Research Note: Antimicrobial resistance of *Campylobacter* species isolated from chickens near Ulaanbaatar city, Mongolia

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ABSTRACT There has been no report on the prevalence of *Campylobacter* spp. in farm animals in Mongolia. To uncover the prevalence of *Campylobacter* spp. in chickens in Mongolia and their antimicrobial resistance, in this study, we isolated and characterized *Campylobacter* spp. from chickens in Mongolia. We collected 71 cloacal swabs of chickens from 5 farms including 4 layer farms and one broiler farm near Ulaanbaatar city and isolated 25 *Campylobacter jejuni* and 6 *Campylobacter coli* isolates. All isolates were resistant to tetracycline, and 3 *C. coli* isolates were resistant to erythromycin. The *C. coli* isolates possessed either the *erm*(B) gene or nucleotide substitution at nt 2,075 of 23S rDNA, both of which are known to be associated with erythromycin resistance. Sixteen of the 31 *C. jejuni*/*C. coli* isolates (51.6%) were resistant to nalidixic acid and fluoroquinolones. All the fluoroquinolone-resistant isolates possessed amino acid substitution from threonine to isoleucine at codon 86 (nucleotide substitution: ACA to ATA). Multilocus sequence typing and phylogenetic analyses showed a variation in *C. jejuni*/*C. coli* in chickens in Mongolia. In addition, some of the *C. jejuni* isolates seemed to be phylogenetically close to isolates in Asian and Oceanian countries. This is the first report on the characterization of antimicrobial resistance of *Campylobacter* spp. in farm animals in Mongolia and is valuable for implementation of measures for a prudent use of antimicrobials in farm animals.

Key words: antimicrobial resistance, *Campylobacter*, fluoroquinolone, Mongolia

INTRODUCTION

Campylobacter is a gram-negative, microaerophilic bacterium, which exists in the intestine of homoiothermal animals. Members of the genus *Campylobacter* are the leading pathogens of foodborne bacterial infections in industrialized countries. *Campylobacter jejuni*/*Campylobacter coli* causes acute gastrointestinal diseases with symptoms such as abdominal pain, diarrhea, and fever. Consumption of undercooked poultry is the major cause of human infections. Furthermore, contaminated water, undercooked beef and pork, and milk are indicated to cause campylobacteriosis in humans. Antimicrobial treatment is not usually recommended because campylobacteriosis is usually self-limiting; however, treatment with macrolides and fluoroquinolones (**FQ**) may be recommended for patients

with severe or prolonged symptoms (Blaser and Engberg, 2008).

Since the late 1980s, there has been an increase in FQ-resistant *C. jejuni*/*C. coli* isolates in livestock concurrently with the use of FQ for the treatment of food-producing animals (Luangtongkum et al., 2009). A single point mutation at nt 257 (C to T) of the *gyrA* gene, which results in amino acid substitution at codon 86 (Thr to Ile) in GyrA, confers a high FQ resistance to *Campylobacter* (Engberg et al., 2001). Therefore, the use of FQ may easily select FQ-resistant *Campylobacter*. The acquisition of FQ resistance was reported not to impair the colonization of *C. jejuni* in chickens in the absence of FQ selection pressure (Luo et al., 2005). Thus, FQ-resistant *Campylobacter* may persist for a long time even after the use of FQ is terminated (Price et al., 2007).

There are no epidemiological data on human campylobacteriosis or the prevalence and antimicrobial resistance of *Campylobacter* spp. in livestock in Mongolia. Broiler farms are not well established in Mongolia. However, there are layer farms that use formulated feed either produced locally or imported, which might contain antimicrobials for growth promotion. Antimicrobial resistance is a worldwide concern, and the prudent use of antimicrobials and a reduction in the use of medically important antimicrobials in food-producing animals are strongly recommended to reduce the risk of antimicrobial-resistant bacteria on public health. In Mongolia, campylobacteriosis in humans has not been officially reported so far owing to the lack of accurate diagnosis for *Campylobacter* infection in human medicine. Consumption of chicken meat is increasing in Mongolia. Basically, there is no culture of eating under-cooked or raw chicken meat. Eggs are consumed in a variety of situations, but they are basically cooked. However, nowadays, because of the increasing Westernization of food culture particularly in urban areas, chicken meat and eggs are used in a variety of dishes including ready-to-eat foods such as salads. Thus, there is a potential risk of occurrence of foodborne *Campylobacter* infection from chicken origin in Mongolia. This study aimed to uncover the prevalence of *Campylobacter* spp. in chickens in Mongolia and their antimicrobial resistance.

MATERIALS AND METHODS

Sampling

Layer farms are clustered in Khan-Uul district, located just west of Ulaanbaatar. There are few industrialized broiler farms in Mongolia. One broiler farm is in Nalaikh district, located just east of Ulaanbaatar. Cloacal swabs of chickens from 4 layer farms and one broiler farm in Mongolia were collected in 2015 (Table 1). Sterilized cotton swabs were used for sampling. We collected cloacal swabs from more than 1-yr-old layers and less than 42-day-old broilers. Cloacal swabs were directly placed in tubes containing 5 mL of Brucella broth (Becton Dickinson, Franklin Lakes, NJ) containing 1× Preston selective supplement (Oxoid, Basingstoke, UK) and 5% lysed horse blood (Nippon Bio-Test Laboratories Inc., Asaka, Japan) (Preston broth). Immediately after collection, they were transported in cold transport containers with ice packs. Within 3 h after the sampling, incubation was started at 42°C.

Isolation of Campylobacter spp.

Cloacal swabs in Preston broth were incubated for 24 h at 42°C under microaerobic conditions using AnaeroPack-MicroAero (Mitsubishi Gas Chemical Co. Inc., Tokyo, Japan). Subsequently, one loop of the culture was inoculated in Campylobacter Blood-Free Selective Agar Base (Oxoid) containing 1× charcoal cefoperazone deoxycholate agar (**CCDA**) selective supplement (Oxoid) (modified CCDA agar) and incubated for 48 h at 37°C under microaerobic conditions. Colonies on the modified CCDA agar plates were picked up using sterilized needles and inoculated into Campylobacter Blood-Free Selective Agar Base (Oxoid) without CCDA supplement to analyze the growth under microaerobic and anaerobic conditions. AnaeroPak-Anaero (Mitsubishi Gas Chemical Co. Inc.) was used for anaerobic culture. Bacteria that grew under microaerobic conditions, but seldom under anaerobic conditions, were selected and subjected to Gram staining.

Identification of Campylobacter spp.

Bacteria grown in Brucella broth were diluted with Mili-Q water to a ratio of 1:10, and DNA was extracted by boiling. Debris was removed by centrifugation at 18,000× *g* for 5 min. Multiplex PCR targeted for the *cdtB* and *cdtC* gene (Asakura et al., 2008) was used for identification of *C. jejuni*/*C. coli*/*C. fetus*. Multiplex PCR was carried out using the AmpliTaq Gold GC360 Master Mix (Thermo Fisher Scientific, Waltham, MA) using 95°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and elongation at 72°C for 40 s. The 16S rDNA fragment was amplified using primers 5F (5′-TTGGAGAGTTT-GATCCTGGCTC-3′) and 810R (5′-GGCGTGGACTTCCAGGGTATCT-3′) at 95°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and elongation at 72°C for 40 s. Species of the isolates were also analyzed by Microflex matrix-assisted laser desorption ionization (**MALDI**) time-of-flight mass spectrometry using the MALDI Biotyper Compass (Bruker Daltonics, Bremen, Germany). Matrix-assisted laser desorption ionization mass profiles were analyzed using a Biotyper Compass library (version 9.0.0.0; Bruker Daltonics).

Table 1. Summary of the isolation of *Campylobacter* spp. from chickens in Mongolia.

Farm[2] ID	Layer/ broiler	No. of samples	No. of C. spp. isolates	No. of *C. jejuni* isolates[1]							No. of *C. coli* isolates[1]						
				Total	TET^R	TET^R–FQ^R	TET^{R-} EM^R–FQ^R	Total TET^R	Total FQ^R	Total EM^R	Total	TET^R	TET^R–FQ^R	TET^{R-} EM^R–FQ^R	Total TET^R	Total FQ^R	Total EM^R
A	Layer	15	5	3	1	2	0	3	2	0	2	2	0	0	2	0	0
B	Layer	17	13	12	9	3	0	12	3	0	1	0	0	1	1	1	1
C	Layer	12	2	0	0	0	0	0	0	0	2	0	0	2	2	2	2
D	Layer	12	11	10	3	7	0	10	7	0	1	0	1	0	1	1	0
E	Broiler	15	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Total	71	31	25	13	12	0	25	12	0	6	2	1	2	6	4	3
			43.7%[3]	35.2%[3]	52.0%[4]	50.0%[4]	0%[4]	100%[4]	50.0%[4]	0%[4]	8.5%[3]	33.3%[5]	16.7%[5]	50.0%[5]	100%[5]	66.7%[5]	50.0%[5]

[1]TET^R: tetracycline resistant; TET^R-FQ^R: tetracycline and fluoroquinolone resistant; TET^R-EM^R-FQ^R: tetracycline, erythromycin, and fluoroquinolone resistant.
[2]Sizes of layer farms A, B, C, and D were 150,000, 18,000, 25,000, 4,000 layers, respectively, whereas the size of the broiler farm was 80,000 broilers.
[3]Percentages to the total sample.
[4]Percentages to the total *C. jejuni* isolates.
[5]Percentages to the total *C. coli* isolates.

Antimicrobial Resistance Test

Antimicrobial resistance of the isolates was analyzed using the E-test. E-test strips of tetracycline (**TET**), erythromycin (**EM**), nalidixic acid (**NA**), ciprofloxacin (**CPFX**), and norfloxacin were obtained from Biomerieux (France, Marcy l'Etoile). The turbidity of isolates grown in Brucella broth was adjusted to approximately 0.5 MacFarland standard, and the culture was spread on Mueller-Hinton agar with 5% lysed horse blood using a cotton swab. E-test strips were placed on the agar plate and incubated for 48–72 h at 37°C under microaerobic conditions. Minimum inhibitory concentrations (**MIC**) were determined in at least 2 independent experiments. *C. jejuni* ATCC33560 (kindly provided by Dr. Yutaka Tamura, Rakuno Gakuen University, Japan) was used for quality control of the E-test. We determined the antimicrobial resistance as per the Clinical and Laboratory Standards Institute (**CLSI**) M45 third edition for EM ($\geq$32 μg/mL), CPFX ($\geq$4 μg/mL), and TET ($\geq$16 μg/mL) and as per the Japanese Veterinary Antimicrobial Resistance Monitoring System for NA ($\geq$32 μg/mL). For norfloxacin, we used MIC_{50} ($\geq$64 μg/mL) deduced from data obtained in this study.

Genetic and Molecular Analyses

The presence of the *tet*(O) gene, which confers TET resistance to *Campylobacter*, was analyzed as previously described by Mazi et al., 2008. The presence of the *ermB* gene, which is responsible for EM resistance, was analyzed by PCR using the primer set reported by Zhang et al., 2016. Nucleotide substitutions at nts 2,074 and 2,075 of 23S rDNA, which are known to be associated with macrolide resistance, were analyzed using the method described by Vacher et al., 2003. The *gyrA* gene fragments were amplified using primers gyrA 135F and gyrA 553R that amplify the 419-bp gene fragment of the *gyrA* gene to analyze codon 86 (Bakeli et al., 2008). Nucleotide sequences of the amplified fragments were determined using the BigDye terminator 3.1 cycle sequencing kit and 3130-Avant Genetic Analyzer (Applied Biosystems, Waltham, MA).

Multilocus Sequencing Typing and Phylogenetic Analyses

Multilocus sequencing typing (**MLST**) was carried out as per the *Campylobacter* Multilocus Sequence Type website (https://pubmlst.org/campylobacter/) by nucleotide sequencing of 7 housekeeping genes: *aspA* (409 base pairs [bp], nt 668–1,077), *glnA* (457 bp, nt 242–698), *gltA* (399 bp, nt 321–719), *glyA* (403 bp, nt 392–794), *tkt* (439 bp, nt 247–685), *uncA* (482 bp, nt 676–1,157), and *pgm* (435 bp, nt 436–870). The nucleotide sequences were combined, and a total of 3,024 nts were used for phylogenetic analysis using Molecular Evolutionary Genetics Analysis, version 7.0 (Kumar et al., 2016).

RESULTS AND DISCUSSION

Isolation of Campylobacter spp. From Chickens in Mongolia

Seventy-one chicken cloacal swabs were collected from the 5 farms, and 31 *Campylobacter* spp. were isolated from the 4 layer farms (43.7%), whereas no *Campylobacter* spp. were isolated from the broiler farm (Table 1). *Campylobacter* spp. were defined as microaerophilic, gram-negative, and catalase- and oxidase-positive species. Multiplex PCR and nucleotide sequences of 16S rDNA revealed that 25 and 6 isolates were identified as *C. jejuni* and *C. coli*, respectively (Table 2). Species of isolates determined genetically were completely identical to the species that were determined using the MALDI Biotyper.

Antimicrobial Resistance of C. jejuni/C. coli Isolates

All isolates were highly resistant to TET (Table 1). The lowest MIC recorded was 32 μg/mL, and the majority exhibited an MIC >256 μg/mL (Table 2). PCR amplification of the *tet*(O) gene showed that 29 of the 31 TET-resistant *C. jejuni*/*C. coli* isolates possessed the *tet*(O) gene (Table 2). Macrolides are the primary choice for treating patients with severe or prolonged campylobacteriosis. Three of the 6 *C. coli* isolates were resistant to EM (Tables 1 and 2). Two isolates (15J-C2 and 15J-C3) carried the *erm*(B) gene that has been found in EM-resistant *C. coli* in China (Zhang et al., 2016). The other isolate (15M-B10) harbored A-to-G nucleotide substitution at nt 2,075 of 23S rDNA, which confers macrolide resistance (Vacher et al., 2003) (Table 2). No EM-resistant *C. jejuni* was isolated in this study. Sixteen *C. jejuni*/*C. coli* isolates (51.6%), including 12 *C. jejuni* (38.7%) and 4 *C. coli* (12.9%) isolates, were highly resistant to NA and FQ (Table 1). The CPFX-resistant *C. jejuni*/*C. coli* (CLSI breakpoint: $\geq$4 μg/mL) isolate showed an MIC higher than 16 μg/mL, the TET-resistant *C. jejuni*/*C. coli* (CLSI breakpoint: $\geq$16 μg/mL) isolate showed an MIC higher than 32 μg/mL, and the NA-resistant *C. jejuni*/*C. coli* (JVARN breakpoint: $\geq$32 μg/mL) isolate showed an MIC higher than 256 μg/mL (Table 2). Consistent with the results of the E-test, all the quinolone/FQ-resistant *C. jejuni*/*C. coli* isolates possessed codon 86-Ile of GyrA (Table 2), which is caused by a single-nucleotide substitution at nt 257 (A<u>C</u>A to A<u>T</u>A).

Multilocus Sequencing Typing and Phylogenetic Analyses

To characterize the molecular genetic properties of the isolates, we selected 11 *C. jejuni* isolates and 5 *C. coli* isolates based on the farms, the scale of the farms, number of isolates in each farm, and antibiograms for MLST analysis (Table 2). The sequence type (**ST**) of the *C.*

Table 2. Antimicrobial resistance of *C. jejuni*/*C. coli* isolates.

Sample ID[1]	Species	EM	TET	NA	NFLX	CPFX	*tet*(O) PCR	*erm*B PCR	23S rDNA[2]	GyrA codon 86	ST	CC
		MIC (μg/mL)									MLST[3]	
15M-A3	*C. jejuni*	1.5	>256	>256	>256	>32	+	−	−	Ile	UA	UA
15M-A5	*C. jejuni*	2	>256	1.5	1	0.19	+	−	−	Thr	−	−
15M-A12	*C. jejuni*	1.5	>256	>256	>256	>32	+	−	−	Ile	UA	UA
15M-B1	*C. jejuni*	4	>256	>256	>256	>32	+	−	−	Ile	7250	UA
15M-B2	*C. jejuni*	3	>256	4	0.75	0.75	+	−	−	Thr	49	49
15M-B3	*C. jejuni*	4	>256	>256	>256	>32	+	−	−	Ile	UA	UA
15M-B5	*C. jejuni*	0.25	>256	3	2.5	0.125	+	−	−	Thr	−	−
15M-B7	*C. jejuni*	1.5	>256	1.5	1.5	0.19	+	−	−	Thr	6393	21
15M-B11	*C. jejuni*	2	64	1.5	0.5	0.125	+	−	−	Thr	−	−
15M-B13	*C. jejuni*	3	>256	3	1	0.19	+	−	−	Thr	−	−
15M-B14	*C. jejuni*	1.5	>256	1.5	1.0	0.25	+	−	−	Thr	−	−
15M-B17	*C. jejuni*	4	>256	>256	>256	>32	+	−	−	Ile	7250	UA
15M-B18	*C. jejuni*	1	64	1	0.38	0.25	+	−	−	Thr	UA	UA
15M-B19	*C. jejuni*	3	128	2	0.5	0.19	+	−	−	Thr	−	−
15M-B20	*C. jejuni*	2	>256	1.5	1	0.19	+	−	−	Thr	−	−
15J-D1	*C. jejuni*	0.75	128	>256	>256	>32	+	−	−	Ile	22	22
15J-D2	*C. jejuni*	4	>256	>256	>256	>32	+	−	−	Ile	−	−
15J-D3	*C. jejuni*	0.5	32	2	0.125	0.125	+	−	−	Thr	464	464
15J-D5	*C. jejuni*	3	>256	>256	>256	>32	+	−	−	Ile	−	−
15J-D6	*C. jejuni*	0.5	>256	NT	>256	NT	+	−	−	Ile	−	−
15J-D7	*C. jejuni*	2	>256	>256	>256	>32	+	−	−	Ile	UA	UA
15J-D8	*C. jejuni*	1.5	>256	>256	>256	>32	+	−	−	Ile	−	−
15J-D10	*C. jejuni*	2	>256	>256	>256	>32	+	−	−	Ile	−	−
15J-D11	*C. jejuni*	1	64	1	0.25	0.64	+	−	−	Thr	−	−
15J-D12	*C. jejuni*	2	>256	0.125	0.5	2	+	−	−	Thr	−	−
15M-A6	*C. coli*	4	>256	2	1.5	0.19	+	−	−	Thr	−	−
15M-A9	*C. coli*	2	>256	2	0.38	0.125	−	−	−	Thr	3753	828
15M-B10	*C. coli*	>256	96	>256	64	16	−	−	nt 2075 A>>G	Ile	898	828
15J-C2	*C. coli*	>256	>256	>256	>256	>32	+	+	−	Ile	872	828
15J-C3	*C. coli*	>256	>256	>256	>256	>32	+	+	−	Ile	872	828
15J-D4	*C. coli*	1.5	256	>256	>256	>32	+	−	−	Ile	898	828

Abbreviations: CPFX, ciprofloxacin; EM, erythromycin; MLST, multilocus sequencing typing; NA, nalidixic acid; NFLX, norfloxacin; TET, tetracycline.

[1]15M and 15J mean year (2015) and month (M, May; J, July) of sampling, whereas A to D mean farms. Numbers in the last indicate individual chickens.
[2]nt 2075 A>>G: nucleotide substitution (A to G) at nt 2,075; −: no nucleotide substitution.
[3]ST: sequence type; CC: clonal complex; UA: unassigned; −: not tested.

jejuni isolates, 15M-B1 and 15M-B17 (ST7250), 15M-B2 (ST49), 15M-B7 (ST6393), 15J-D1 (ST22), and 15J-D3 (ST464), could be determined. Data on 4 clonal complexes (**CC**) (CC21, CC22, CC49, and CC464) were deposited worldwide. To confirm the genetic diversity of *C. jejuni* isolates in Mongolia, the nucleotide sequences of the 7 genes for MLST analysis were combined, and a phylogenetic tree was constructed (Figure 1). Consistent with the ST and CC distribution, *C. jejuni* isolates from chickens in Mongolia were found to be genetically diverse. Phylogenetic analysis showed that the 15M-A3 isolate whose ST or CC could not be assigned was close to ST6077 (Figure 1). *C. jejuni* with ST6077 and similar allele-type profiles (6 alleles were matched), such as ST7251, ST8913, and ST9975, have been isolated in China (*Campylobacter* Sequence Typing database). The 15J-D7 isolate was found to be close to ST4258; *C. jejuni* with ST4258 and similar allele type profiles (6 alleles were matched) have been isolated in Thailand (ST2276) and China (ST4323, ST7185, ST7250, and so on) (*Campylobacter* Sequence Typing database). Although the ST could not be determined, 5 allele types of the 15M-A12 isolate were matched to theisolates in CC 574, such as ST7357 (Figure 1), ST2145, ST2279, ST2895, and ST6915, which were mainly deposited from Asian and Oceanian countries

and a few from European countries. These results suggest a geographical dependent distribution of *C. jejuni* to some extent. Phylogenetic analysis also showed that different types of *C. jejuni* existed on the same farm, for example, 15M-A3 and 15M-A12 isolates in farm A and 15M-B2 and other isolates in farm B.

The ST of *C. coli* isolates were identified as ST872, ST898, and ST3753 (Table 2). Although the present study had a limited number of *C. coli* isolates, ST898 appeared phylogenetically distinguishable from ST872 and ST3753. In addition, there were 3 different antimicrobial resistance types, TET-resistant, TET- and FQ-resistant, and TET-, FQ-, and EM-resistant *C. coli* (Tables 1 and 2). These results suggest the diversity of *C. coli* in Mongolia.

The average occurrence of TET-resistant *C. jejuni*/*C. coli* isolated from broilers in member states of the European Union was reportedly 50.7%. Several countries such as Italy and Spain showed a very high TET-resistant *C. jejuni*/*C. coli* occurrence, whereas the occurrence level in the Nordic countries remained low (EFSA and ECDC, 2018). This is probably due to the difference in the use of antibiotics in farm animals among these countries. Here, all the *C. jejuni*/*C. coli* isolates were TET resistant, suggesting the use of TET in layer farms. Thus, in August 2019, a questionnaire survey was
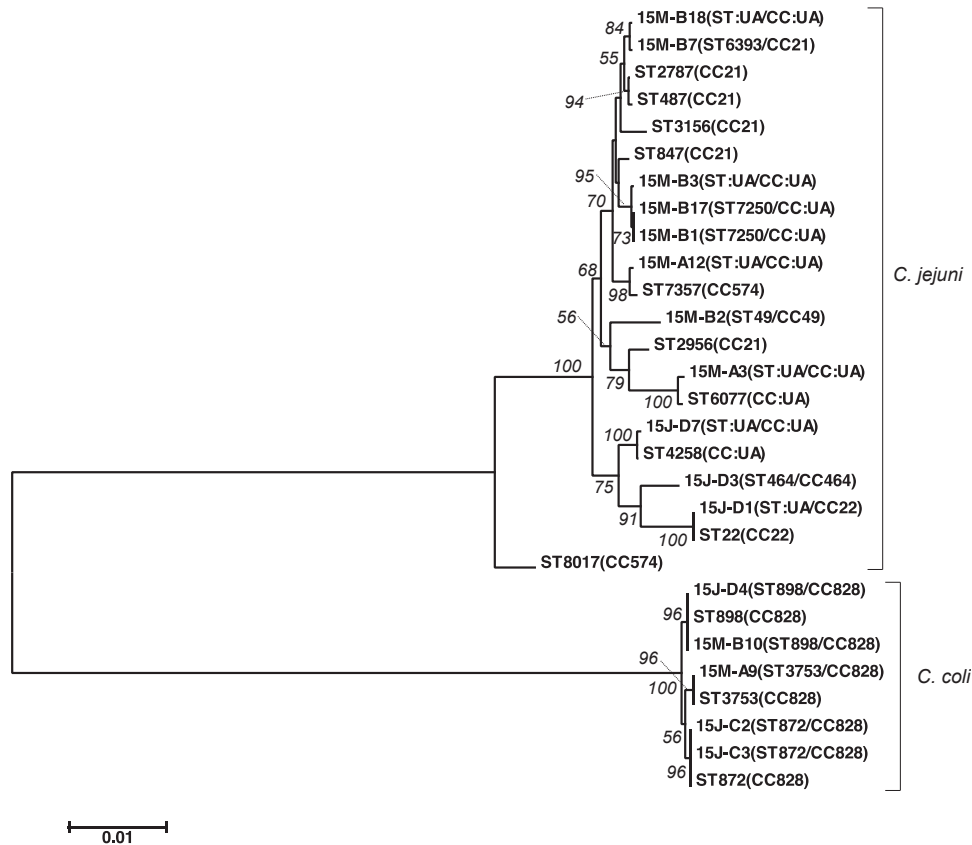
Figure 1. Phylogenetic analysis of *C. jejuni* and *C. coli* isolated from chickens in Mongolia. Nucleotide sequences of a total of 3,024 nt from *aspA*, *glnA*, *gltA*, *glyA*, *tkt*, *uncA*, and *pgm* were used for phylogenetic analysis. Nucleotide sequences of ST22 (CC22), ST487 (CC21), ST847 (CC21), ST2956 (CC21), ST2787 (CC21), ST3156 (CC21), ST4258 (CC: unassigned [UA]), ST6077 (CC: UA), ST7357 (CC574), and ST8017 (CC574) for *C. jejuni* and of ST872 (CC828), ST989 (CC828), and ST3753 (CC828) for *C. coli* were obtained from the *Campylobacter* Sequence Typing database. The percentages (>50%) of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) are shown italic numbers next to the branches.

carried out in the 7 layer farms including 4 farms, where *C. jejuni/C. coli* was isolated in 2015, to understand the situation of antimicrobial use in chicken farms. All the farms did not know if their formulated feeds, whether imported or locally produced, contained antimicrobials for growth promotion. Five of the 7 farms used TET for treatment of sick chickens, and they administered those antibiotics by adding to feed or drinking water from 2 to 7 d. Although the use of TET for growth promotion cannot be excluded, the results of the questionnaire suggest that the use of TETs in poultry resulted in high levels of TET-resistant *C. jejuni/C. coli* from chickens in Mongolia. Two *C. coli* isolates, 15M-A9 and 15M-B10, were negative for the *tet*(O) gene as per PCR. The heterogeneity of the *tet*(O) gene might explain the lack of amplification (Hormeño et al., 2020); further genetic analysis is required to clarify this point.

The high percentage of FQ resistance in *C. jejuni/C. coli* in layer farms in Mongolia suggests the use of FQ for treatment of chickens. However, no evidence for the use of FQ was obtained from the results of the questionnaire. It is suggested that *C. jejuni* acquires enhanced fitness for in vivo colonization in the absence of FQ by the single-nucleotide substitution at nt 257 of the *gyrA* gene (Luo et al., 2005). The slow rate of declining FQ-resistant *C.*

jejuni/C. coli was reported after treatment had ceased (Humphrey et al., 2007; Price et al., 2007). No trend in decreasing CPFX-resistant *C. jejuni* levels in European countries was observed except in Slovenia (EFSA and ECDC, 2018), although the use of FQ in food-producing animals has been restricted. These facts suggest that once FQ-resistant *C. jejuni* infect, they may colonize the host for a long period owing to little fitness cost to FQ resistance in *C. jejuni* in the absence of FQ. The spread of FQ-resistant *C. jejuni/C. coli* by clonal expansion and/or horizontal gene transfer (Sproston et al., 2018), even without the confirmation of the use of FQ as per our questionnaire, also accounts for the high percentage of FQ-resistant *C. jejuni/C. coli* isolates from chickens in Mongolia.

It is reported that the prevalence of EM resistance in *C. jejuni* is lower than that in *C. coli* (Bolinger and Kathariou, 2017). Consistent with this, 3 EM-resistant isolates were identified as *C. coli*, but no EM-resistant *C. jejuni* were isolated in the present study. One EM-resistant *C. coli* (15M-B10) possessed nucleotide substitution at nt 2,075 of 23S rDNA, which is the common mechanism for conferring macrolide resistance (Vacher et al., 2003); the other 2 isolates harbored the *erm*(B) gene, the product of which confers EM resistance by demethylating a single adenine

residue of 23S rRNA (Zhang et al., 2016). *C. coli* carrying *erm*(B) are thought to emerge in 2011–2012 by the horizontal gene transfer and are largely confirmed in China (Wang et al., 2014); subsequently, only a few *erm*(B)-carrying *C. jejuni*/ *C. coli* isolates were found in European countries and the USA (Chen et al., 2018; Elhadidy et al., 2019). The detection of *erm*(B)-carrying *C. coli* (15J-C2 and 15J-C3 in Table 2) in Mongolia suggests a wider distribution of the *erm*(B) gene in *C. coli.* ST872 is one of the major EM-resistant *C. coli* ST, and *erm*(B)-carrying ST872 has been isolated in China (Wang et al., 2014).

To the best of our knowledge, this is the first report on the isolation and state of antimicrobial resistance of *C. jejuni*/ *C. coli* in farm animals from Mongolia. Although there are no epidemiological data on human campylobacteriosis in Mongolia, the presence of multidrug-resistant *C. jejuni*/ *C. coli* in farm animals indicates the necessity of countermeasures for prudent use of antimicrobials in farm animals.

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DISCLOSURES

The authors declare no conflicts of interest.

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