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RESEARCH ARTICLE

Genetic Relatedness and Molecular Characterization of Multidrug-Resistant *Campylobacter jejuni* Isolated from Humans and Poultry in Korea

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ABSTRACT

The widespread use of antimicrobials in poultry production has led to the emergence of multidrug-resistant (MDR) *Campylobacter jejuni* (*C. jejuni*), which can be directly transmitted from poultry to humans. This study aimed to investigate the antimicrobial resistance patterns and genetic relatedness of MDR *C. jejuni* isolates obtained from human and poultry using whole-genome sequencing (WGS). A total of 154 *C. jejuni* isolates were obtained, including 100 isolates from human samples and 54 from poultry samples. Antimicrobial susceptibility testing was performed using minimum inhibitory concentrations. We conducted WGS for MDR *C. jejuni* isolates using Oxford Nanopore platforms, followed by multiple bioinformatics analyses. Multilocus sequence typing profiles were used to characterize the genomes. Among all 154 *C. jejuni* isolates, high resistance rates were observed for ciprofloxacin (93.5%; 144/154), nalidixic acid (93.5%; 144/154), and tetracycline (40.9%; 63/154). Based on the MDR definition, 47 isolates were classified as MDR and selected for further genomic analysis. The most frequently observed MDR profile was resistance to fluoroquinolones, quinolones, and tetracyclines with human isolates exhibiting greater variability in their resistance patterns. The clonal complexes (CC) identified as overlapping in both poultry and human isolates included CC-21 and CC-45. Nine pTet-like plasmids were identified, all of which contained analogous sets of genes and a complete type IV secretion system. Whole-genome analysis of MDR *C. jejuni* strains isolated from humans and poultry revealed both genetic diversity and conserved genomic features, highlighting their evolutionary relatedness and potential for zoonotic transmission. Detection of plasmids carrying both antimicrobial resistance and virulence genes underscores the need for heightened surveillance to prevent zoonotic transmission.

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INTRODUCTION

Campylobacter species are among the most important intestinal pathogens and constitute the primary etiological agents of acute bacterial gastroenteritis in humans worldwide (Facciolà, 2017). Among *Campylobacter* species, *Campylobacter jejuni* (*C. jejuni*) is the pathogenic species most frequently implicated in human infections, often associated with diarrhea, fever, and abdominal pain (Kim *et al.*, 2019). The natural reservoir of *Campylobacter* is poultry, with humans becoming infected primarily through the handling and consumption of contaminated poultry meat (García-Fernández *et al.*, 2025).

A wide range of antimicrobials are used in veterinary medicine for both therapeutic and prophylactic purposes (Enshaie *et al.*, 2025). The misuse of these antimicrobials has accelerated the emergence of antimicrobial-resistant bacteria, especially multidrug-resistant (MDR) *Campylobacter* (Sharifi, Bakhshi, and Najar-peerayeh, 2021). The emergence of MDR *Campylobacter* constitutes a significant public health issue given the zoonotic nature of the pathogen and its potential for transmission through the

food chain (Cooper *et al.*, 2024; Sharifi, Bakhshi, and Najar-peerayeh, 2021). Therefore, numerous countries are continuously monitoring and investigating antimicrobial resistance (AMR) in pathogenic bacteria at a national level (Delpy *et al.*, 2024).

Campylobacter is commonly isolated from a variety of food-producing animals, with poultry regarded as the primary reservoir (García-Fernández *et al.*, 2025). It has been detected in over 40% of retail chicken samples, and chickens have been implicated in 65% of human *Campylobacter* enteritis cases with a known etiology (Cho *et al.*, 2023). In addition to chicken, *Campylobacter* has also been frequently detected in duck meat, with a recent meta-analysis in Korea reporting a prevalence of 70.46% in duck meat (Je *et al.*, 2023). Poultry meat and internal organs have been identified as sources of *Campylobacter* contamination in various countries, and human infections have been linked to the consumption of such products (Manzanares-Pedrosa *et al.*, 2025). In Korea, *Campylobacter* has been recognized as one of the leading foodborne pathogens over the past decade, underscoring its continued public health importance (Je *et al.*, 2023).

Campylobacter exhibits substantial genetic diversity because of frequent recombination events that confer various virulence and adaptation traits (Epping, Antão, and Semmler, 2021; Wilson *et al.*, 2009). Additionally, horizontal gene transfer can lead to the acquisition of virulence and AMR determinants (Costa and Iraola, 2019; Zhao *et al.*, 2025), with some elements occasionally integrated into the chromosome (Crespo *et al.*, 2012). Considering these factors, MDR *C. jejuni* strains can be transmitted directly to humans, and elucidating their reservoirs and transmission routes is essential for understanding their epidemiology (Truccollo *et al.*, 2021). Although several studies have investigated the distribution of AMR in *Campylobacter* isolates from either poultry or human (Gahamanyi *et al.*, 2021; Kim *et al.*, 2024), studies concurrently investigating MDR *C. jejuni* isolates from both human and poultry in Korea remain limited. Accordingly, this study aimed to examine the AMR patterns and genetic relatedness of MDR *C. jejuni* isolates obtained from human and poultry sources using whole-genome sequencing (WGS).

MATERIALS AND METHODS

Sample collection: The collection of human fecal samples was exempted from review by the Institutional Review Board (IRB) of Seegene Medical Foundation, with the approval number SMF-IRB-2022-027. A total of 100 human-derived *C. jejuni* isolates were obtained from fecal samples submitted for diagnostic testing at hospitals across Korea from 2022 to 2023. The isolates were derived from sporadic enteritis cases, and only one isolate per patient was included in the analysis. Fifty-four *C. jejuni* (34 from chicken meat and 20 from duck meat) were isolated from 165 chicken and 85 duck meat samples collected from retail stores across six regions in Korea: Seoul, Gyeonggi, Chungcheong, Gyeongsang, Jeolla and Gangwon. Antimicrobial susceptibility testing was performed for all 154 *C. jejuni* isolates. All poultry meat samples were domestically produced and collected from 2023 to 2024.

***Campylobacter* spp. isolation and identification:** Human fecal samples were enriched in Preston *Campylobacter* selective enrichment broth (Oxoid, UK) supplemented with horse blood and selective additives, followed by incubation at 42°C for 48h under microaerophilic conditions. Enriched cultures were streaked onto modified charcoal cefoperazone deoxycholate agar (mCCDA; Oxoid, UK) and incubated at 42°C for 48h. Chicken and duck meat samples were homogenized in buffered peptone water, enriched in Preston broth at 42°C under microaerophilic conditions, and subsequently plated onto mCCDA. Presumptive *Campylobacter* isolates were identified using MALDI Biotyper® (Bruker, USA).

Antimicrobial resistance testing: All *C. jejuni* isolates were investigated for AMR by determining the minimum inhibitory concentrations of eight antimicrobial agents using a commercial microdilution tool, following the manufacturer's instructions. The antimicrobials tested included macrolides (MACs; azithromycin and erythromycin), quinolones (QNs; nalidixic acid), fluoroquinolones (FQs; ciprofloxacin), phenicols (PHs; florfenicol), tetracyclines (TETs; tetracycline), aminoglycosides (gentamicin), and lincosamides (LINCs; clindamycin). The susceptibility results were interpreted based on the epidemiological cutoff values provided by the U.S. National Antimicrobial Resistance Monitoring System (NARMS) for *Campylobacter* isolates (https://www.cdc.gov/narms/antibiotics-tested.html; accessed on 30 November 2024) (Kim *et al.*, 2024). Resistance was determined according to the NARMS 2019 recommendations for *C. jejuni*. In addition, the isolates that exhibited resistance to three or more antimicrobial groups were considered MDR (Magiorakos *et al.*, 2012). Among the 154 *C. jejuni* isolates tested for antimicrobial susceptibility, 47 isolates, including 24 human-derived and 23 poultry-derived isolates, were identified as MDR. All recovered MDR isolates were selected for WGS.

Detection of virulence factors: Virulence factors were detected by PCR using gene-specific primer sets. Genomic DNA was extracted from *C. jejuni* isolates using the conventional boiling method and used as the PCR template. The presence of virulence genes, including *cdtA*, *cdtB*, *cdtC*, *cadF*, *dnaJ*, *ciaB*, and *pldA*, was examined according to previously described PCR protocols (Talukder *et al.*, 2008).

Biofilm assay: The biofilm assay was performed as previously described (Zhang *et al.*, 2017), with minor modifications. Overnight cultures were adjusted to an OD_{600} of 0.1 in Brucella medium supplemented with 10% defibrinated sheep blood, transferred to 24-well polystyrene plates, and incubated at 42°C under microaerophilic conditions for 48h. Biofilms were stained with 0.5% crystal violet, eluted with an alcohol/acetone solution (80:20, v/v), and quantified by measuring absorbance at OD_{590}. Biofilm-forming ability was classified as non-, weak, moderate, or strong based on ODcut values, as previously defined (Islam *et al.*, 2023).

Whole-genome sequencing: Genomic DNA of the 47 MDR isolates was extracted using the DNeasy Blood &

Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. WGS was performed using a Nanopore MinION platform (Oxford Nanopore Technologies, UK). Long-read libraries were prepared using the Native Barcoding Kit 96 V14 (Oxford Nanopore Technologies), according to the manufacturer's instructions. Long-read sequencing was performed on an Mk1B MinION platform with a FLO-MIN114 flow cell using MinKNOW v24.11.10 (Oxford Nanopore).

Bioinformatic analysis: Reads were filtered using FiltLong v0.2.1 and Porechop v0.2.1, and *de novo* assembly was performed using Flye v2.9 with default settings optimized for long-read data (Kolmogorov *et al.*, 2019). The assembly N50 value was 8.06 kb. Genome annotation was performed using Prokka v1.14.5 (Seemann, 2014). Annotated GFF3 files were used for pangenome analysis with Roary v3.13.0 (Page *et al.*, 2015). ABRicate v0.8.10 was used in combination with ResFinder v2.1.1 (https://cge.food.dtu.dk/services/ResFinder/, accessed on 25 April 2025). AMR profiles, virulence factors, and biofilm phenotypes were visualized using the iTOL software (v7) (https://itol.embl.de/, accessed on 1 May 2025). Comparative genomic analysis was conducted using EasyFig v2.2.5 (Sullivan, Petty, and Beatson, 2011).

To investigate the genetic basis underlying biofilm formation, biofilm phenotypes were integrated with pan-genome data for association analysis. Biofilm phenotypes were converted into binary traits (biofilm-forming=1, non-biofilm-forming=0) and used as input for Scoary (https://github.com/AdmiralenOla/Scoary, accessed on 15 November 2025). Gene presence-absence data obtained from the pan-genome analysis were used to assess associations between genes and biofilm phenotypes using Fisher's exact test. The odds ratio (OR) was used as an effect size measure, and genes with an OR>1 and a naive P-value<0.05 were selected as candidate genes showing potential positive associations with biofilm formation. Selected candidate genes were functionally annotated using eggNOG-mapper v2 (http://eggnog-mapper.embl.de/, accessed on 28 November 2025) and further classified based on KEGG pathway annotations (https://www.kegg.jp/kegg/mapper/reconstruct.html, accessed on 28 November 2025). All MDR *C. jejuni* genomic sequences were compared against the *Campylobacter jejuni* PubMLST database to assign sequence types (ST) and clonal complexes (CC), with novel alleles and STs submitted to the database when identified. Phylogenetic relationships were inferred using PhyloViz v2.0 (https://www.phyloviz.net/, accessed on 1 April 2025).

RESULTS

Antimicrobial susceptibilities of *C. jejuni* isolates from humans and poultry: The overall isolation rate of *C. jejuni* from poultry was 21.6% (54/250), with detection rates of 20.6% (34/165) in chicken meat and 23.5% (20/85) in duck meat. A high prevalence of resistance to ciprofloxacin, nalidixic acid, and TET was observed among the overall isolates. Among the 100 human-derived *C. jejuni* isolates, resistance to nalidixic acid (92.0%; 92/100), ciprofloxacin (92.0%; 92/100), and TET (32.0%; 32/100) was observed. Among the 54 poultry-derived *C. jejuni* isolates, resistance to nalidixic acid (96.3%; 52/54), ciprofloxacin (96.3%; 52/54), and TET (57.4%; 31/54) was observed. Of the total 154 *C. jejuni* isolates, 47 (24 from humans and 23 from poultry) were classified as MDR, showing resistance to 3-5 classes of antimicrobial agents (Table 1). All 47 recovered MDR isolates were subsequently subjected to WGS.

Table 1: Antimicrobial resistance class pattern distribution for 47 MDR *C. jejuni* isolates from humans and poultry.

No. of classes	Antimicrobial resistance class pattern[1]	No. (%) of isolates		
		Human (n=24)	Poultry (n=23)	Total (n=47)
3	FQs-QNs-TETs	20 (83.3)	23 (100.0)	43 (91.4)
3	FQs-MACs-QNs	1 (4.2)	0 (0.0)	1 (2.1)
4	FQs-PHs-QNs-TETs	2 (8.3)	0 (0.0)	2 (4.3)
5	FQs-LINCs-MACs-QNs-TETs	1 (4.2)	0 (0.0)	1 (2.1)

[1]FQs, Fluoroquinolones; LINCs, Lincosamides; MACs, Macrolides; PHs, Phenicols; QNs, Quinolones; TETs, Tetracyclines.

MLST of multidrug-resistant *C. jejuni* from humans and poultry: Nineteen STs were identified among the 47 MDR *C. jejuni* isolates obtained in this study (Table 2). These STs were subsequently classified into 10 distinct CCs, with the most prevalent CC being CC-21 (n=13; 27.7%). ST-12630, belonging to CC-354, was the most frequent ST identified in this study (n = 7; 14.9%). ST-50 (n=4, 8.5%) was the most prevalent MLST type among human-derived isolates, whereas ST-12630 (n=6, 12.8%) predominated in isolates derived from poultry meat. The STs identified as overlapping in both poultry and human-derived isolates included ST-45, ST-298, ST-572, ST-3456, ST-4253, and ST-12630. ST-14790, a novel ST identified in this study, was detected in two poultry-derived MDR *C. jejuni* isolates and was not assigned to any known CC. These isolates exhibited the FQs-QNs-TETs multidrug resistance pattern. Both ST-14790 isolates harbored the *gyrA* (T86I) mutation associated with fluoroquinolone resistance and carried bla_{OXA}-465. Notably, bla_{OXA}-465 was detected only in these two isolates among the strains analyzed in this study.

As shown in Fig. 1, the minimum spanning tree was generated based on the 7-locus MLST profiles of MDR *C. jejuni* isolates derived from human and poultry sources. CC-21 and CC-45 formed major clusters containing isolates from both human and poultry.

Whole genome based antimicrobial resistance, pangenome analysis, and biofilm characterization: Whole-genome analysis of MDR *C. jejuni* isolates revealed their AMR genes, virulence gene profiles, and strain-specific biofilm phenotypes, as shown in Fig. 2. Two distinct phylogenetic clusters were observed (groups A and B). Group A exhibited genetic homogeneity with consistent resistance gene profiles, whereas group B represented a more heterogeneous cluster characterized by greater variability in resistance determinants and virulence gene content. The *gyrA* (T86I) mutation, which is associated with fluoroquinolone resistance, was detected in all FQ-resistant isolates except CBUSHCJ0008. β-lactamase genes of the

blaOXA family were highly prevalent among the isolates. TET resistance was observed in all isolates except one; however, the *tet*(O) gene was present in 58.3% (14/24) of human-derived isolates and 65.2% (15/23) of poultry-derived isolates. In total, 25 of the 47 MDR *C. jejuni* isolates (53.2%) were identified as biofilm formers, whereas 22 isolates (46.8%) were classified as non-biofilm formers. Among the biofilm-forming isolates, 21 isolates were found to be weak biofilm formers (21/47, 44.7%), and two isolates each were moderate and strong biofilm formers (2/47, 4.3%).

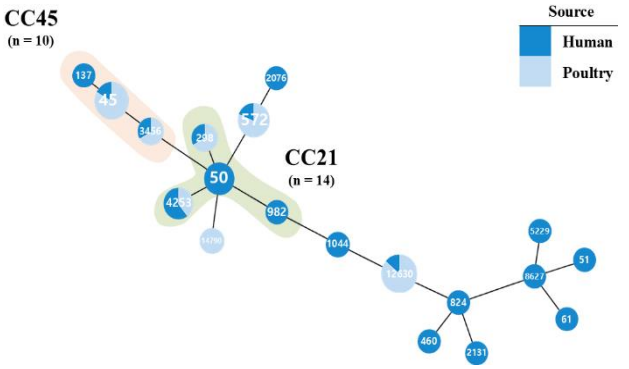

Fig. 1: Minimum spanning tree of MDR *C. jejuni* isolates (based on 7-locus MLST profiles). Each node represents one ST. The size of the node is related to the number of isolates. Branch length between nodes indicates genetic distance based on the nucleotide differences among seven housekeeping genes of *Campylobacter*. The colors of nodes represent source of *C. jejuni*: Blue, Human; light blue, Poultry. The green shaded area represents STs belonging to CC-21, whereas the orange shaded area represents STs belonging to CC-45.

Pan-genome analysis of candidate genes potentially associated with biofilm formation: The pan-genome-wide association study (Pan-GWAS) identified eight candidate genes showing potential positive associations with biofilm formation in MDR *C. jejuni* (Table 3). KEGG annotation indicated that these candidate genes were assigned to metabolic and genetic information processing pathways. Among these eight candidate genes, four encoded hypothetical proteins with no known functional annotation. Of the genes with known functions, *polA* encoded DNA polymerase I, and *hxuB* was annotated as part of a heme/hemopexin transport system. Additionally, group_273 and group_1029 were indicated to be involved in lipid metabolism and energy production, respectively.

Table 2: Distribution of sequence types and clonal complexes of 47 MDR *C. jejuni* isolates from humans and poultry.

Clonal Complex (CC)	ST[1]	No. of isolates			Total Rate
		Human (n=24)	Poultry (n=23)	Total (n=47)	
CC21 (n=13)	4253	3	2	5	27.7%
	298	1	2	3	
	50	4	-	4	
	982	1	-	1	
CC45 (n=10)	45	1	5	6	21.3%
	3456	1	2	3	
	137	1	-	1	
CC354 (n=7)	12630	1	6	7	14.9%
CC206 (n=5)	572	1	4	5	10.6%
CC658 (n=2)	1044	2	-	2	4.3%
CC61 (n=1)	61	1	-	1	2.1%
CC257 (n=1)	824	1	-	1	2.1%
CC353 (n=1)	2076	1	-	1	2.1%
CC443 (n=1)	51	1	-	1	2.1%
CC460 (n=1)	460	1	-	1	2.1%
CC464 (n=1)	8627	1	-	1	2.1%
NT[2] (n=2)	14790	-	2	2	4.3%
NT (n=1)	2131	1	-	1	2.1%
NT (n=1)	5229	1	-	1	2.1%

[1]ST, Sequence type; [2]NT, non-typable Clonal Complex.

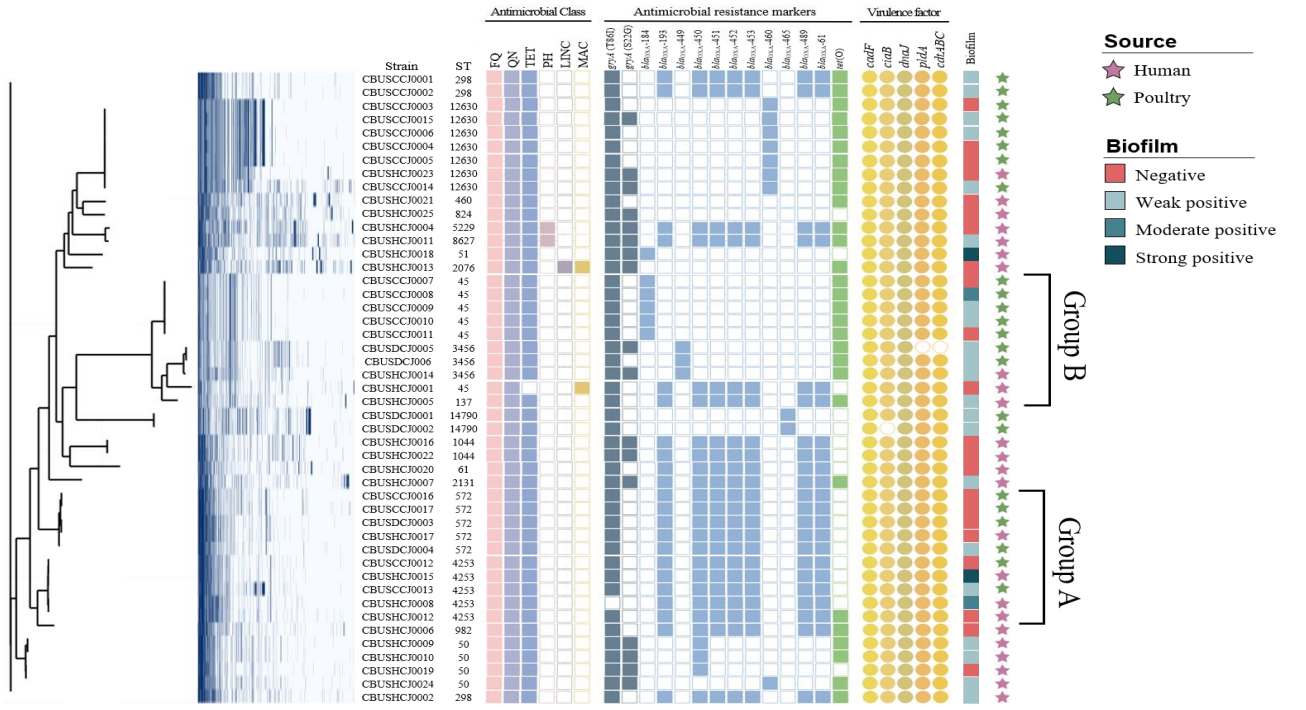

Fig. 2: Genomic epidemiology and biofilm phenotypes of 47 MDR C. jejuni isolates from humans and poultry. FQ, Fluoroquinolones; LINC, Lincosamides; MAC, Macrolides; PH, Phenicols; QN, Quinolones; TET, Tetracyclines. Two clades are designated as groups A and B. Sequence types are indicated on the labels of each isolate. The presence of antimicrobial resistance markers, virulence factors, and biofilm phenotypes is visualized using colored squares and circles.

Molecular characterization of the pTet-like plasmids: In total, 17 plasmids were identified among the 47 MDR *C. jejuni* isolates analyzed. Of the 47 MDR *C. jejuni* isolates, nine isolates carried the pTet-like plasmid (Fig. 3). Seven of the nine pTet-like plasmids contained *tet*(O). All human-derived pTet-like plasmids encoded *tet*(O), whereas the 42-kb plasmids CBUSCCJ0016 and CBUSCCJ0017, both recovered from poultry, lacked *tet*(O) but maintained most of the typical pTet-like plasmid backbone. The gene arrangement of the type IV secretion system (T4SS) was conserved across all pTet-like plasmids. The CBUSHCJ0021 plasmid included type VI secretion system (T6SS)-related genes, such as *vasB*, *vasA*, *vipB*, *vipA*, and *impA*.

Table 3: Functional annotation and KEGG pathway classification of candidate genes showing potential positive associations with biofilm formation.

Gene	Production	Function	KEGG pathway	OR[1]	Naive p-value	Bonferroni-corrected p-value
polA	DNA polymerase I	DNA replication and repair	Genetic Information Processing	4.57	0.019	1
group_27	Heme/hemopexin transporter protein *HxuB*	Intracellular trafficking and secretion	-	5.63	0.041	1
group_273	hypothetical protein	Lipid metabolism & secondary metabolite processes	Metabolism	5.85	0.015	1
group_1029	hypothetical protein	Energy production and conversion	-	4.98	0.029	1
group_69	hypothetical protein	Unknown function	-	4.74	0.019	1
group_324	hypothetical protein	Unknown function	-	5.54	0.009	1
group_1405	hypothetical protein	Unknown function	-	5.63	0.041	1
group_1976	hypothetical protein	Unknown function	-	5.63	0.041	1

[1]OR, Odds ratio

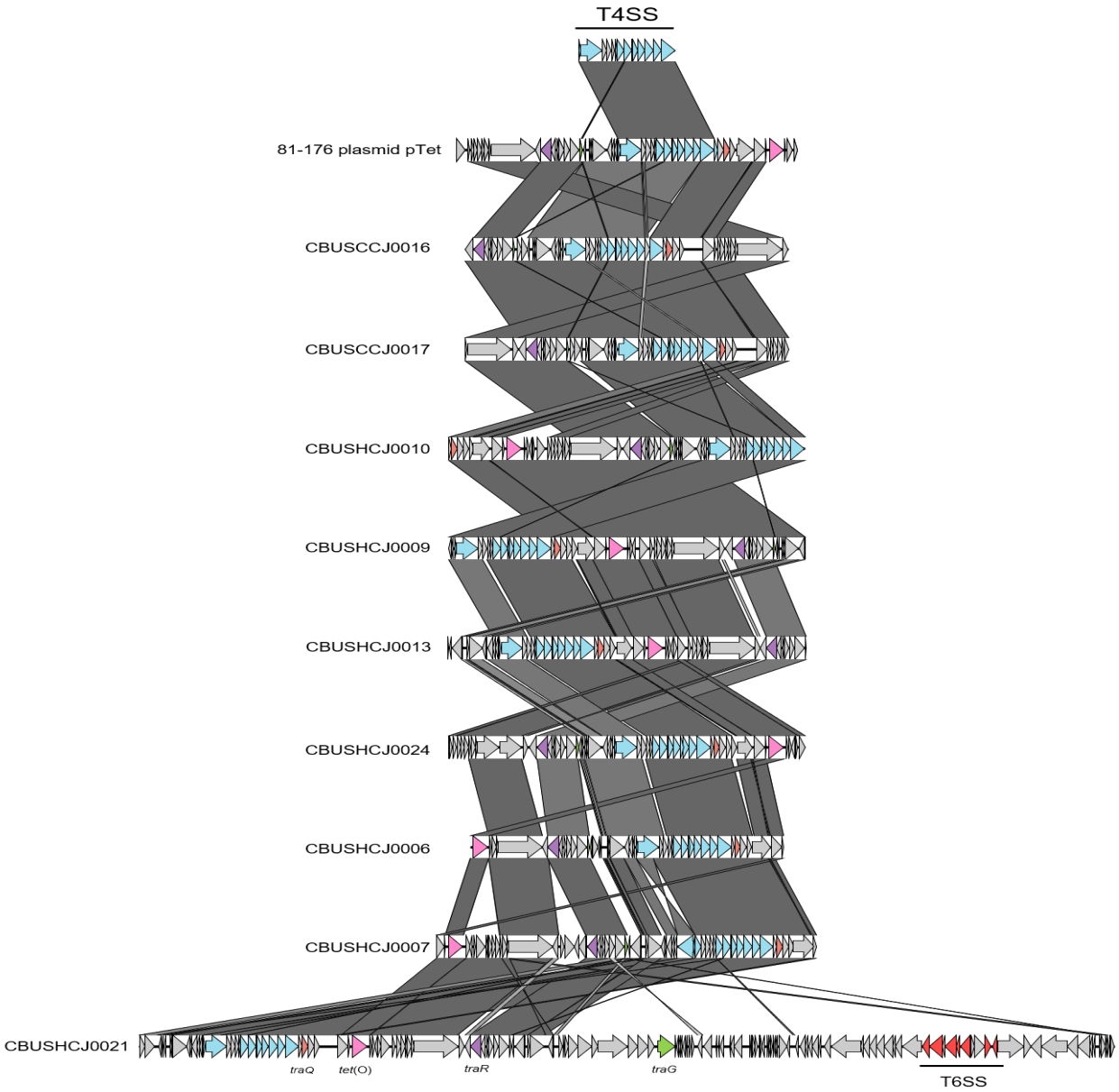

Fig. 3: Comparative alignment of pTet-like plasmids from MDR *C. jejuni* isolates from human and poultry. The pTet plasmids were used as references to the plasmid pTet from *C. jejuni* strain 81-176 (NC_008790). The *tet*(O) genes and conjugation transfer elements are shown. The T6SS regions were identified by reference to *C. jejuni* strain 14980A chromosome (CP017029.1).

DISCUSSION

C. jejuni is an important foodborne zoonotic pathogen commonly found in poultry and is capable of causing significant gastrointestinal illnesses in humans through the consumption of contaminated poultry products (García-Fernández *et al.*, 2025). In this study, a high rate of resistance to FQs and QNs was observed in all MDR *C. jejuni* isolates. Although resistance in all poultry isolates was confined to FQs, QNs and TETs, additional resistance to MACs, PHs and LINCs was observed in human isolates. Notably, the predominant MDR pattern observed in *C. jejuni* isolates was FQs-QNs-TETs, accounting for 91.4% of the total. This observation is consistent with previous reports on MDR *C. jejuni*, in which FQs-QNs-TETs were most frequently identified (Elhadidy *et al.*, 2020). Furthermore, although all poultry isolates exhibited resistance patterns to the same three classes of antimicrobials (FQs-QNs-TETs), human isolates demonstrated more diverse resistance combinations. In the Korean poultry industry, the use of antimicrobials tends to concentrate on specific classes (Lim *et al.*, 2024, 2014), whereas in human clinical settings, a wider variety of antimicrobial classes is used (Kim *et al.*, 2018). This contrast in antimicrobial exposure may explain the emergence of the more complex and diverse MDR patterns observed in human-derived *C. jejuni* isolates.

MLST is widely used for determining the genetic relatedness and population structure of bacterial isolates. Nineteen STs and eleven CCs were identified among MDR *C. jejuni* isolates from both humans and poultry in this study. The most prevalent CC among all human and poultry isolates was identified as CC-21 (27.7%), followed by CC-45 (21.3%). CC-21 is one of the most frequently identified CCs in Korea and has been commonly isolated from both human and poultry in other Asian countries (Asakura *et al.*, 2013; Zhang *et al.*, 2020; Kim *et al.*, 2024). In the present study, a considerable number of isolates belonging to the host-generalist lineages CC-21 and CC-45 were identified, suggesting that these lineages are capable of colonizing a wide range of reservoirs. This overlap between human and poultry isolates in dominant CCs is consistent with previous studies suggesting that poultry may serve as a potential transmission route for human *C. jejuni* infection (Oh *et al.*, 2017).

The minimum spanning tree based on MLST profiles (Fig. 1) showed close clustering of STs within the same CCs, suggesting genetic relatedness among them. Among the STs detected in both sources, isolates assigned to ST-45, ST-298, ST-3456, and ST-4253 formed closely grouped clusters. ST-572 and ST-12630 were also detected in both human and poultry isolates. In the MLST-based tree, ST-572 was located near other STs detected in both human and poultry isolates, whereas ST-12630 appeared to be more distantly positioned from these STs, suggesting relatively lower genetic relatedness. As in previous studies, ST-12630 was positioned relatively distantly in the phylogenetic analysis (Kim *et al.*, 2024). This suggests that, although ST-12630 was detected in both human and poultry isolates, its distinct position in the MLST-based tree may reflect a more divergent lineage rather than close genetic relatedness to other STs shared by the two sources. This may indicate a unique transmission route or a separate ecological adaptation.

Pangenome analysis provides a comprehensive view of the genetic diversity and AMR profiles of MDR *C. jejuni* isolates from both human and poultry. In this study, the isolates were divided into two groups (A and B) for comparative analysis. Group A comprised isolates belonging to ST-572 and ST-4253. Despite having different STs, these isolates clustered closely in the phylogenetic tree and exhibited highly similar profiles, including AMR genes of the same type. In Group B, one of the ST-45 isolates formed an independent cluster that was phylogenetically closer to ST-137 and ST-3456 than to other ST-45 isolates, and shared a similar resistance profile. These genomic similarities among distinct STs may reflect possible horizontal gene transfer or adaptation to similar environmental conditions. Pangenome analysis not only reveals genomic characteristics that are undetectable through MLST but also offers insights into the evolution, transmission dynamics, and AMR potential of *C. jejuni*.

TET is a widely used antimicrobial worldwide because of its low cost and ease of administration (Jahantigh *et al.*, 2020). However, sales of veterinary TET-class antimicrobials in Korea decreased between 2011 and 2020 (Kim *et al.*, 2024). Although TET is infrequently used in humans, our study showed a high overall resistance rate (97.8%) to TET among MDR *C. jejuni* isolates from both human and poultry, indicating that nearly all isolates exhibited resistance. According to domestic reports, TET resistance rates between 2009 and 2013 were 74.6% for human isolates and 81.0% for animal isolates (Cha *et al.*, 2014). These findings suggest that, although the use of TET antimicrobials has significantly decreased, resistance rates remain high. This may be attributable to the persistence of resistance genes acquired through previous misuse. Consequently, TET resistance can be maintained over extended periods even in the absence of direct selective pressure, underscoring the importance of regulating antimicrobial usage to prevent the emergence and persistence of resistance.

Overall, a high correlation was observed between phenotypic resistance to tetracycline and quinolones and the presence of resistance-associated genes or nucleotide polymorphisms (Fig. 2). Nevertheless, some discrepancies between genotype and phenotype were observed. In this study, 17 tetracycline-resistant isolates did not harbor *tet*(O). In addition, one fluoroquinolone-resistant isolate exhibited phenotypic resistance despite lacking the *gyrA* T86I mutation. These discrepancies suggest that the resistance phenotypes observed in these isolates may not be fully explained by the resistance genes or mutations and may be explained by the existence of efflux pump mechanisms or other unidentified resistance determinants (Marotta *et al.*, 2019).

Biofilm formation plays a critical role in bacterial survival and host infection by being induced in response to environmental stresses, and is closely associated with AMR, genetic typing, and virulence (Dufour, Leung, and Lévesque, 2012; Weaver *et al.*, 2012). In our study, most biofilm-forming *C. jejuni* isolates exhibited a weak biofilm phenotype. This result is consistent with previous reports showing that *C. jejuni* forms weaker biofilms than other bacterial species (Teh, Lee, and Dykes, 2014). However, the predominance of weak biofilm formation does not necessarily indicate a limited capacity for environmental

survival. Besides forming biofilms independently, *C. jejuni* can also attach to food or abiotic surfaces and reside within pre-established biofilms of other bacterial species (Teh, Lee, and Dykes, 2014). Such interactions may protect *C. jejuni* from environmental stressors, allowing cells within biofilms to survive for prolonged periods under aerobic conditions (Ma *et al.*, 2022).

Strong biofilm formation was observed in two human-derived isolates, even though the overall tendency was toward weak biofilm formation. *C. jejuni* is transmitted to humans along the food chain and needs to be capable of surviving exposure to oxygen, nutrient limitation, and other environmental stresses during transmission (Kim *et al.*, 2015). Under such selective pressures, strains that are better adapted to stressful environments may be more likely to survive during transmission along the food chain and be transmitted to humans (Mouftah *et al.*, 2021). In this regard, strong biofilm formation observed in human-derived *C. jejuni* strains may suggest that strains with enhanced biofilm-forming capacity were preferentially selected and transmitted under such selective pressures. Therefore, strong biofilm formation may enhance tolerance to antimicrobial agents and resistance to host defense mechanisms, thereby making treatment more challenging and highlighting the need for increased caution (Høiby *et al.*, 2010).

Exploratory Pan-GWAS identified eight candidate genes showing potential positive associations with biofilm formation in MDR *C. jejuni*, which were present in different combinations among strains exhibiting a biofilm-forming phenotype. Among these candidate genes, *polA* encodes DNA polymerase I, which is involved in DNA replication-associated processes and multiple DNA repair pathways in bacteria under DNA-damaging conditions, thereby contributing to bacterial survival and potentially influencing biofilm formation (Sharma and Smith, 1987; Hagensee and Moses, 1989; Sayers, 1994). Moreover, *hxuB*, as a component of the *hxu* system, plays an important role in heme-derived iron acquisition from the heme-hemopexin complex, with iron known to promote biofilm formation (Price and Skaar, 2025). Zhou *et al*. (2023) reported that biofilm formation is not determined by a single gene but instead reflects the interactions of multiple genes. However, the roles of genes involved in this process in *C. jejuni* remain poorly characterized, indicating the need for further investigation (Melo *et al.*, 2017). Improving our understanding of these genetic factors may help elucidate the mechanisms that enable *C. jejuni* to survive in diverse environments and may ultimately guide the development of therapeutic strategies against *C. jejuni* infection. Because of the limited sample size and the exploratory nature of the Pan-GWAS analysis, these findings should be interpreted with caution. Further large-scale genomic analysis and functional validation are required to confirm the roles of these candidate genes in *C. jejuni* biofilm formation.

The pTet family plasmid is the most prevalent plasmid type in *C. jejuni* and is considered to play a key role in the dissemination of AMR and virulence-associated genes (Morita *et al.*, 2023). The pTet-like plasmids shared high sequence similarity and conserved structural features with the reference pTet plasmid, including the typical pTet backbone and conjugation-associated T4SS region, as described previously (Garcia-Fernandez *et al.*, 2024). In the present study, nine pTet-like plasmids were identified, including two poultry and seven human-derived *C. jejuni* isolates. Notably, high sequence homology was observed between the pTet-like plasmids from poultry and human isolates, suggesting potential inter-host transmission or a shared evolutionary background. Moreover, T4SS, which utilizes a needle-like apparatus to directly transport effector molecules from bacterial pathogens to eukaryotic host cells (Gabbert *et al.*, 2023), was identified in all pTet-like plasmids. In particular, *traG*, *traR*, and *traQ*, which were identified in all pTet-like plasmids analyzed in this study (Robinson *et al.*, 2021; He *et al.*, 2025), are core genes involved in conjugative transfer. These findings suggest that pTet-like plasmids retain conjugative potential across host species, thereby facilitating the horizontal transfer of AMR and virulence genes between humans and poultry.

T6SS in *C. jejuni* plays multiple roles in virulence and environmental adaptation (Katz *et al.*, 2023). In this study, the pTet-like plasmid identified in CBUSHCJ0021 contained a large insertion region that encodes the T6SS. Plasmids of the pTet family containing T6SS have been identified in multiple *C. jejuni* isolates (Morita *et al*., 2023), and their acquisition has been linked to increased hemolytic activity (Marasini *et al*., 2020). These results imply that the integration of the T6SS into pTet-like plasmids harboring T4SS elements may facilitate the carriage and horizontal dissemination of virulence-associated genes (Wei *et al.*, 2014).

Genome assemblies in this study were generated using Oxford Nanopore long-read sequencing data only, without short-read sequencing or additional post-assembly polishing. Therefore, potential sequencing errors may influence SNP-level accuracy and fine-scale genomic comparisons. Caution is warranted when interpreting minor nucleotide-level variations. Future studies using hybrid assembly with short-read sequencing or high-accuracy long-read sequencing platforms may improve genome resolution and SNP-level accuracy.

In addition, although pTet-like plasmids were identified in several *C. jejuni* isolates based on sequence similarity to previously reported plasmids, comprehensive plasmid-level comparative analysis and experimental validation of plasmid transferability were not performed in this study. Therefore, the mobility and dissemination potential of these plasmids should be interpreted with caution.

Conclusions: In conclusion, this study emphasizes the need for comprehensive surveillance of MDR *C. jejuni* to mitigate its potential zoonotic transmission to humans. Whole-genome analyses revealed both genetic diversity and similarities among MDR *C. jejuni* strains isolated from human and poultry. Furthermore, the identification of mobile genetic elements, including pTet-like plasmids carrying AMR and virulence-associated genes, suggests that these elements may contribute to the dissemination of such traits among strains. These findings support the need for enhanced monitoring and control strategies targeting poultry reservoirs to reduce the potential dissemination of antimicrobial-resistant and virulent *C. jejuni* strains.

Availability of data and materials: All raw sequencing data generated and analyzed in this study have been

deposited in the NCBI Sequence Read Archive (SRA) under the BioProject accession numbers PRJNA1288030, PRJNA1309317, and PRJNA1320532.

Authors Contribution: HA conceived and designed the study. HA, DR, MK, SC, YG and SK performed the experiments. HA and MK analyzed the data. KD, SK and YP reviewed and edited the manuscript. KD and KS supervised the study. HA wrote the manuscript. All authors read and approved the final manuscript.

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