Antimicrobial Resistance Profiles, Virulence Genes, and Genetic Diversity of Thermophilic Campylobacter Species Isolated From a Layer Poultry Farm in Korea

Noel Gahamanyi1,2, Dae-Geun Song1, Kye-Yoon Yoon1, Leonard E. G. Mboera2, Mecky I. Matee3, Dieudonné Mutangana4, Raghabendra G. Amachawadi5, Erick V. G. Komba2* and Cheol-Ho Pan1,6*

1 Natural Product Informatics Research Center, KIST Gangneung Institute of Natural Products, Gangneung, South Korea, 2 SACIDS Foundation for One Health, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania, 3 School of Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, 4 College of Science and Technology, University of Rwanda, Kigali, Rwanda, 5 Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, Manhattan, KS, United States, 6 Division of Bio-Medical Science and Technology, KIST School, Korea University of Science and Technology, Seoul, South Korea

Thermophilic Campylobacter species are among the major etiologies of bacterial enteritis globally. This study aimed at assessing the antimicrobial resistance (AMR) profiles, virulence genes, and genetic diversity of thermophilic Campylobacter species isolated from a layer poultry farm in South Korea. One hundred fifty-three chicken feces were collected from two layer poultry farms in Gangneung, South Korea. The Campylobacter species were isolated by cultural techniques, while PCR and sequencing were used for species confirmation. Antimicrobial susceptibility testing for six antimicrobials [ciprofloxacin (CIP), nalidixic acid (NAL), sitafloxacin (SIT), erythromycin (ERY), tetracycline (TET), and gentamicin (GEN)] was carried out by broth microdilution. Three AMR and nine virulence genes were screened by PCR. Genotyping was performed by flaA-restriction fragment length polymorphism (RFLP) and multilocus sequence typing (MLST). Of the 153 samples, Campylobacter spp. were detected in 55 (35.9%), with Campylobacter jejuni and Campylobacter coli being 49 (89.1%) and six (10.9%), respectively. High-level resistance was observed for CIP (100%), NAL (100%), and TET (C. jejuni, 93.9%; C. coli: 83.3%). No resistance was observed for SIT. The missense mutation (C257T) in gyrA gene was confirmed by sequencing, while the tet(O) gene was similar to known sequences in GenBank. The rate of multidrug-resistant (MDR) strains was 8.2%, and they all belonged to C. jejuni. All Campylobacter isolates possessed five virulence genes (cdtB, cstI, flaA, cadF, and dnaJ), but none possessed ggt, while the rates for other genes (craA, ciaB, and pldA) ranged between 33.3 and 95.9%. The flaA-RFLP yielded 26 flaA types (C. jejuni: 21 and C. coli: five), while the MLST showed 10 sequence types (STs) for C. jejuni and three STs for C. coli, with
INTRODUCTION

Globally, Campylobacter is the leading cause of bacterial gastroenteritis (Alaboudi et al., 2020). Campylobacter jejuni and Campylobacter coli are the species of clinical significance, being accountable for more than 95% of human campylobacteriosis (Moore et al., 2005; Fitzgerald, 2015). Globally, 96 million cases of diarrhea each year are due to Campylobacter (Havelaar et al., 2015). In Europe, Campylobacter was ranked as the second (next to Salmonella) etiological agent of outbreaks associated with water and food poisoning in 2018 (Klančnik et al., 2020). Contrary to European countries, reports on human campylobacteriosis in Asian countries including South Korea are limited, possibly due to low disease prevalence or the sporadic nature of infections (Kim et al., 2019; Wei et al., 2019).

Human campylobacteriosis requires antimicrobial therapy only in case of complications and in immuno-compromised people (Wieczorek and Osek, 2013; Sproston et al., 2018). Over the years, increasing rates of Campylobacter strains that are resistant to the drugs of choice [fluoroquinolones (FQs) and macrolides] and alternative therapies [gentamicin (GEN) and tetracycline (TET)] have been reported (Blaser and Engberg, 2008; Koolman et al., 2015), making antimicrobial resistant (AMR) Campylobacter strains a public health concern (Mourkas et al., 2019; Windiasti et al., 2019). The resistance to antimicrobials is partly due to their misuse in both human medicine and livestock production (Elisha et al., 2017; Sproston et al., 2018). For instance, different quinolone antibiotics have been extensively used in poultry raising, which led to an accelerated number of quinolone-resistant strains of Campylobacter from chicken and humans (Sproston et al., 2018).

In Korea, FQ use in livestock was banned since 2010, with a prediction to curb the increased resistance in the future (Ku et al., 2011). However, recently, FQ-resistant Campylobacter strains have been isolated from poultry and duck meat (Kim et al., 2019). Due to the increased resistance to quinolones throughout the world (Tang et al., 2017), erythromycin (ERY) has emerged as the recommended drug for treating human campylobacteriosis (Giannatale et al., 2019). Recently, sitafloxacin (SIT), a novel FQ drug, proved to be effective against various FQ-resistant pathogens including Campylobacter (Changkwanyeun et al., 2016; Chen et al., 2020), and could be a promising drug. The persistence of FQ resistance in Campylobacter strains could be linked to the continued use of ciprofloxacin (CIP) in human medicine, international trade, travel, and use of FQs in animal husbandry along with the circulation of resistant isolates among different reservoirs.

Poultry can carry Campylobacter, and chicken intestines are regarded as reservoirs for thermophilic Campylobacter species based on optimal conditions (high body temperature) favoring their growth (Sibanda et al., 2018). Previous reports have associated an increase in human campylobacteriosis cases with the increase in chicken consumption (Oh et al., 2017). Chickens' ceca alone are usually colonized by C. jejuni to levels above $10^9$ colony-forming unit (CFU)/ml, posing a risk to humans (Humphrey et al., 2007, 2014). Furthermore, Campylobacter can stay in feces and litter for many days, and the use of these byproducts as fertilizers would aggravate the dissemination of the pathogens (Kassem et al., 2016). Campylobacter persistence in chicken farms and slaughterhouses is a hazard to the consumers because it is transmitted along the whole production chain up to the final product (Kim et al., 2019; Ramires et al., 2020). While the literature on broiler chicken is extensive, studies on the epidemiology of Campylobacter species from layer farms are limited (Kassem et al., 2016).

Campylobacter species are equipped with virulome which is used in attachment, establishment, invasion, and production of toxins, contributing to their increased occurrence and epidemiology compared to other enteric bacteria (Bolton, 2015; Otigbe et al., 2018). However, the mechanisms associated with Campylobacter pathogenicity are not fully understood (Nguyen et al., 2016). C. jejuni is known to cause Guillain–Barré syndrome, characterized by acute and progressive neuromuscular paralysis, mediated by sialyltransferases (cdtII) (Koga et al., 2006; Humphrey et al., 2007; González-Hein et al., 2013). Sialic acid confers immune avoidance to C. jejuni, as a mutant lacking lipooligosaccharide sialic acid residues showed greater immunoreactivity and decreased serum resistance (Kreling et al., 2020). The CDT complex, another important factor in Campylobacter, codes for the cytolethal distending toxin with cdtB acting as the catalytic site, and in the nucleus, cdtB induces cell cycle arrest and leads to apoptosis of both immune and epithelial cells in the intestines (Jain et al., 2008). It has been reported that C. jejuni cdtB mutants had reduced extra-intestinal invasiveness (Yamasaki et al., 2006) and bowel disturbances (Pokkunuri et al., 2012). A study carried out in Poland showed that Campylobacter strains lacking cdtB and cdtC were non-cytotoxic, confirming their roles in toxin production (Wysok et al., 2020). The presence of ggt contributes to the colonization potential of C. jejuni in chicken and mice intestines (Barnes et al., 2007). The flaA gene contributes to Campylobacter pathogenesis as it is involved in colonization, motility, auto-agglutination, and biofilm formation (Guerry, 2007). Mutation experiments highlighted the role of flaA in chicken colonization (Bolton, 2015). Campylobacter species also possess other genes that are resistant to quinolones and TET, the virulence potential, and the diverse genotypes among Campylobacter strains isolated from the layer poultry farm.

Keywords: Campylobacter, quinolones, antimicrobial resistance, flaA RFLP, multilocus sequence typing, poultry, Korea
associated with adhesion (cadF and pldA), invasion (ciaB), thermo-tolerance (dnaJ) (Pillay et al., 2020), and stress response (csrA) (Fields and Thompson, 2008). Studies have shown that Campylobacter strain mutants for cadF and ciaB exhibited a reduced attachment and invasion of INT 407 cell line along with a decline of survival potential (Kreling et al., 2020; Ramires et al., 2020).

Molecular typing methods are important not only in distinguishing bacteria at the species and subspecies levels but also in source attribution of Campylobacter strains (Eberle and Kiess, 2012; Lydekaitei and Kudirkienė, 2020). Although source attribution aiming at quantifying the contribution of different reservoirs, pathways, exposures, and risk factors to the burden of human illness is difficult (Wagenaar et al., 2013), it is estimated that 80% of human campylobacteriosis are attributed to Campylobacter of poultry origin (Wagenaar et al., 2013; Mulder et al., 2020). Multilocus sequence typing (MLST), based on seven housekeeping genes (HKGs), is the gold-standard method used for epidemiological surveillance (Harrington et al., 2003; Lydekaitei and Kudirkienė, 2020). Data on MLST of Campylobacter strains in Asia are limited (Nguyen et al., 2016), but previous studies in Korea and Japan have shown the predominance of CC-460, CC-607, CC-21, and CC-45 in poultry and human isolates (Wei et al., 2014; Ozawa et al., 2016; Oh et al., 2017). MLST data are expected to give accurate phylogenetic estimation, typing, and strain relatedness (Alaboudi et al., 2020). Whole-genome sequencing (WGS) might become the preferred typing method in the future, but still, there is a need for a consensus upon bioinformatics pipelines and tools for processing WGS data (Duarte et al., 2016b).

Considering the persistence of FQ-resistant Campylobacter strains even in the absence of antimicrobial use and the fact that SIT has a different structure compared to other FQs, we hypothesized that Campylobacter species from chicken are still resistant to both ciprofloxacin and nalidixic acid (NAL) but sensitive to SIT. Furthermore, we think that the same sequence types (STs) are circulating in poultry in Korea and the region. Based on the virulence potential of Campylobacter and the favorable environment offered by chicken, it is most probable that Campylobacter species of poultry origin are hypervirulent and could be of concern. The present study aimed at assessing the antimicrobial resistance profiles, virulence genes, and genetic diversity of thermophilic Campylobacter species isolated from a layer poultry farm in Korea.

MATERIALS AND METHODS

Sample Collection

Fresh chicken fecal samples were purposively collected from two layer poultry farms located in Gangneung city, Republic of Korea in June 2020. The first farm uses an intensive poultry farming system with around 800 1-year-old chickens dispatched into battery cages inside a closed house. The second farm is a small one that is not for commercial purposes, where around 30 1-year-old chickens are enclosed in a cage subdivided into two blocks by a fence. A total of 133 (from the first farm) and 20 (from the second farm) pen floor fecal samples were collected using sterile swabs and transported to the laboratory under refrigeration (ice) within 1 h.

Campylobacter Isolation and Antimicrobial Susceptibility Testing

Upon arrival at the laboratory, the feces were inoculated onto modified charcoal cefoperazone deoxycholate agar (mCCDA) (Oxoid Ltd., Basingstoke, Hampshire, England) containing the Campylobacter mCCDA-selective supplement, SR155E (Oxoid Ltd., Basingstoke, Hampshire, England). Incubation was done as previously described (Kurekci et al., 2012) at 37°C for 48 h under microaerophilic conditions generated by CampyGen™ gas sachets (Oxoid, Basingstoke, England, United Kingdom). Typical colonies of Campylobacter, which are grayish, flat, moistened, and with a tendency to spread (Al-Edany et al., 2015), were sub-cultured on Mueller Hinton Agar supplemented with 5% defibrinated horse blood (MHS) and incubated at 37°C for 48 h under microaerophilic conditions (Kurekci et al., 2012). Campylobacter isolates were preserved at −80°C in Mueller Hinton broth containing 25% glycerol (v/v).

Antimicrobial susceptibility testing was performed by broth microdilution. The isolates were tested against quinolones, namely ciprofloxacin (CIP), NAL (0.25–512 µg/ml), SIT (0.03–16 µg/ml); macrolide, ERY (0.06–64 µg/ml); and aminoglycoside, GEN (0.06–64 µg/ml), and TET (0.125–1,024 µg/ml). Apart from SIT purchased from AdooQ BioScience (Irvine, CA, United States), the other antimicrobials were supplied by Sigma-Aldrich (St. Louis, MO, United States). CIP and ERY were dissolved in 0.1 N HCl and 70% ethanol, respectively. GEN and TET were dissolved in water, while NAL and SIT were dissolved in dimethyl sulfoxide (DMSO). Except for the antibiotics dissolved in DMSO, other solutions of antibiotics were filter-sterilized before being used.

Preserved Campylobacter isolates were inoculated onto MHS (Oxoid Ltd, Basingstoke, Hampshire, England) and incubated at 37°C for 48 h under microaerophilic conditions (Kurekci et al., 2012). A sub-culture was performed on the same media and the same conditions to get well-grown pure colonies free from glycerol. For antimicrobial susceptibility assays, suspensions corresponding to 0.5 McFarland standard (1.5 × 10^8 CFU/ml) were prepared using normal saline, and the final concentration in a 96-well plate was 2–5 × 10^6 CFU/ml. The minimal inhibitory concentration (MIC) was determined by checking the absorbance at λ_{600} nm on a microplate reader (Synergy HT; BioTek Instruments Inc., Winooski, VT, United States) and confirmed by the addition of iodonitrotetrazolium chloride to 96-well plates as previously described (Klančnik et al., 2009). The MIC was designated as the lowest concentration of the antimicrobial leading to a significant decrease (>90%) in inoculum viability after 48 h as previously described with modification on incubation time (Burt, 2004). The minimal bactericidal concentration (MBC) was determined as previously described (Dholvitayakhun and Trachoo, 2012; Duarte et al., 2016a). The concentration at which no bacterial growth was noticed after 48 h of incubation was regarded as MBC. The
The PCR mixture (25 µl) contained 12.5 µl of 2X Master Mix (Thermo Fisher Scientific, Seoul, South Korea), 1 µl of primer (10 µM), 1.5 µl of template DNA (20 µg/ml), and 7 µl of sterile deionized water. The cycling conditions were one cycle of 95°C for 5 min, 35 cycles each of 94°C for 30 s, 55°C for 45 s, and 72°C for 45 s, and a final extension at 72°C for 5 min using MiniAmp Plus Thermal Cycler (Applied Biosystems, MA, United States). The PCR products were held at 4°C before analysis.

For the genes associated with antibiotic resistance [tet(O), gyrA, and cmeB] and virulence (cstII, cdbB, flaA, ggt, csrA, cadF, ciaB, pldA, and dnuA], the PCR was performed using specific primers (Supplementary Table 1). After electrophoresis, bands of PCR products (Figure 1) were observed on a Dual UV Transilluminator (Core Bio System, Huntington Beach, CA, United States) under ultraviolet light. The bands of the amplification products were compared to the 100-bp DNA ladder (Dyne bio, Seongnam-si, Republic of Korea). The PCR products of antibiotic resistance genes were purified with AMPure XP beads (Beckman Coulter, Fullerton, CA, United States) and sequenced by the Sanger method at SolGent (Solutions for Genetic Technologies, Daejeon, Republic of Korea).

**flaA-Restriction Fragment Length Polymorphism**

Genetic diversity was first analyzed by flaA-restriction fragment length polymorphism (RFLP) using 25-µl PCR reactions (Harrington et al., 2003; Wieczorek and Osek, 2008). The flaA amplicon (1.7 kb) was digested for 6 h at 37°C using HpyF3I restriction enzyme (Thermo Scientific, Waltham, MA, United States), and the fragments were separated using 2.5% agarose gel (Lonza Inc., Rockland, ME, United States) in Tris–acetate–EDTA buffer at 90 V for 90 min. The bands were photographed with iBright™ CL1000 Imaging System (Thermo Fisher Scientific, Seoul, Republic of Korea). The dye 100-bp and 1-kb DNA ladders (Dyne bio, Seongnam-si, Republic of Korea) were used as standards for molecular size determination.

**Multilocus Sequence Typing Analysis**

Multilocus sequence typing was performed as previously described (Dingle et al., 2001; Giannatale et al., 2019) using primers available from the Campylobacter MLST website. Briefly, the seven HKGs (aspa (aspartase), gltA (glutamine synthetase), gltB (citrate synthase), ggt (serine hydroxymethyltransferase), pyn (phosphor glucomutase), tkt (transketolase), and uncA (ATP synthase)) were amplificated from genomic DNA. For C. jejuni, two rounds of PCR were performed (nested PCR), while for C. coli one set of primers was used. The PCR conditions were denaturation at 94°C for 5 min, 35 cycles of 94°C for 30 s, 60°C for 45 s, and 72°C for 45 s, and then a final extension at 72°C for 5 min. The purification of amplicons was performed by AMPure XP beads (Beckman Coulter, Fullerton, CA, United States) as per the manufacturer's recommendations and sequenced by the Sanger method at SolGent (Solutions for Genetic Technologies, Daejeon, Republic of Korea).

**Data Analysis**

GraphPad Prism 8.4.0 (GraphPad Software, La Jolla, CA, United States) was used to compute the descriptive statistics (detection rate, proportions, and frequencies of different attributes). The flaA restriction profiles were analyzed by pairwise comparisons and cluster analysis using the Dice correlation coefficient and the unweighted pair group method with arithmetic mean clustering algorithm in BIOMUTERICCS V8.0 (Applied Maths, Sint-Martens-Latem, Belgium). The optimization and position tolerance (1%) for band analysis and a cutoff of 100% were used. BioEdit software (version 7.2.6.1) was used to edit, align, and analyze the DNA chromatograms (Hall, 1999). A BLAST search was performed to compare consensus sequences [gyrA and tet(O)] with those from the GenBank database. Then, our sequences were submitted to get the corresponding accession numbers. Standard sensitive strains (L04566.1 and U63413.1) and resistant strains (KX982339.1 and MT176401.1) for gyrA were used for comparison. For the gyrA gene, the comparison was performed with Clustal Omega (Madeira et al., 2019). Amino acid sequences were deduced from the DNA sequences using the ExPASyTranslate tool (Gasteiger et al., 2003). Alleles, STs, and clonal complexes (CCs) were assigned by submitting the sequence data to the MLST database (see text footnote 2) (Maiden, 2006). A minimum spanning tree of C. jejuni and C. coli STs was created from MLST allelic profiles using BIOMUTERICCS 8.0 (Applied Maths NV, Saint-Martens-Latem, Belgium).

**RESULTS**

Out of 153 fecal samples, the detection rate of Campylobacter spp. was 35.9% (55), with C. jejuni and C. coli being 89.1% (49) and 10.9% (six), respectively. None of the 20 fecal samples from the second farm was positive for Campylobacter.

1http://www.eucast.org/

2http://pubmlst.org/campylobacter
Antimicrobial Susceptibility Testing

All Campylobacter isolates were screened for antimicrobial susceptibility to six antimicrobials, and they showed high-level resistance to CIP, NAL, and TET. Resistance to CIP and NAL was 100%, while resistance to TET was 93.9% for C. jejuni and 83.3% for C. coli (Table 1). Four (8.2%) C. jejuni strains were multidrug-resistant (MDR), but none of the C. coli was MDR. Of the four MDR isolates, two were resistant to CIP, NAL, TET, and ERY, while the other two were resistant to CIP, NAL, TET, and GEN. The presence of tet(O) and mutation in gyrA were confirmed by PCR (Figure 1), but all strains did not show bands for the multidrug efflux pump gene (cmeB). Sequencing revealed the presence of a missense mutation (C257T) in the quinolone resistance determining region of gyrA gene, causing resistance to quinolones along with other silent mutations. There was 100% sensitivity to SIT, while 4.1% of the C. jejuni isolates were resistant to both ERY and GEN (Table 1). The MBC values were as follows: CIP, 64–256 µg/ml; NAL, 128–512 µg/ml; TET, 2–1,024 µg/ml; SIT, 0.25–1 µg/ml; ERY, 1–32 µg/ml; and GEN, 1–256 µg/ml.

Upon submission of the gyrA and tet(O) sequences to the GenBank database, the following accession numbers have been assigned: MW067325–MW067370 (Table 2). The main mutation in gyrA gene is the missense mutation (C257T) associated with the codon change from ACA to ATA (C. jejuni) and ACT to ATT (C. coli) leading to T86I substitution. However, silent mutations were also found. It was noticed that TET-resistant strains possessed the tet(O) gene, which was confirmed by sequencing. The BLAST search showed similarity with known tet(O) gene sequences in GenBank.

Virulence Genes

The presence of selected virulence genes (cstII, cdtB, flaA, ggt, csrA, cadF, ciaB, pldA, and dnaJ) was checked by PCR (Figure 1). We found that all isolates possessed cdtB, cstII, flaA, cadF, and

---

**TABLE 1 | Antimicrobial resistance data for both Campylobacter jejuni and Campylobacter coli species.**

| Antimicrobial Class | Species | Resistance (%) | Number of isolates at the indicated minimal inhibitory concentration (µg/ml) |
|--------------------|---------|----------------|--------------------------------------------------------------------------------|
|                    |         |                | 0.06 | 0.13 | 0.25 | 0.5 | 1   | 2   | 4   | 8   | 16  | 32  | 64  | 128 | 256 | 512 |
| CIP                | C. jejuni | 100            | 0    | 0    | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 36  | 10  | 2   | 0   | 0   |
| Fluoroquinolone    | C. coli  | 100            | 0    | 0    | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 4   | 2   | 0   | 0   | 0   |
| SIT                | C. jejuni | 0              | 2    | 33   | 11   | 2   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
|                    | C. coli  | 0              | 0    | 0    | 5    | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| NAL                | C. jejuni | 100            | 0    | 0    | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 25  | 17  | 6   | 0   |
| Quinolone          | C. coli  | 100            | 0    | 0    | 0    | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| ERY                | C. jejuni | 4.1            | 0    | 0    | 1    | 38  | 3   | 2   | 3   | 1   | 1   | 0   | 0   | 0   | 0   | 0   | 0   |
| Macrolide          | C. coli  | 0              | 0    | 0    | 0    | 1   | 0   | 4   | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| GEN                | C. jejuni | 4.1            | 0    | 1    | 11   | 27  | 8   | 0   | 0   | 0   | 0   | 0   | 0   | 2   | 0   | 0   | 0   |
| Aminoglycoside     | C. coli  | 0              | 0    | 0    | 0    | 2   | 1   | 3   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 0   |
| Tetracycline       | C. jejuni | 93.9           | 0    | 0    | 0    | 2   | 1   | 0   | 0   | 0   | 0   | 0   | 3   | 10  | 22  | 10  | 1   |
|                    | C. coli  | 83.3           | 0    | 0    | 0    | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 0   | 1   | 2   | 2   | 0   |
TABLE 2 | Accession numbers for DNA gyrA and tet(O) resistance genes.

| Isolate | DNA gyrA accession number | tet(O) accession number |
|---------|--------------------------|-------------------------|
| CJ5     | MW067325                 | MW067326                |
| CJ8     | MW067327                 | MW067328                |
| CJ17    | MW067329                 | MW067350                |
| CJ21    | MW067330                 | MW067351                |
| CJ28    | MW067331                 | MW067352                |
| CJ32    | MW067332                 | MW067353                |
| CJ37    | MW067333                 | MW067354                |
| CJ42    | MW067334                 | MW067355                |
| CJ46    | MW067335                 | MW067356                |
| CJ48    | MW067336                 | MW067357                |
| CJ50    | MW067337                 | MW067358                |
| CJ51    | MW067338                 | MW067359                |
| CJ52    | MW067339                 | MW067360                |
| CJ53    | MW067340                 | MW067361                |
| CJ54    | MW067341                 | MW067362                |
| CJ55    | MW067342                 | MW067363                |
| CJ56    | MW067343                 | MW067364                |
| CJ60    | MW067344                 | MW067365                |
| CJ71    | MW067345                 | MW067366                |
| CC13    | MW067346                 | MW067367                |
| CC45    | MW067347                 | MW067388                |
| CC47    | MW067348                 | MW067369                |
| CC2     | MW067349                 | MW067370                |
| CC1     | MT947449                 | MT967270                |

CJ, Campylobacter jejuni; CC, Campylobacter coli.

dnaJ, but none showed the presence of ggt. The percentages for csrA, ciaB, and pldA were 73.5, 95.9, and 98% for C. jejuni and 66.7, 33.3, and 33.3% for C. coli, respectively (Figure 2).

The flaA Polymorphism

Campylobacter jejuni (n = 37) and C. coli (n = 5) were digested with HpyF3I, yielding six to 10 fragments of DNA. There were 26 fla-A types (C. jejuni: 21 and C. coli: five). For C. jejuni, there was a predominant cluster at the top of the dendrogram. For C. coli, isolate numbers 45 and 47 clustered together; the other isolates had different patterns (Figure 3).

Multilocus Sequence Typing

Twenty-four isolates (C. jejuni: 19; C. coli: five), selected based on flaA RFLP profiles to maximize the diversity, were genotyped by MLST. C. jejuni isolates were matched with 10 STs grouped into five CCs. However, three C. jejuni isolates had new combinations of previously described alleles but could not be matched with any of the existing STs. Upon submission to the database (see text footnote 2) for ST assignment, the isolates CJ42 (id: 106369), CJ52 (id: 106370), and CJ71 (id: 106371) were assigned to ST-10645, ST-10647, and ST-10648, respectively. Of the 10 STs, ST-3611 was the main one with five isolates, followed by ST-460 with four isolates. The CCs with a higher number of isolates were CC-607 with nine isolates and CC-460 with five isolates. For C. coli (five isolates), three STs were found, with ST-5935 being the most prevalent (three isolates), and all the five isolates belonged to CC-1150 (Table 3).

The minimum spanning tree of C. jejuni and C. coli STs was created from MLST allelic profiles using BIONUMERICS 8.0 (Applied Maths NV, Saint-Martens-Latem, Belgium). The tree shows 78 STs grouped into 12 previously characterized CCs, including both STs identified in this study (13) and 65 STs reported in the literature as predominant in Campylobacter strains of human and poultry origins. ST-460 and ST-10613, belonging to CC-460, are clustered together, and they share six of the seven HKGs, with the only difference being in glnA allele. The STs 6238, 607, and 3611 form another cluster belonging to the same CC-607 at the center of the tree. ST-51 belonging to CC-443 is located far from the other STs identified in this study. Of the three newly assigned STs, it can be concluded that ST-10645 is closely related to ST-8994, both belonging to CC-52, while ST-10647 is closely related to ST-3611, both belonging to CC-607. We also included STs that have been previously identified in Campylobacter strains isolated from poultry, human, and cattle fecal samples from the database (see text footnote 2). It can be concluded that CCs of this study are closely related to other CCs (257, 353, and 354) and distanced from CC-45 and CC-21 commonly reported in Campylobacter strains of poultry.
origin in Asia and elsewhere. The STs for *C. coli* from this study all belong to CC-1150 along with other STs obtained from the database (Figure 4).

**DISCUSSION**

Chicken contamination or infection by *Campylobacter* at the farm level usually affects the whole poultry production chain from farm to fork (Giannatale et al., 2019; Tang et al., 2020), and suitable interventions have to be adopted to reduce transmission from poultry to humans (Alter et al., 2011). The detection rate of *Campylobacter* was 35.9%, with *C. jejuni* being predominant (89.1%) compared to *C. coli* (10.9%). The detection rate was slightly higher compared to the rate previously reported for layers in the United States (Rama et al., 2018) but lower than the rates reported in the Netherlands (Schets et al., 2017) and Sri Lanka (Kalupahana et al., 2013). *C. jejuni* is reported to be...
The predominant species causing human campylobacteriosis, and our results concur with the literature (Han et al., 2007; Wei et al., 2014). However, exceptions have been reported, where *C. coli* was the predominant or the only isolated species (Marinou et al., 2014). However, exceptions have been reported, where *C. coli* was the predominant species causing human campylobacteriosis, and *Campylobacter* CC1; CC13; CC45 5935 3 1150 CJ5; CJ50 51 2 443

| Species | Isolate ID | Sequence type | Total number | Clonal complex |
|---------|------------|---------------|--------------|----------------|
| *Campylobacter jejuni* | CJ5; CJ50 | 51 | 2 | 443 |
| | CJ8; CJ51; CJ54; CJ60 | 460 | 4 | 460 |
| | CJ56 | 10613 | 1 | |
| | CJ17; CJ32; CJ37; CJ46; CJ53 | 3611 | 5 | 607 |
| | CJ21; CJ48 | 607 | 2 | |
| | CJ55 | 6238 | 1 | |
| | CJ52 | 10647 | 1 | |
| | CJ28 | 8994 | 1 | 52 |
| | CJ42 | 10645 | 1 | |
| | CJ71 | 10648 | 1 | 446 |
| *Campylobacter coli* | CC1; CC13; CC45 | 5935 | 3 | 1150 |
| | CC2 | 8164 | 1 | |
| | CC47 | 1121 | 1 | |

The low resistance to ERY and GEN shown by the isolates of this study has also been reported in different countries like Korea, Vietnam, and China (Carrique-Mas et al., 2014; Wei et al., 2014; Zhang et al., 2020). Resistance to ERY has been low and stable in China (Zhang et al., 2020), the United States, and across Europe (Tang et al., 2017). The limited resistance to ERY may be partly explained by a slower process of developing resistant strains when exposed to ERY and reduced survival of resistant strains (Luangtongkum et al., 2009, 2012). Resistance to GEN has also been relatively low as it is used for treating systemic infections (Lynch et al., 2020).

The *Campylobacter* species virulome contributes to their pathogenicity (Han et al., 2019). In this study, *cstII, cdIB, flaA, cadF, and dna* were detected in all isolates. The detection rates were similar to a previous report in Korea (Oh et al., 2017) but higher than those reported in South Africa and Chile (González-Hein et al., 2013; Otigbue et al., 2018; Pillay et al., 2020). The outer membrane phospholipase A (*pldA*) was observed more in *C. jejuni* than in *C. coli* (Figure 2), which corroborates the study in South Africa (Pillay et al., 2020), while for *ciaB*, our findings are above those of a previous report in Korea (Oh et al., 2017) and contrast with the study in South Africa (Pillay et al., 2020). The presence of *cadF* and *ciaB* facilitates the adhesion and internalization of *Campylobacter* in cellular models (Ramires et al., 2020). The detection rate for *csrA* in *C. jejuni* was slightly higher than the rate in *C. coli*, but *csrA* was lacking in *Campylobacter* strains from South Africa (Otigbue et al., 2018). None of our isolates expressed *ggt*. The latter was reported to be only 5.5% in Chile (González-Hein et al., 2013), but our findings contrast with the high values (30.9–43.2%) reported in Finland (Gonzalez et al., 2009). The difference could be associated with the complexity of the colonization process involving several genes and the use of strains from a single sampling site (González-Hein et al., 2013). Furthermore, MDR and virulent *C. jejuni* strains have
been found more in summer than in winter, suggesting the role played by climate in the expression of some genes (Kim et al., 2019). We also evoke that several virulence genes are plasmid-mediated, which may affect their presence in different strains (Oh et al., 2017). The virulence genes reported in this study have been previously reported in *Campylobacter* strains isolated from humans (González-Hein et al., 2013; Oh et al., 2017), highlighting the potential virulence of these *Campylobacter* strains in causing human infections.

The *flaA*-RFLP typing showed a considerable diversity of *Campylobacter* strains despite being from the same farm. In our study, 26 types were found by *flaA* typing of 42 *Campylobacter* isolates. In another study in Korea, 30 *flaA* types were reported for 100 *C. jejuni* from chicken (Han et al., 2007), while 19 *flaA* types were reported for 100 *C. jejuni* from Grenada, Puerto Rico, and Alabama (Behringer et al., 2011). The *flaA* typing is suitable for laboratories dealing with a small number of isolates as it is cheaper and reproducible (Behringer et al., 2011). However, the drawback of the *flaA* typing is increased recombination events in the *flaA* gene, which modifies RFLP profiles (Harrington et al., 1997). Furthermore, the *flaA* typing focuses only on a single gene from a considerable genome, and there is a lack of inter-laboratory comparison of obtained results (Nguyen et al., 2016) due to the absence of *flaA* database. Therefore, a combination of several typing methods is recommended (Wieczorek and Osek, 2008).

Molecular typing techniques showed that 80% of human campylobacteriosis is associated with *Campylobacter* of poultry origin (Newell et al., 2011). In this study, the MLST revealed 10 STs for *C. jejuni* and three STs for *C. coli*. Three STs were new but could fit in existing clonal complexes. The predominant STs (3611 and 460) have been previously isolated in *C. jejuni* from poultry, while ST-51 was found in *C. jejuni* of both chicken and human origins in various regions of Korea (Oh et al., 2017). In Korea, in addition to the CCs identified in this study, other CCs including CC-48, CC-21, and CC-45 (Figure 4) have been recovered from *Campylobacter* isolates of poultry and human origins (Wei et al., 2014; Oh et al., 2017). The predominance of these clonal complexes and STs could be associated with environmental factors (geography and climate) and increasing poultry consumption in Korea (Oh et al., 2017). Globally, CC-45 has been found in various hosts, including poultry, cattle, dogs, wild birds, penguins, and it was also isolated from environmental samples (Cody et al., 2012; Shin et al., 2013), while CC-607 is largely associated with chicken and humans as reported from the United Kingdom, France, Canada, Thailand, and Uruguay (Cody et al., 2012; Duarte et al., 2014; Guyard-Nicodème et al., 2015). ST-45 and ST-50, predominant in Korea, are also common
in *C. jejuni* from chickens in Europe (Wieczorek et al., 2020b). In 2019, ST-21, ST-50, and ST-137 have been isolated from wild ducks, indicating their widespread distribution (Wei et al., 2019). Three of the 10 STs (607, 443, and 51) were found in Japan and China (Ozawa et al., 2016; Ma et al., 2017), while CC-460 and CC-607 have also been reported in samples from humans and cattle, indicating their threat to public health (Ozawa et al., 2016; Kiatsomphob et al., 2019; Wei et al., 2019). CC-460 and CC-607 are thought to be virulent as they both possess many virulence genes. In Israel, type VI secretion system (T6SS), implicated in virulence, metabolism, AMR, and contributing to host adaptation, has been found in both CCs (Rokney et al., 2018). In Japan, CC-21 is known as the prevalent clonal complex in human-derived *C. jejuni* isolates sharing a common genetic background and similar antimicrobial susceptibilities with *C. jejuni* strains from chicken (Ohishi et al., 2017). CC-21 (ST-50 and ST-21) could be of interest in the region as it has been found in Korea, Japan, and China from humans, poultry, and cattle (Ozawa et al., 2016; Oh et al., 2017; Kiatsomphob et al., 2019; Wei et al., 2019; Zhang et al., 2020). However, further studies are needed to confirm this hypothesis. Except for ST-257, other STs (ST-21, ST-48, and ST-353) identified in Korea have been recovered from *C. jejuni* of human origin in the United Kingdom, Brazil, and China (Gomes et al., 2019). It is known that the predominance of certain genotypes of *C. jejuni* depends on several factors such as animal reservoirs, zoonotic transmission, rates of recombination events, food source, and the first time when a given genotype is recorded in the country (Rokney et al., 2018).

Although resistance to quinolones and TET was not a particularity of certain STs, a strong correlation between CC-460, CC-607, CC-45, CC-48, CC-21, and resistance to both TET and quinolones in *C. jejuni* strains is not new (Cody et al., 2012; Shin et al., 2013; Guyard-Nicodème et al., 2015; Zhang et al., 2020). In Korea, *C. jejuni* strains of human origin with ST-607, ST-137, ST-45, ST-21, and ST-48 were found to be MDR (Shin et al., 2013). Two isolates of *C. jejuni* belonged to CC-443, which is suggested to harbor antibiotic-resistant and pathogenic *C. jejuni* strains (Kim et al., 2019). There is limited literature associating virulence genes to specific CCs and STs; thus, this study highlights the virulence potential of the presented *Campylobacter* isolates. For *C. coli*, ST-1121 has been reported from broilers in China (Tang et al., 2020). We suggest carrying out WGS to have a detailed picture of the pathogenicity by analyzing all the housekeeping, virulence, and AMR genes. The WGS would also enlighten the evolutionary pathways of the *Campylobacter* spp. used in this study to inform better practices that may lead to a reduction of campylobacteriosis cases.

The limitation of this study was the restricted access to various poultry farms across the city which would have given a broad picture of the prevalence, AMR profiles, and genotypes associated with *Campylobacter* in Gangneung. *Campylobacter* strains from the current study proved to be highly diverse considering the number of obtained STs and CCs. This is in agreement with other published studies reporting the possibility of getting several genotypes in a single poultry flock, suggesting different exposure sources via horizontal transmission and/or genetic drifts within the *Campylobacter* population (Alter et al., 2011; El-Adawy et al., 2013).

**CONCLUSION**

This study highlights the role of layers as a reservoir of *Campylobacter* spp., harboring various AMR and virulence genes. The genotyping highlighted that *C. jejuni* isolates were more diverse than *C. coli* as analyzed by MLST. The MLST revealed that CC-607 (ST-3611) and CC-460 (ST-460) were the predominant ones, while three STs were newly assigned. ERY, GEN, and SST need to be appropriately used to prevent or delay the increasing resistance in *Campylobacter* species. The isolates of this study may present a potential hazard to public health based on their AMR profiles, virulence genes, and genotyping data.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**ETHICS STATEMENT**

Ethical review and approval was not required for the animal study.

**AUTHOR CONTRIBUTIONS**

NG, C-HP, and EK conceived the study. NG carried out the experiments, analyzed and interpreted the data, and wrote the manuscript. D-GS and K-YY substantially contributed to the analysis of the results. LM, MM, DM, and RA substantially revised the manuscript. All authors read and approved the final version of the manuscript.

**FUNDING**

This work was financially supported by the Ministry of Oceans and Fisheries, South Korea (grant no. 20170488) and the Partnership for Skills in Applied Sciences, Engineering, and Technology – Regional Scholarship and Innovation Fund (PASET-RSIF) in collaboration with the government of the Republic of Korea, and the KIST intramural grant (2Z06483).

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2021.622275/full#supplementary-material
REFERENCES

Abraham, S., Sahibzada, S., Hewson, K., Laird, T., Abraham, R., Pavic, A., et al. (2020). Emergence of fluoroquinolone-resistant Campylobacter jejuni and Campylobacter coli among Australian chickens in the absence of fluoroquinolone use. Appl. Environ. Microbiol. 86:e02765-19. doi: 10.1128/AEM.02765-19

Alaboudi, A. R., Malkawi, I. M., Osaili, T. M., Abu-Basha, E. A., and Guitian, J. (2020). Prevalence, antibiotic resistance and genotypes of Campylobacter jejuni and Campylobacter coli isolated from chickens in Irbid governorate, Jordan. Intern. J. Food Microbiol. 327:108656. doi: 10.1016/j.ijfoodmicro.2020.108656

Al-Edany, A. A., Khudor, M. H., and Radhi, L. Y. (2015). Isolation, identification and toxigenic aspects of Campylobacter jejuni isolated from slaughtered cattle and sheep at Basrah city. Microbiology 3.14, 316–327.

Alter, T., Weber, R. M., Hamedy, A., and Glünder, G. (2011). Carry-over of thermophilic Campylobacter spp. between sequential and adjacent poultry flocks. Vet. Microbiol. 147, 99–105. doi: 10.1016/j.vetmic.2010.06.005

Barnes, I. H. A., Bagnall, M. C., Browning, D. D., Thompson, S. A., Manning, G., and Newell, D. G. (2007). γ-Glutamyl transpeptidase has a role in the persistent colonization of the avian gut by Campylobacter jejuni. Microbiol. Pathog. 43, 198–207.

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

Blaser, M. J., and Engberg, J. (2008). “Clinical aspects of Campylobacter jejuni and Campylobacter coli infections,” in Campylobacter, 3ed Edn, eds I. Nachamkin, C. M. Szymanski, and M. J. Blaser (Washington, DC: ASM Press), 99–121. doi: 10.1128/9781555815554.ch6

Bolton, D. J. (2015). Campylobacter virulence and survival factors. Food Microbiol. 48, 99–108.

Burt, S. (2004). Essential oil: their antibacterial properties and potential applications in foods—a review. Intern. J. Food Microbiol. 94, 223–253. doi: 10.1016/j.ijfoodmicro.2004.03.022

Carrique-Mas, J. J., Bryant, J. E., Cuong, N. V., Hoang, N. V. M., Campbell, J., Hoang, N. V., et al. (2014). An epidemiological investigation of Campylobacter in pig and poultry farms in the Mekong delta of Vietnam. Epidemiol. Infect. 142, 1425–1436. doi: 10.1017/S0950268813002410

Changkwaneyeun, R., Usui, M., Kongsoi, S., Yokoyama, K., Kim, H., Suthienkul, O., et al. (2015). Characterization of Campylobacter jejuni DNA gyrase as the target of quinolones. J. Infect. Chemother. 21, 604–609. doi: 10.1016/j.jiac.2015.05.003

Changkwaneyeun, R., Yamaguchi, T., Kongsoi, S., Changkaew, K., Yokoyama, K., Kim, H., et al. (2016). Impact of mutations in DNA gyrase genes on quinolone resistance in Campylobacter jejuni. Drug Test. Anal. 8, 1071–1076. doi: 10.1002/dta.1937

Chen, C. K., Cheng, I., Chen, Y. H., and Lai, C. C. (2020). Efficacy and safety of satiufloxacin in the treatment of acute bacterial infection: a meta-analysis of randomized controlled trials. Antibiotics 9, 1–9. doi: 10.3390/antibiotics9030106

Cody, A. J., McCarthy, N. M., Wimalarathna, H. L., Colles, F. M., Clark, L., Bowler, I. C. J. W., et al. (2012). A longitudinal 6-Year study of the molecular epidemiology of clinical Campylobacter isolates in Oxfordshire, United Kingdom. J. Clin. Microbiol. 50, 3193–3201. doi: 10.1128/JCM.01086-12

Dholvitayakun, A., and Trachoo, N. (2012). Antibacterial activity of ethanol extract from Some Thai medicinal plants against Campylobacter jejuni. Nat. Product Res. 6, 113–116.

Dingle, K. E., Colles, F. M., Wareing, D. R. A., Ure, R., Fox, A. J., Bolton, F. E., et al. (2001). Multilocus sequence typing system for Campylobacter jejuni. J. Clin. Microbiol. 39, 14–23. doi: 10.1128/JCM.39.1.14-23.2001

Duque, A., Luis, A. Oleastros, M., and Domingues, F. C. (2016a). Antioxidant properties of coriander essential oil and linalool and their potential to control Campylobacter spp. Food Control 61, 115–122. doi: 10.1016/j.foodcont.2015.09.033
Han, X., Guan, X., Zeng, H., Li, J., Huang, X., Wen, Y., et al. (2019). Prevalence, antimicrobial resistance profiles and virulence-associated genes of thermophilic Campylobacter spp. isolated from ducks in a Chinese slaughterhouse. Food Control 104, 157–166. doi: 10.1016/j.foodcont.2019.04.038

Hao, H., Sander, P., Iqbal, Z., Wang, Y., Cheng, G., and Yuan, Z. (2016). The risk of some veterinary antimicrobial agents on public health associated with antimicrobial resistance and their molecular basis. Front. Microbiol. 7:1626. doi: 10.3389/fmicb.2016.01626

Harrington, C. S., Moran, L., Ridley, A. M., Newell, D. G., and Madden, R. H. (2003). Inter-laboratory evaluation of three flagellin PCR/RFLP methods for typing Campylobacter jejuni and C. coli: the CAMPYNET experience. J. Appl. Microbiol. 95, 1321–1333. doi: 10.1046/j.1365-2672.2003.01201.x

Klančnik, A., Šimunović, K., Šternička, M., Ramaz, D., Smole Možina, S., and Bucar, F. (2020). Anti-adhesion activity of phytochemicals to prevent Campylobacter jejuni biofilm formation on abiotic surfaces. Phytochem. Rev. 1–30. doi: 10.1007/s11101-020-09669-6

Koga, M., Gilbert, M., Takahashi, M., Li, J., Kooke, S., Hirata, K., et al. (2006). Comprehensive analysis of bacterial risk factors for the development of Guillain-Barré syndrome after Campylobacter jejuni enteritis. J. Infect. Dis. 193, 547–555.

Koolman, L., Whyte, P., Burgess, C., and Bolton, D. (2015). Distribution of virulence-associated genes in a selection of Campylobacter isolates. Foodborne Pathog. Dis. 12, 424–432. doi: 10.1089/fpd.2014.1883

Kreiling, V., Falcome, F. H., Kehrenberg, C., and Hensel, A. (2020). Campylobacter sp.: pathogenicity factors and prevention methods—new molecular targets for innovative antivirulence drugs? Appl. Microbiol. Biotechnol. 104, 10409–10436. doi: 10.1007/s00253-020-10974-5

Ku, B. K., Kim, H. J., Lee, Y. J., Kim, Y. I., Choi, J. S., Park, M. Y., et al. (2011). Genetic Characterization and antimicrobial susceptibility of Campylobacter Spp. isolated from domestic and imported chicken meats and humans in Korea. Foodborne Pathog. Dis. 8, 381–386. doi: 10.1089/fpd.2010.0680

Kurekci, C., Bishop-Hurley, S. L., Vercove, P. E., Durm, Z., Al Jassim, R. A. M., and McSweeney, C. S. (2012). Screening of Australian plants for antimicrobial activity against Campylobacter jejuni. Phytother. Res. 26, 186–190. doi: 10.1002/ptr.3526

Lee, J., Jeong, J., Lee, H., Ha, J., Kim, S., Choi, Y., et al. (2017). Antibiotic susceptibility, genetic diversity, and the presence of toxin producing genes in Campylobacter jejuni isolates from poultry. Intern. J. Environ. Res. Public Health 14, 1–11. doi: 10.3390/ijerph14111400

Linton, D., Lawson, A. J., Owen, R. J., and Stanley, J. (1997). PCR detection, identification to species level, and fingerprinting of Campylobacter jejuni and Campylobacter coli direct from diarrheic samples. J. Clin. Microbiol. 35, 2568–2572.

Luangtongkum, T., Jeon, B., Plummer, P., Logue, C. M., and Zhang, Q. (2009). Antibiotic resistance in Campylobacter: emergence, transmission and persistence. Fut. Microbiol. 4, 189–200. doi: 10.2217/fmb.07.18

Luangtongkum, T., Shen, Z., Sung, W. V., Sahin, O., Jeon, B., Liu, P., et al. (2012). Impaired fitness and transmission of macrolide-resistant Campylobacter jejuni in its natural host. Antimicrob. Agents Chemother. 56, 1300–1308. doi: 10.1128/AAC.00516-11

Lydekaïtène, V. L., and Kudirkiene, E. (2020). Prevalence and genotypyng diversity of C. jejuni isolated from Broilers and their environment using flaA-RFLP typing and MLST analysis. Ann. Anim. Sci. 20, 485–501. doi: 10.2478/aosa-2020-0008

Lynch, C. T., Lynch, H., Burke, S., Hawkins, K., Buttimmer, C., Mc Carly, C., et al. (2020). Antibiotic resistance determinants circulating among thermophilic Campylobacter isolates recovered from broilers in Ireland over a one-year period. Antibiotics 9, 1–20. doi: 10.3390/antibiotics9060308

Ma, H., Su, Y., Ma, L., Ma, L., Li, P., Du, X., et al. (2017). Prevalence and Characterization of Campylobacter jejuni isolated from Retail Chicken in Tianjin, China. J. Food Protect. 80, 1032–1040. doi: 10.4315/0362-028X-JFP-16-561

Madeira, F., Park, Y. M., Lee, J., Buso, N., Gut, T., Madhusoodan, N., et al. (2019). The EMBL-EBI search and sequence analysis tools API in 2019. Nucleic Acids Res. 47, W636–W641. doi: 10.1093/nar/gkx268

Maiden, M. C. J. (2006). Multilocus sequence typing of bacteria. Annu. Rev. Microbiol. 60, 561–588. doi: 10.1146/annurev.micro.59.030804.121325

Marinou, I., Bersimis, S., Ioannidis, A., Nicolaou, C., Mitroussia-Ziouva, A., Legakis, N. J., et al. (2012). Identification and antimicrobial resistance of Campylobacter species isolated from animal sources. Front. Microbiol. 3:58. doi: 10.3389/fmicb.2012.00058

Moore, J. E., Corcoran, D., Dooley, J. S. G., Fanning, S., Lucey, B., Matsuda, M., et al. (2003). Campylobacter. Vet. Res. 36, 351–382. doi: 10.1051/vetres:2005012

Mourkas, E., Florez-Cuadrado, D., Pascoe, B., Calland, J. K., Bayliss, S. C., Mageiros, L., et al. (2019). Gene pool transmission of multidrug resistance
among Campylobacter from livestock, sewage and human disease. Environ. Microbiol. 21, 4597–4613. doi: 10.10111/1462-2990.14760

Mulder, A. C., Franz, E., de Rijk, S., Versluis, M. A. J., Coipan, C., Buiz, K., et al. (2020). Tracing the animal sources of surface water contamination with Campylobacter jejuni and Campylobacter coli. Water Res. 187, 116421. doi: 10.1016/j.watres.2020.116421

Newell, D. G., Elvers, K. T., Dofper, D., Hansson, J., Jones, P., James, S., et al. (2011). Biosecurity-based interventions and strategies to reduce Campylobacter spp. on poultry farms. Appl. Environ. Microbiol. 77, 8605–8614. doi: 10.1128/AEM.01090-10

Nguyen, T. N. M., Hotzel, H., Tran, H. T., Le, M. T. H., Pavlova, M., Alexandrova, E., Donkov, G., et al. (2020). Decrease in fluoroquinolone use in French poultry and pig farms. Front. Microbiol. 9:2002. doi: 10.3389/fmicb.2019.02002

Sproston, E. L., Wimalarathna, H. M. L., and Sheppard, S. K. (2018). Trends in fluoroquinolone resistance in Campylobacter. Microb. Genom. 4:198. doi: 10.1099/mgen.0.001918

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wei, B., Cha, S. Y., Kang, M., Roh, J. H., Seo, H. S., Yoon, R. H., et al. (2014). Antimicrobial susceptibility trends and mechanisms in the foodborne pathogen, Campylobacter. Ann. Health Res. Rev. 18, 87–98. doi: 10.1017/S146625317000135

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wei, B., Cha, S. Y., Kang, M., Roh, J. H., Seo, H. S., Yoon, R. H., et al. (2014). Antimicrobial susceptibility trends and mechanisms in the foodborne pathogen, Campylobacter. Ann. Health Res. Rev. 18, 87–98. doi: 10.1017/S146625317000135

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wei, B., Cha, S. Y., Kang, M., Roh, J. H., Seo, H. S., Yoon, R. H., et al. (2014). Antimicrobial susceptibility trends and mechanisms in the foodborne pathogen, Campylobacter. Ann. Health Res. Rev. 18, 87–98. doi: 10.1017/S146625317000135

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.

Wagenaar, J. A., French, N. P., and Havelaar, A. H. (2013). Preventing Campylobacter at the source: why is it so difficult? Clin. Infect. Dis. 57, 1600–1606.
structure and role in diarrheal disease. *Toxin Rev.* 25, 61–88. doi: 10.1080/15569540300320938

Yamazaki-Matsune, W., Taguchi, M., Seto, K., Kawahara, R., Kawatsu, K., Kumeda, Y., et al. (2007). Development of a multiplex PCR assay for identification of *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis*. *J. Med. Microbiol.* 56, 1467–1473.

Zhang, L., Li, Y., Shao, Y., Hu, Y., Lou, H., Chen, X., et al. (2020). Molecular characterization and antibiotic resistant profiles of *Campylobacter* species isolated from poultry and diarrheal patients in Southeastern China 2017-2019. *Front. Microbiol.* 11:1244. doi: 10.3389/fmicb.2020.01244

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Gahamanyi, Song, Yoon, Mboera, Matee, Mutangana, Amachawadi, Komba and Pan. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.