. **Serhat Hizlisoy:** Writing – original draft. **Nurhan Ertas Onmaz:** Writing – original draft. **Yeliz Yildirim:** Writing – original draft. **Zafer Gonulalan:** Writing – original draft. **K. Semih Gumussoy:** Writing – original draft.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Conflict of interest statement

The authors declare that they have no competing interests.

Peer review

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Data availability statement

The data supporting our findings, such as targeted sequencing data is available on NCBI (https://blast.ncbi.nlm.nih.gov).

References

Abay, S., Kayman, T., Otlu, B., Hizlisoy, H., Aydin, F. & Ertas, N. (2014). Genetic diversity and antibiotic resistance profiles of *Campylobacter jejuni* isolates from poultry and humans in Turkey. *International Journal of Food Microbiology*, **178**, 29–38.

Abebe, E., Gugsa, G. & Ahmed, M. (2020). Review on major food-borne zoonotic bacterial pathogens. *Journal of Tropical Medicine*, **2020**, 1–19.

Ahmed, H.A., El Hofy, F.I., Ammar, A.M., Abd El Tawab, A.A. & Hefny, A.A. (2015). ERIC-PCR genotyping of some *Campylobacter jejuni* isolates of chicken and human origin in Egypt. *Vector-Borne and Zoonotic Diseases*, **15**, 713–717.

Alobaidy, H.A.K., Ayvaz, A., Sağiroğlu, P. & Atalay, M.A. (2024). Diagnosis of *Campylobacter* spp. isolates and their antimicrobial susceptibility patterns. *Indian Journal of Microbiology*, https://doi.org/10.1007/s12088-024-01199-5

Alonso, R., Mateo, E., Churruca, E., Martinez, I., Girbau, C. & Fernández-Astorga, A. (2005). MAMA-PCR assay for the detection of point mutations associated with high-level erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli* strains. *Journal of Microbiological Methods*, **63**, 99–103.

Ammar, A.M., Abd El-Hamid, M.I., El-Malt, R.M. *et al.* (2021). Molecular detection of fluoroquinolone resistance among multidrug-, extensively drug-, and pan-drug-resistant *Campylobacter* species in Egypt. *Antibiotics*, **10**, 1342.

Aslantaş, Ö. (2019). Isolation and molecular characterization of thermophilic *Campylobacter* spp. in dogs and cats. https://doi.org/10.9775/kvfd.2018.20952

Aydin, F., Kayman, T., Abay, S. *et al.* (2023). MLST genotypes and quinolone resistance profiles of *Campylobacter jejuni* isolates from various sources in Turkey. *International Journal of Food Microbiology*, **391**, 110137.

Bae, J. & Jeon, B. (2013). Increased emergence of fluoroquinolone-resistant *Campylobacter Jejuni* in biofilm. *Antimicrobial Agents and Chemotherapy*, **57**, 5195–5196.

Bankevich, A., Nurk, S., Antipov, D. *et al.* (2012). SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology*, **19**, 455–477.

Barbut, S. & Leishman, E.M. (2022). Quality and processability of modern poultry meat. In: *Animals*. Pp. 2766, Vol. **12**. Basel, Switzerland: MDPI AG.

Brown, H.L., Reuter, M., Salt, L.J., Cross, K.L., Betts, R.P. & van Vliet, A.H. (2014). Chicken juice enhances surface attachment and biofilm formation of *Campylobacter jejuni*. *Applied and Environmental Microbiology*, **80**, 7053–7060.

Büyükünal, S.K. (2017). İstanbul'da satışa sunulan piliç etlerinde termotolerant *Campylobacter* spp. prevalansı ve antibiyotik dirençliliği. *İstanbul Üniversitesi Veteriner Fakültesi Dergisi*, **43**, 98–109.

Casagrande Proietti P, Guelfi G, Bellucci S, De Luca S, Di Gregorio S, Pieramati C, Franciosini MP (2020). Beta-lactam resistance in *Campylobacter coli* and *Campylobacter jejuni* chicken isolates and the association between blaOXA-61 gene expression and the action of β-lactamase inhibitors. *Veterinary Microbiology*, **241**:108553.

This reference was utilised for information about a study conducted for *C. jejuni* worldwide. This study was cited for information about the ampicillin resistance of *C. jejuni* isolates.

Chen, C.Y., Clark, C.G., Langner, S. *et al.* (2020). Detection of antimicrobial resistance using proteomics and the comprehensive antibiotic resistance database: a case study. *Proteomics. Clinical Applications*, **14**, 1800182.

CLSI (2022). *CLSI, M100-Performance Standards for Antimicrobial Susceptibility Testing*, 28th edn. Clinical and Laboratory Standards Institute: Wayne, PA.

Cokal, Y., Caner, V., Sen, A., Cetin, C. & Karagenc, N. (2009). *Campylobacter* spp. and their antimicrobial resistance patterns in poultry: an epidemiological survey study in Turkey. *Zoonoses and Public Health*, **56**, 105–110.

Dasti, J.I., Groß, U., Pohl, S., Lugert, R., Weig, M. & Schmidt-Ott, R. (2007). Role of the plasmid-encoded tet (O) gene in tetracycline-resistant clinical isolates of *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Medical Microbiology*, **56**, 833–837.

Dramé, O., Leclair, D., Parmley, E.J. *et al.* (2020). Antimicrobial resistance of *Campylobacter* in broiler chicken along the food chain in Canada. *Foodborne Pathogens and Disease*, **17**, 512–520.

Elhadidy, M., Ali, M.M., El-Shibiny, A. *et al.* (2020). Antimicrobial resistance patterns and molecular resistance markers of *Campylobacter jejuni* isolates from human diarrheal cases. *PLoS One*, **15**, e0227833.

Engberg, J., Aarestrup, F.M., Taylor, D.E., Gerner-Smidt, P. & Nachamkin, I. (2001). Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. Coli*: resistance mechanisms and trends in human isolates. *Emerging Infectious Diseases*, **7**, 24–34.

Gahamanyi, N., Song, D.G., Yoon, K.Y. *et al.* (2021). Antimicrobial resistance profiles, virulence genes, and genetic diversity of thermophilic *Campylobacter* species isolated from a layer poultry farm in Korea. *Frontiers in Microbiology*, **12**, 622275.

Gerace, E., Mancuso, G., Midiri, A., Poidomani, S., Zummo, S. & Biondo, C. (2022). Recent advances in the use of molecular methods for the diagnosis of bacterial infections. *Pathogens*, **11**, 663.

Gharbi, M., Béjaoui, A., Hamda, C.B., Ghedira, K., Ghram, A. & Maaroufi, A. (2022). Distribution of virulence and antibiotic resistance genes in *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler chickens in Tunisia. *Journal of Microbiology, Immunology and Infection*, **55**, 1273–1282.

Gibreel, A., Tracz, D.M., Nonaka, L., Ngo, T.M., Connell, S.R. & Taylor, D.E. (2004). Incidence of antibiotic resistance in *Campylobacter jejuni* isolated in Alberta, Canada, from 1999 to 2002, with special reference to tet (O)-mediated tetracycline resistance. *Antimicrobial Agents and Chemotherapy*, **48**, 3442–3450.

Gurevich, A., Saveliev, V., Vyahhi, N. & Tesler, G. (2013). QUAST: quality assessment tool for genome assemblies. *Bioinformatics*, **29**, 1072–1075.

Guyard-Nicodème, M., Rivoal, K., Houard, E. *et al.* (2015). Prevalence and characterization of *Campylobacter jejuni* from chicken meat sold in French retail outlets. *International Journal of Food Microbiology*, **203**, 8–14.

Hedayatianfard, K., Akhlaghi, M. & Sharifiyazdi, H. (2014). Detection of tetracycline resistance genes in bacteria isolated from fish farms using polymerase chain reaction. In: *Veterinary Research Forum*. Pp. 269, Vol. **5**. Urmia, Iran: Faculty of Veterinary Medicine, *Urmia Undiversity*.

Hizlisoy, H., Al, S., Yıldırım, Y. *et al.* (2020). Virulence genes, antibiotic susceptibility profiles and molecular characterization of *Campylobacter* species isolated from different slaughterhouses. *Mikrobiyoloji Bülteni*, **54**, 11–25.

Hizlisoy, H., Sagiroglu, P., Barel, M. *et al.* (2023). *Campylobacter jejuni* and *Campylobacter coli* in human stool samples: antibiotic resistance profiles, putative virulence determinants and molecular characterization of the isolates. *World Journal of Microbiology and Biotechnology*, **39**, 353.

Houf, K., De Zutter, L., Van Hoof, J. & Vandamme, P. (2002). Assessment of the genetic diversity among arcobacters isolated from poultry products by using two PCR-based typing methods. *Applied and Environmental Microbiology*, **68**, 2172–2178.

Iovine, N.M. (2013). Resistance mechanisms in *Campylobacter jejuni*. *Virulence*, **4**, 230–240.

ISO 10272 (1995). *Microbiology of food and animal feeding stuffs-horizontal method for detection of thermotolerant Campylobacter*. Geneva, Switzerland: The International Organization for Standardization.

Jeon, B., Muraoka, W., Sahin, O. & Zhang, Q. (2008). Role of Cj1211 in natural transformation and transfer of antibiotic resistance determinants in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy*, **52**, 2699–2708.

Kaakoush, N.O., Castaño-Rodríguez, N., Mitchell, H.M. & Man, S.M. (2015). Global epidemiology of *Campylobacter* infection. *Clinical Microbiology Reviews*, **28**, 687–720.

Kayman, T., Abay, S., Aydin, F. & Şahin, O. (2019). Antibiotic resistance of *Campylobacter jejuni* isolates recovered from humans with diarrhoea in Turkey. *Journal of Medical Microbiology*, **68**, 136–142.

This reference was cited for general information about the campylobacters. This reference was used for being a novel study conducted in Turkiye.

Kim, J.C., Oh, E., Kim, J. & Jeon, B. (2015). Regulation of oxidative stress resistance in *Campylobacter jejuni*, a microaerophilic foodborne pathogen. *Frontiers in Microbiology*, **6**, 751.

Klančnik, A., Šimunović, K., Sterniša, M., Ramić, D., Smole Možina, S. & Bucar, F. (2021). Anti-adhesion activity of phytochemicals to prevent *Campylobacter jejuni* biofilm formation on abiotic surfaces. *Phytochemistry Reviews*, **20**, 55–84.

Krueger, F. (2019). trim galore: a wrapper tool around Cutadapt and FastQC to consistently apply quality and adapter trimming to FastQ files. Available from: https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/ [Accessed March 2023]

Lapierre, L., Gatica, M.A., Riquelme, V. *et al.* (2016). Characterization of antimicrobial susceptibility and its association with virulence genes related to adherence, invasion, and cytotoxicity in *Campylobacter jejuni* and *Campylobacter coli* isolates from animals, meat, and humans. *Microbial Drug Resistance*, **22**, 432–444.

Larsen, M.V., Cosentino, S., Rasmussen, S. *et al.* (2012). Multilocus sequence typing of total-genome-sequenced bacteria. *Journal of Clinical Microbiology*, **50**, 1355–1361.

Letunic, I. & Bork, P. (2019). Interactive tree of life (iTOL) v4: recent updates and new developments. *Nucleic Acids Research*, **47** (W1), W256–W259.

Lim, S.K., Moon, D.C., Chae, M.H. *et al.* (2016). Macrolide resistance mechanisms and virulence factors in erythromycin-resistant *Campylobacter* species isolated from chicken and swine feces and carcasses. *Journal of Veterinary Medical Science*, **78**, 1791–1795.

Liu, B., Zheng, D., Jin, Q., Chen, L. & Yang, J. (2019). VFDB 2019: a comparative pathogenomic platform with an interactive web interface. *Nucleic Acids Research*, **47**(D1), D687–D692.

Lluque, A., Riveros, M., Prada, A., Ochoa, T.J. & Ruiz, J. (2017). Virulence and antimicrobial resistance in *Campylobacter* spp. from a Peruvian pediatric cohort. *Scientifica*, **2017**, 2017.

Mikulić, M., Humski, A., Njari, B., Ostović, M., Duvnjak, S. & Cvetnić, Ž. (2016). Prevalence of thermotolerant *Campylobacter* spp. in chicken meat in Croatia and multilocus sequence typing of a small subset of *Campylobacter jejuni* and *Campylobacter coli* isolates. *Food Technology and Biotechnology*, **54**, 475–481.

Mousavi, S., Schmidt, A.M., Escher, U. *et al.* (2020). Carvacrol ameliorates acute campylobacteriosis in a clinical murine infection model. *Gut Pathogens*, **12**, 1–16.

Nowaczek, A., Urban-Chmiel, R., Dec, M. *et al.* (2019). *Campylobacter* spp. and bacteriophages from broiler chickens: characterization of antibiotic susceptibility profiles and lytic bacteriophages. *Microbiology Open*, **8**, e00784.

Obeng, A.S., Rickard, H., Sexton, M., Pang, Y., Peng, H. & Barton, M. (2012). Antimicrobial susceptibilities and resistance genes in *Campylobacter* strains isolated from poultry and pigs in Australia. *Journal of Applied Microbiology*, **113**, 294–307.

Ohadi, E., Bakhshi, B., Kalani, B.S., Talebi, M. & Irajian, G. (2022). Transcriptome analysis of biofilm formation under aerobic and microaerobic conditions in clinical isolates of *Campylobacter* spp. *Research in Veterinary Science*, **142**, 24–30.

Oyarzabal, O.A., Backert, S., Williams, L.L. *et al.* (2008). Molecular typing, serotyping and cytotoxicity testing of *Campylobacter jejuni* strains isolated from commercial broilers in Puerto Rico. *Journal of Applied Microbiology*, **105**, 800–812.

Quino, W., Caro-Castro, J., Hurtado, V., Flores-León, D., Gonzalez-Escalona, N. & Gavilan, R.G. (2022). Genomic analysis and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* in Peru. *Frontiers in Microbiology*, **12**, 4181.

Ranjbar, R., Tabatabaee, A., Behzadi, P. & Kheiri, R. (2017). Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) genotyping of *Escherichia coli* strains isolated from different animal stool specimens. *Iranian Journal of Pathology*, **12**, 25. PMID: 29760750; PMCID: PMC5938721–34.

This reference was used for general information about the ERIC-PCR. This reference was cited for giving information about some advantages of using ERIC-PCR.

Salem, A., Abou El Roos, N. & Nassar, Y. (2019). Molecular and conventional methods for detection of *Campylobacter* spp. in retail poultry cuts, offal and chicken products. *Benha Veterinary Medical Journal*, **36**, 71–76.

Santos-Ferreira, N., Alves, Â., Cardoso, M.J. *et al.* (2021). Cross-contamination of lettuce with *Campylobacter* spp. via cooking salt during handling raw poultry. *PLoS One*, **16**, e0250980.

Seemann, T. (2014). Prokka: rapid prokaryotic genome annotation. *Bioinformatics*, **30**, 2068–2069.

Sierra-Arguello, Y.M., Morgan, R.B., Perdoncini, G., Lima, L.M., Gomes, M.J.P. & Nascimento, V.P.D. (2015). Resistance to β-lactam and tetracycline in *Campylobacter* spp. isolated from broiler slaughterhouses in southern Brazil. *Pesquisa Veterinaria Brasileira*, **35**, 637–642.

Staji, H., Birgani, S.F. & Raeisian, B. (2018). Comparative clustering and genotyping of *Campylobacter jejuni* strains isolated from broiler and Turkey feces by using RAPD-PCR and ERIC-PCR analysis. *Annals of Microbiology*, **68**, 755–762.

Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, **30**, 1312–1313.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008). A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, **57**, 758–771.

Stepanović, S., Vuković, D., Dakić, I., Savić, B. & Švabić-Vlahović, M. (2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *Journal of Microbiological Methods*, **40**, 175–179.

Taylor, D.E. & Tracz, D.M. (2005). *Mechanisms of Antimicrobial Resistance in Campylobacter*. *Campylobacter*: Molecular and Cellular Biology. Pp. 193–204. Norfolk: Horizon Bioscience.

Thames, H.T. & Theradiyil Sukumaran, A. (2020). A review of salmonella and *Campylobacter* in broiler meat: emerging challenges and food safety measures. *Food*, **9**, 776.

Tram, G., Day, C.J. & Korolik, V. (2020). Bridging the gap: a role for *Campylobacter jejuni* biofilms. *Microorganisms*, **8**, 452.

Wang, G., Clark, C.G., Taylor, T.M. *et al.* (2002). Colony multiplex PCR assay for identification and differentiation of *Campylobacter jejuni, C. Coli, C. Lari, C. Upsaliensis*, and *C. Fetus* subsp. *fetus*. *Journal of Clinical Microbiology*, **40**, 4744–4747.

Wang, J., Wang, Z., Zhang, J. *et al.* (2021). Prevalence, antibiotic susceptibility and genetic diversity of *Campylobacter jejuni* isolated from retail food in China. *LWT – Food Science & Technology*, **143**, 111098.

This reference was cited for the suggestion of the reviewers. This reference was cited for the general information about *C. jejuni*.

Wieczorek, K. (2009). Relationship between the molecular typing of *Campylobacter* strains and the prevalence of their virulence genes. *Bulletin of the Veterinary Institute in Puławy*, **53**, 193–198.

Wirz, S.E., Overesch, G., Kuhnert, P. & Korczak, B.M. (2010). Genotype and antibiotic resistance analyses of *Campylobacter* isolates from ceca and carcasses of slaughtered broiler flocks. *Applied and Environmental Microbiology*, **76**, 6377–6386.

Yildirim, M., İstanbulluoğlu, E. & Ayvali, B. (2005). Prevalence and antibiotic susceptibility of thermophilic *Campylobacter* species in broiler chickens. *Turkish Journal of Veterinary and Animal Sciences*, **29**, 655–660.

Zhang, X., Zhou, Q., Tang, M. *et al.* (2021). Aminoglycoside resistance and possible mechanisms in *Campylobacter* spp. isolated from chicken and swine in Jiangsu, China. *Frontiers in Microbiology*, **12**, 716185.

Zhong, X., Wu, Q., Zhang, J. *et al.* (2020). *Campylobacter jejuni* biofilm formation under aerobic conditions and inhibition by ZnO nanoparticles. *Frontiers in Microbiology*, **11**, 207.

Zirnstein, G., Li, Y., Swaminathan, B. & Angulo, F. (1999). Ciprofloxacin resistance in *Campylobacter jejuni* isolates: detection of *gyrA* resistance mutations by mismatch amplification mutation assay PCR and DNA sequence analysis. *Journal of Clinical Microbiology*, **37**, 3276–3280.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. Detailed virulence profiles of H22 isolate.

