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

Zolzaya Byambajav,* Erdenebat Bulgan,*,† Yuji Hirai,† Momoko Nakayama,‡ Misaki Tanaka,‡ Yurika Nitta,‡ Akio Suzuki,‡ Takashi Umemura,‡ Bold Altankhuu,# Alimaa Tsagaan #, Batbaatar Vanaabaatar,# Erdenebaatar Janchiydorj,# Nyam-Osor Purevdorj,‡ Narantuya Ayushjav,*,# Takeshi Yamasaki,‡ and Motohiro Horiuchi‡,#

*Ulaanbaatar Veterinary Department, Laboratory of Veterinary Sanitation and Hygiene, Ulaanbaatar 16050, Mongolia; †Ulaanbaatar Veterinary Department, Laboratory of Diagnostic and Surveillance, Ulaanbaatar 16050, Mongolia; ‡Laboratory of Veterinary Hygiene, Faculty of Veterinary Medicine, Graduate School of Infectious Diseases, Hokkaido University, Sapporo 060-0818 Japan; ‡Project Office of Japan International Cooperation Agency, Mongolian University of Life Science, Ulaanbaatar 17024, Mongolia; #Laboratory of Infectious Disease and Immunology, Institute of Veterinary Medicine, Mongolian University of Life Science, Ulaanbaatar 11000, Mongolia; ‡School of Veterinary Medicine, Mongolian University of Life Science, Ulaanbaatar 17024, Mongolia; and ‡Global Station for Zoonosis Control, Global Institute for Collaborative Research and Education, Hokkaido University, Sapporo 001-0020, Japan

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: A Ca A). 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

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*Corresponding author: horiuchi@vetmed.hokudai.ac.jp

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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. Antibiotic 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 is 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 undercooked 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. AnaeroPack-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 rRNA fragment was amplified using primers 5F (5’-TTGGAGAGTT-GATCCTGGCTC-3’) and 810R (5’-GCGTGACTTCCAGGTATCT-3’) at 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. 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 ID | Layer/broiler | No. of samples | No. of *C. jejuni* isolates | No. of *C. coli* isolates | Total TET<sup>R</sup> | Total TET<sup>R</sup>-FQ<sup>R</sup> | Total TET<sup>R</sup>-EM<sup>R</sup>-FQ<sup>R</sup> | Total FQ<sup>R</sup> | Total EM<sup>R</sup> | Total TET<sup>R</sup> | Total TET<sup>R</sup>-FQ<sup>R</sup> | Total TET<sup>R</sup>-EM<sup>R</sup>-FQ<sup>R</sup> | Total FQ<sup>R</sup> | Total EM<sup>R</sup> |
|---------|---------------|----------------|----------------------------|---------------------------|------------------------|-------------------------------|--------------------------------|----------------|----------------|----------------|-------------------------------|--------------------------------|----------------|----------------|
| A       | Layer         | 15             | 5                          | 3                         | 1                      | 2                             | 0                           | 3              | 2              | 0              | 2                             | 0                           | 2              | 0              |
| B       | Layer         | 17             | 13                         | 12                        | 9                      | 3                             | 0                           | 12                          | 3              | 0              | 1              | 0                             | 0                           | 1              | 1              |
| C       | Layer         | 12             | 2                          | 0                         | 0                      | 0                             | 0                           | 0                           | 0              | 0              | 2              | 0                             | 0                           | 2              | 2              |
| D       | Layer         | 12             | 11                         | 10                        | 3                      | 7                             | 0                           | 10                          | 7              | 0              | 1              | 0                             | 1                           | 0              | 1              |
| E       | Broiler       | 15             | 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              |

1TET<sup>R</sup>: tetracycline resistant; TET<sup>R</sup>-FQ<sup>R</sup>: tetracycline and fluoroquinolone resistant; TET<sup>R</sup>-EM<sup>R</sup>-FQ<sup>R</sup>: tetracycline, erythromycin, and fluoroquinolone resistant.
2Sizes 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.
3Percentages to the total sample.
4Percentages to the total *C. jejuni* isolates.
5Percentages 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.

Antimicrobial Resistance 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: ≥4 μg/mL) isolate showed an MIC higher than 16 μg/mL, the TET-resistant C. jejuni/C. coli (CLSI breakpoint: ≥16 μg/mL) isolate showed an MIC higher than 32 μg/mL, and the NA-resistant C. jejuni/C. coli (JVARN breakpoint: ≥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 (ACA to ATA).

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 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).

Multiple Sequence Typing and Phylogenetic Analyses

Multiple sequence 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), glmA (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).
| Sample ID | Species     | EM  | TET | NA  | NFLX | CPFX | tet(O) PCR | ermB PCR | 23S rDNA2 | GyrA codon 86 | ST | CC |
|-----------|-------------|-----|-----|-----|------|------|------------|-----------|------------|----------------|----|----|
| 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  | +          | –         | –          | Thr 7250       | UA | –  |
| 15M-B2    | C. jejuni   | 3   | >256| 4   | 1.0  | 0.75 | +          | –         | 4          | 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    | 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 | >256| 3   | 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: nucleotide substitution (A to G) at nt 2075; –: no nucleotide substitution.

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
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 \textit{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 \textit{erm}(B)-carrying \textit{C. jejuni}/\textit{C. coli} isolates were found in European countries and the USA (Chen et al., 2018; Elhadidy et al., 2019). The detection of \textit{erm}(B)-carrying \textit{C. coli} (15J-C2 and 15J-C3 in Table 2) in Mongolia suggests a wider distribution of the \textit{erm}(B) gene in \textit{C. coli}. ST872 is one of the major EM-resistant \textit{C. coli} ST, and \textit{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 \textit{C. jejuni}/\textit{C. coli} in farm animals from Mongolia. Although there are no epidemiological data on human campylobacteriosis in Mongolia, the presence of multidrug-resistant \textit{C. jejuni}/\textit{C. coli} in farm animals indicates the necessity of countermeasures for prudent use of antimicrobials in farm animals.

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Nucleotide sequences that were not identical to those in Campylobacter Sequence Typing database, \textit{pgm} genes for 15M-A3, 15M-A12, and 15J-D1 and \textit{glnA} gene for 15M-A3, have been submitted to DDBJ (DNA Data Bank of Japan) and have been assigned the accession numbers, LC582535, LC582536, LC582534, and LC582537, respectively.

**DISCLOSURES**

The authors declare no conflicts of interest.

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