Emergence of Genetic Diversity and Multi-Drug Resistant Campylobacter jejuni From Wild Birds in Beijing, China

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Campylobacter jejuni (C. jejuni) is considered as an opportunistic zoonotic pathogen that may cause gastroenteritis in humans and other animals. Wild birds may be as potential vectors of C. jejuni around urban and suburban areas. Here, 520 samples were collected from 33 wild bird species in urban and suburban areas, Beijing. In total 57 C. jejuni were isolated from seven species. It was found that Nineteen (33.33%, 19/57) isolates were resistant to at least one of 11 antibiotics, especially streptomycin (36.84%) and four isolates resistant to all. Nineteen (33.33%, 19/57) isolates were multi-drug resistance. Multilocus sequence typing (MLST) analysis of the isolates showed that 36 different sequence types (STs) belonged to four Cional complexes and unassigned. Twenty STs (55.56%) and six alleles among them were first detected. Virulence genes including flaA, cadF, and the cytolethal distending toxin (CDT) gene cluster, were detected in all isolates, but truncated cdt gene clusters only detected in the isolates from the crow, daurian jackdaw and silver pheasant. In conclusion, it was the first detection of C. jejuni involved truncated cdt gene clusters from the silver pheasant. These wild birds around urban and suburban areas may pose potential public health problems as reservoir vectors of C. jejuni.

Keywords: emergence, Campylobacter jejuni, wild birds, MLST, multi-resistance, CDT gene cluster

INTRODUCTION

Campylobacter jejuni is a gram-negative spiral rod bacterium that causes gastroenteritis in humans and other animals. In 2016, a total of 246,307 confirmed cases of human campylobacteriosis were reported in the European Union (EU), representing almost 70% of all the reported human cases of zoonoses (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2017).
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significantly increasing trend over the period 2008–2016. Compared with European countries, reports on campylobacteriosis in Asian countries, including China are limited, and the campylobacteriosis prevalence in humans is generally low (Wang et al., 2015).

The clinical presentation of campylobacteriosis includes watery or bloody diarrhea lasting for a median duration of 6 days, with 80% of patients having cramps and fever (Friedman et al., 2004). In some cases, the infection can lead to extra-intestinal complications and severe autoimmune disorders, such as pancreatitis, cholecystitis, obstructive hepatitis, Guillain-Barré syndrome (GBS), and Miller Fisher syndrome. Generally, the patients with campylobacteriosis are self-limited and disappear after 1 week without any specific treatment. However, in some relatively severe cases, antimicrobial chemotherapy is required. Macrolides and quinolones are commonly used as first-line therapies, and tetracycline, doxycycline, and chloramphenicol are alternative drugs (Ma et al., 2017).

Resistant strains and multi-drug resistance (MDR) strains are increasingly reported in humans and animals which may be induced by increasing use of antibiotics in humans, domestic animals and poultry (Franciçek et al., 2012; Karaiosk and Giamarello, 2014; Alsan et al., 2015). *C. jejuni* has a high capacity to transfer genetic elements that lead to the combination of different strains. This characteristic may allow of *C. jejuni* transfer antibiotic resistance genes easily (de Boer et al., 2002; Wilson et al., 2003; Avrain et al., 2004). The abuse of antibiotics could increase the selection pressure and decrease the effectiveness of antibiotics further (Klena et al., 2004; Laxminarayan et al., 2013).

In *Campylobacter*, mutations in the 23S rRNA genes shown to contribute to macrolide resistance (Payot et al., 2006). *C. jejuni* resistance to tetracycline is usually associated with the tet(O) gene, which is carried on transmissible plasmids or located chromosomally. Ribosomal protein – Tet(O), which encoded by tet(O) gene can confer the resistance by displacing tetracycline from its primary binding site on the ribosome (Connell et al., 2003; Gibreel et al., 2004b). *C. jejuni* resistance to fluoroquinolones is mainly associated with mutations in the DNA gyrase gene (gyrA) (Smith and Fratamico, 2010). Accordingly, it is necessary to monitor the antibiotic resistance and research the antibiotic resistance mechanism of *C. jejuni* in wildlife.

The process of infection involved in adhesion, colonization, invasion and toxin production, especially, flaA genes, cadF genes, and cdt gene clusters are necessary for cell pathology and virulence in humans (Wei et al., 2018). These three virulence genes are frequently researched in isolates from various hosts, especially humans and poultry. However, little is known about these virulence genes in wild bird isolates (Wei et al., 2018).

The consumption of feces-contaminated raw or undercooked poultry has been identified as an important transmission vehicle for human campylobacteriosis (Wei et al., 2018). However, there is evidence that non-food-borne exposure of *C. jejuni* may contribute to the burden of illness as well. Thus contamination of the environment by domestic and wild birds feces may constitute an additional risk for human infection via environmental water or direct contact with them (French et al., 2009). Some studies suggested that *C. jejuni* is a commensal microorganism in the intestine of many wild and domestic animals, particularly avian species, and they can be natural reservoirs of *C. jejuni* (Oravcova et al., 2014). Furthermore, migratory birds may play a direct
Anatidae Swan Goose (*Anser*)
Anatidae Bar-headed Goose (*Anser*)
Anatidae Black Swan (*Cygnus*)

**RESULTS**

**The Prevalence of *Campylobacter jejuni***

In total, 520 samples were collected from 33 species and 12 sites during the 4 months in Beijing, China (Figure 1). *C. jejuni* was isolated from 57 samples (10.96%, 57/520) including seven species. In the positive *C. jejuni* samples, 24.19% (15/62) were from crow (*Corvus* sp.), 51.67% (31/60) were from daurian jackdaw (*Corvus dauricus*), 14.29% (1/7) were from silver pheasant (*Lophura nycthemera*), 8.57% (6/70) were from mallard (*Anas platyrhynchos*), 6.25% (1/16) were from mandarin duck (*Aix galericulata*), 12.5% (1/8) were from black swan (*Cygnus atratus*) and 7.69% (2/26) were from crow (*Corvus pica*). Crows (*Corvus* sp.) and Daurian jackdaws (*Corvus dauricus*) had significantly higher positive rates than other species (*P < 0.007*). Mallard (*Anas platyrhynchos*), mandarin duck (*Aix galericulata*) and black swan (*Cygnus atratus*) showed a relatively low positive rate between 6.25 and 12.5%. Silver Pheasant (*Lophura nycthemera*), family Phasianidae showed a similar positive rate as chicken, which is one of the most important reservoirs of *C. jejuni*. Rock pigeon (*Columba livia*), had relatively lower positive rate than others. This is the first time that *C. jejuni* has been isolated from black swan (*Cygnus atratus*) (Table 1).

**Table 1 | Prevalence of *Campylobacter jejuni* isolates from different species.**

| Family           | Species                  | Number | Isolation rate (%) | Family           | Species                  | Number | Isolation rate (%) |
|------------------|--------------------------|--------|--------------------|------------------|--------------------------|--------|--------------------|
| Accipitridae     | European Sparrowhawk     | 3      | 0                  | Falconidae       | Eurasian Kestrel         | 19     | 0                  |
|                  | (Accipiter nisus)        |        |                    |                  | (Falco tinnunculus)      |        |                    |
| Accipitridae     | Gray-faced Buzzard       | 1      | 0                  | Falconidae       | Eurasian Hobby           | 7      | 0                  |
|                  | (Butastur indicus)       |        |                    |                  | (Falco subbuteo)         |        |                    |
| Accipitridae     | Eurasian Goshawk         | 1      | 0                  | Falconidae       | Peregrine Falcon         | 2      | 0                  |
|                  | (Accipiter gentilis)     |        |                    |                  | (Falco peregrinus)       |        |                    |
| Accipitridae     | Common Buzzard           | 7      | 0                  | Falconidae       | Red feet falcon          | 2      | 0                  |
|                  | (Buteo buteo)            |        |                    |                  | (Falco amurensis)        |        |                    |
| Anatidae         | Mallard                  | 70     | 9%                 | Gruidae          | Crane                    | 22     | 0                  |
|                  | (*Anas platyrhynchos*)   |        |                    |                  | (Grus grus)              |        |                    |
| Anatidae         | Mandarin duck            | 16     | 6.25%              | Laridae          | Herring Gull             | 46     | 0                  |
|                  | (*Aix galericulata*)     |        |                    |                  | (Larus argentatus)       |        |                    |
| Anatidae         | Black Swan               | 8      | 12.50%             | Paridae          | Sparrow                  | 50     | 0                  |
|                  | (*Cygnus atratus*)       |        |                    |                  | (Passer montanus)        |        |                    |
| Anatidae         | Swan Goose               | 10     | 0                  | Phasianidae      | Silver Pheasant          | 7      | 14.29%             |
|                  | (*Anser cygnoides*)      |        |                    |                  | (Lophura nycthemera)     |        |                    |
| Anatidae         | Bar-headed Goose         | 7      | 0                  | Phasianidae      | Common Peafowl           | 31     | 0                  |
|                  | (*Anser indicus*)        |        |                    |                  | (Pavo cristatus)         |        |                    |
| Anatidae         | Mute Swan                | 4      | 0                  | Strigidae        | European Scops Owl       | 12     | 0                  |
|                  | (*Cygnus olor*)          |        |                    |                  | (Otus scops)             |        |                    |
| Anatidae         | Swan                    | 3      | 0                  | Strigidae        | Long-eared Owl           | 2      | 0                  |
|                  | (*Cygnus sp.*)           |        |                    |                  | (Asio otus)              |        |                    |
| Ardeidae         | Gray Heron               | 23     | 0                  | Strigidae        | Little Owl               | 1      | 0                  |
|                  | (*Ardea cinerea*)        |        |                    |                  | (Athene noctua)          |        |                    |
| Columbidae       | Rock Pigeon              | 26     | 7.69%              | Strigidae        | Eagle owl                | 2      | 0                  |
|                  | (*Columba livia*)        |        |                    |                  | (Ninox scutulata)        |        |                    |
| Corvidae         | Crow                    | 62     | 24%                | Strigidae        | Tawny Owl                | 1      | 0                  |
|                  | (*Corvus sp.*)           |        |                    |                  | (Strix aluco)            |        |                    |
| Corvidae         | Daurian Jackdaw          | 60     | 52%                | Struthionidae    | Common Ostrich           | 3      | 0                  |
|                  | (*Corvus dauricus*)      |        |                    |                  | (Struthio camelus)       |        |                    |
| Corvidae         | Magpie                   | 1      | 0                  | Sturnidae        | Crested Myna             | 7      | 0                  |
|                  | (*Pica pica*)            |        |                    |                  | (Acrocephalus cristatellus) |    |                    |
| Falconidae       | Saker falcon             | 4      | 0                  |                    |                          |        |                    |
|                  | (*Falco cherrug*)        |        |                    |                  |                          |        |                    |
(6.25%), Temple of Heaven Park (THP) (10.00%), Cuihu Park (CP) (27.78%), Beijing Wildlife Rescue Center (BWRC) (5.56%), Beijing Wildlife Park (BWP) (10.00%), Milu Park (MP) (7.14%), and Niukouyu Wetland Park (NWP) (10.00%). The highest was BNU (55%) which located in an urban area, and the lowest was BWRC (5.46%) which located in the suburb area. The average positive rate in urban areas (17.81%) was 2.36 times higher than in suburb areas (7.66%) (Table 2).

**Multilocus Sequence Typing for *Campylobacter jejuni***

To discover the genetic diversity of *C. jejuni* in wild birds and explore the role of wild birds in disease transmission, the genotype of *C. jejuni* isolates from wild birds were tested by multilocus sequence typing (MLST). MLST, which uses seven genes to build a classification system, is a common way to reveal genetic diversity. Fifty-seven isolates were divided into 36 different sequence types (STs) that clustered into four clonal complexes (CCs) and unassigned.

The same bird species could carry a variety of STs from different individuals (Table 3). Twenty-three STs were identified from 31 *C. jejuni* isolates in daurian jackdaws, and 7 STs from 15 isolates in crows. Overall, 52.6% of novel STs were first discovered in the present study (Table 3), especially more than 10 novel STs were from one or more new allelic genes, and six novel alleles were found (aspA484, aspA485, glnA673, glyA754, tkt718, tkt719) (Supplementary Table S1). Moreover, 84.5% (49 strains) of the STs did not belong to any clonal complex (CC). Three STs (ST-692, ST-52, ST-1275) comprised three STs, and explore the role of wild birds in disease transmission, the genotype of *C. jejuni* isolates showed that nine STs (ST-995, ST-991, ST-951, ST-9191) belonged to ST-692 clonal complex and ST-52, ST-952, and ST-1275 complex comprised three STs, respectively (Table 3).

**Table 2** | Prevalence of *Campylobacter jejuni* isolates from different sites.

| Location | Site       | Sample number | Isolation rate (%) | Average isolation rate (%) |
|----------|------------|---------------|--------------------|---------------------------|
| Urban area | BNU        | 20            | 55.00              |                           |
|           | BRRC       | 64            | 0.00               | 17.81                     |
|           | BP         | 16            | 6.25               |                           |
|           | THP        | 40            | 10.00              |                           |
|           | BWRC       | 72            | 5.56               |                           |
|           | CP         | 90            | 27.78              |                           |
|           | BWP        | 10            | 10.00              |                           |
| Suburb area | WDL        | 22            | 0.00               | 7.56                      |
|           | HL         | 7             | 0.00               |                           |
|           | MP         | 98            | 7.14               |                           |
|           | NWP        | 40            | 10.00              |                           |
|           | XR         | 41            | 0.00               |                           |

1 BNU, Beijing Normal University; BP, Beihai Park; THP, Temple of Heaven Park; CR, Cuihu Park; BWRC, Beijing Wildlife rescue center; BWP, Beijing Wildlife Park; MP, Milu Park; NWP, Niukouyu Wetland Park; BRRC, Beijing Raptor rescue center; WDL, Wild Duck Lake; HL, Hongluo Lake; XR, Xiyu Reservoir.

**Phylogenetic Analysis of *Campylobacter jejuni* Strains**

The minimum spanning tree for 57 *C. jejuni* isolates and other reference isolates from the PubMLST database was constructed (Supplementary Figure S1). The genetic diversity of the *C. jejuni* isolates showed that nine STs (ST-995, ST-991, ST-951, ST-9191) belonged to ST-692 clonal complex and ST-52, ST-952, and ST-1275 complex comprised three STs, respectively (Table 3).

**Table 3** | Clonal complex (CC) and sequence type (ST) distribution of *Campylobacter* in wild bird species.

| CC      | ST | Number | Avian species (No. of isolates) | Site     |
|---------|----|--------|-------------------------------|----------|
| 52      | 52 | 1      | Black Swan (1)                | BERC     |
| 692     | 692| 1      | Mandarin Duck (1)             | BP       |
| 991     | 991| 2      | Mallard (2)                   | NWP      |
| 9191    | 9191| 1      | Mallard (1)                   | BWSC     |
| 952     | 9176| 2      | Crow (2)                      | THP      |
| 1275    | 1540| 1      | Daurian Jackdaw (1)           | CP       |
| U       | 448| 4      | Daurian Jackdaw (4)           | MP       |
| 952     | 951 | 1      | Daurian Jackdaw (1)           | CP       |
| 952     | 953 | 2      | Daurian Jackdaw (2)           | CP       |
| 952     | 995 | 1      | Mallard (1)                   | CP       |
| 2367    | 2367| 2      | Pigeon (2)                    | NWP      |
| 3938    | 3938| 1      | Daurian Jackdaw (1)           | CP       |
| 4069    | 4069| 1      | Daurian Jackdaw (1)           | CP       |
| 4382    | 4382| 2      | Crow (2)                      | BNU      |
| 4571    | 4571| 1      | Crow (1)                      | BNU      |
| 6188    | 6188| 1      | Daurian Jackdaw (1)           | CP       |
| 7805    | 7805| 1      | Daurian Jackdaw (1)           | CP       |
| 9175    | 9175| 4      | Crow (4)                      | BNU      |
| 9177    | 9177| 2      | Daurian Jackdaw (2)           | MP/CP    |
| 9178    | 9178| 2      | Daurian Jackdaw (1)           | MP       |
| 9179    | 9179| 1      | Daurian Jackdaw (1)           | CP       |
| 9180    | 9180| 2      | Daurian Jackdaw (2)           | CP       |
| 9181    | 9181| 1      | Daurian Jackdaw (2)           | CP       |
| 9182    | 9182| 1      | Daurian Jackdaw (2)           | CP       |
| 9183    | 9183| 2      | Daurian Jackdaw (2)           | CP       |
| 9184    | 9184| 1      | Daurian Jackdaw (2)           | CP       |
| 9185    | 9185| 2      | Daurian Jackdaw (2)           | CP       |
| 9186    | 9186| 1      | Daurian Jackdaw (2)           | CP       |
| 9190    | 9190| 2      | Mallard (2)                   | BWRC     |
| 9192    | 9192| 2      | Crow (2)                      | BNU      |
| 9194    | 9194| 1      | Daurian Jackdaw (2)           | CP       |
| 9195    | 9195| 1      | Daurian Jackdaw (3)           | CP       |
| 9196    | 9196| 2      | Crow (2)                      | THP/BNU  |
| 9197    | 9197| 2      | Crow (3)                      | THP      |
| 9222    | 9222| 2      | Daurian Jackdaw (1)           | CP       |
|         |     |        | Silver Pheasant (1)           | BWP      |

1 U, Unassigned clonal complex. 2 Novel STs are indicated in bold. 3 In parentheses, the number of isolates from each bird. 4 BNU, Beijing Normal University; BP, Beihai Park; THP, Temple of Heaven Park; CR, Cuihu Park; BWRC, Beijing Wildlife rescue center; BWP, Beijing Wildlife Park; MP, Milu Park; NWP, Niukouyu Wetland Park; BRRC, Beijing Raptor rescue center; WDL, Wild Duck Lake; HL, Hongluo Lake; XR, Xiyu Reservoir.
ST4069) were found in wild birds and human, 5 STs (ST-995, ST-991, ST-2367, ST-52, ST692, ST1540) in wild birds, chicken and humans, 2 STs (ST-448 and ST-995) in monkeys, wild birds and humans, and 2 STs (ST-52 and ST-995) in 4 hosts (wild birds, humans, chicken, dogs/monkeys). Researches previously reported showed that humans and other animals have the same predominant STs, which was suggested that these animals are important reservoirs of human domestically acquired infections (Olkkola et al., 2016). In this study, the isolates from black swan, mandarin duck, mallard, daurian jackdaw, and rock pigeon were found in humans (Table 4). Therefore these results above indicated that the C. jejuni

### Table 4: Sequence types (STs) and their association certain species and area.

| STs  | Species           | Area                                    | STs  | Species           | Area                                    |
|------|-------------------|-----------------------------------------|------|-------------------|-----------------------------------------|
| 52   | Human stool       | United Kingdom/United States/Brazil/Israel/Botswana/Germany/Sweden/Luxembourg/Switzerland/Canada/Japan/The Netherlands/Australia/Greece | 995  | Human stool       | Sweden                                 |
| Sheep| United Kingdom    |                                         | 3938 | Human stool       | Sweden                                 |
| Chicken | United Kingdom       | United States/New Zealand/Spain/Luxembourg/Senegal/Switzerland/Uruguay                          | 4069 | Human stool       | Canada                                 |
| Cattle| United Kingdom    |                                         | 953  | Daurian Jackdaw   | China                                  |
| Xiangjiang River | China             |                                         | 999  | Starling          | United Kingdom                          |
| Black Swan | China             |                                         | 692  | Daurian Jackdaw   | China                                  |
| Human stool | United Kingdom | Wild birds                             | 6188 | Environmental waters | Luxembourg                         |
| Goose | United Kingdom     | Wild birds                             | 7805 | Wild birds        | Finland                                |
| Goose | United Kingdom     | Environmental waters/United States/New Zealand/New Zealand/Canada                                      |
| Chicken | United Kingdom | Wild birds                             |      |                  |                                        |
| Sheep | New Zealand       | Sheep                                   | 9175 | Daurian Jackdaw   | China                                  |
| Environmental waters | Canada | Wild birds                             | 9176 | Daurian Jackdaw   | China                                  |
| Human stool | United Kingdom/ /United States | Wild birds                             | 9177 | Daurian Jackdaw   | China                                  |
| Wild birds | Sweden/New Zealand/Finnland | Wild birds                             | 9178 | Daurian Jackdaw   | China                                  |
| Chicken | United Kingdom     | Wild birds                             | 9179 | Daurian Jackdaw   | China                                  |
| Sheep | New Zealand       | Wild birds                             | 9180 | Daurian Jackdaw   | China                                  |
| Environmental waters | Canada | Wild birds                             | 9181 | Daurian Jackdaw   | China                                  |
| Mallard | China             | Wild birds                             | 9182 | Daurian Jackdaw   | China                                  |
| 1540 | Human stool       | Wild birds                             | 9183 | Daurian Jackdaw   | China                                  |
| Chicken | United Kingdom | Environmental waters/Luxembourg      | 9184 | Daurian Jackdaw   | China                                  |
| Wild birds | United States /Japan | Wild birds                             | 9185 | Daurian Jackdaw   | China                                  |
| Daurian Jackdaw | China | Wild birds                             | 9186 | Daurian Jackdaw   | China                                  |
| 448 | Human stool       | Wild birds                             | 9190 | Mallard           | China                                  |
| Wild birds | United Kingdom /Japan/United States | Wild birds                             | 9191 | Mallard           | China                                  |
| Environmental waters | Canada/The Netherlands/France | Wild birds                             | 9192 | Crow              | China                                  |
| Daurian Jackdaw | China | Wild birds                             | 9193 | Daurian Jackdaw   | China                                  |
| 951 | Human stool       | Wild birds                             | 9194 | Daurian Jackdaw   | China                                  |
| Wild birds | United Kingdom | Wild birds                             | 9195 | Daurian Jackdaw   | China                                  |
| Daurian Jackdaw | China | Wild birds                             | 9196 | Crow              | China                                  |
| 2367 | Human stool       | Wild birds                             | 9197 | Crow              | China                                  |
| Chicken | Germany           | Wild birds                             | 9222 | Daurian Jackdaw/  | China                                  |
| Pigeon | China             | Wild birds                             |      |                  |                                        |

1 Novel STs are indicated in bold. 2 Species isolated campylobacter jejuni in this study are indicated in bold. 3 Areas in china are indicated in bold. 4 BNU, Beijing Normal University; BP, Beihai Park; THP, Temple of Heaven Park; CP, Cuihu Park; BWRC, Beijing Wildlife rescue center; BWF, Beijing Wildlife Park; MP, Milu Park; NWP, Niukouyu Wetland Park; BRRC, Beijing Raptor rescue center; WDL, Wild Duck Lake; HL, Hongluo Lake; Xr, Xiyu Reservoir.

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FIGURE 2 | Frequency of resistance to 11 antibiotics among the 57 Campylobacter jejuni isolates. R represents resistant, I represents intermediate, S represents sensitive. AZI, azithromycin; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin, STR, streptomycin; CHL, streptomycin, FLO, florfenicol; TET, tetracycline, TEL, telithromycin, CLI, clindamycin; ERY, erythromycin.

could transmit between different species (Tables 3, 4 and Supplementary Figure S1).

Antibiotic Resistance of Campylobacter jejuni

The antibiotic resistance profile of C. jejuni isolates was evaluated by 11 antibiotics according to the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2015). Antibacterial resistance revealed that streptomycin (36.84%) was most common, followed by tetracycline (29.82%), gentamicin (29.82%), clindamycin (28.07%), telithromycin (28.07%), florfenicol (26.32%), nalidixic acid (17.54%), and ciprofloxacin (15.79%), azithromycin (14.04%), chloramphenicol (14.04%), erythromycin (7.02%) (Figure 2 and Table 5).

The isolates from different locations and sites showed different antimicrobial profile. In the urban area, the antimicrobial efficiency of three antibiotics commonly used in humans and animals are as follows: streptomycin (62.50%), gentamicin (62.50%), and telithromycin (50.00%) (Table 6). In the suburb area, the rate of antibiotic resistance was low, ranging from 7.32 to 26.83% (Table 6).

Multi-drug resistance of bacteria is also common in this study. Nineteen (33.33%, 19/57) isolates were MDR. In detail, The main antibiotics producing drug resistance were streptomycin, gentamicin, and clindamycin (26.32%) (Figure 3 and Supplementary Table S2).

Antibiotic Resistance Mechanism of Campylobacter jejuni

The characteristics of macrolide resistance associated with the genes were analyzed in 4 resistant isolates and 53 susceptible isolates, and this helps investigate the molecular mechanisms of the macrolide-resistant isolates. The A2075G mutation in the 23S rRNA gene, which is responsible for high-level resistance to macrolide, was not detected in all of the resistant strains and susceptible isolates (Supplementary Figure S2A).

All Campylobacter isolates were also investigated for the presence of the tet(O) gene associated with the resistance to tetracycline. The tet(O) marker was detected in all but three resistant strains (3/17) (Supplementary Figure S2B).

To investigate the molecular mechanisms of the fluoroquinolones resistant isolates, Multiplex PCR was designed, which uses three primers in a single reaction. Only the isolates with the gene mutation generated a product with the reverse primer with mutation and the conserved forward primer, whereas all 57 strains generated the gyrA PCR product with reverse and forward conserved primers. 87.50%(7/8) C. jejuni resistant isolates generated the specific product in MAMA-PCR that indicated the mutation Thr-86-to-Ile (ACA → ATA for C. jejuni), while none of the susceptible strains gave positive results (Supplementary Figure S2C).

All Campylobacter isolates were also investigated for the presence of the aphA-3 gene associated with the resistance to Aminoglycosides. The aphA-3 marker was detected in all but one resistant strains (1/17) (Supplementary Figure S2B).

Determination of the Presence of Virulence Genes

To determine whether virulence differences exist among isolates from different wild birds, we tested the virulence genes including the cdt gene cluster, flaA gene and cadF gene, the gene
| Species (Number) | Aminoglycosides | Tetracyclines | Lincomides | Macrolides Ketolides | Quinolones | Chloramphenicols |
|-----------------|-----------------|---------------|------------|-----------------------|-------------|------------------|
|                 | GEN | STR | TET | CLI | ERY | AZI | TEL | NAL | CIP | CHL | FLO |
| Crow (15)       | 60.00% | 60.00% | 26.67% | 60.00% | 6.67% | 26.67% | 26.67% | 13.33% | 20.00% | 26.67% | 33.33% |
| Daurian Jackdaw (31) | 0.00% | 6.45% | 6.45% | 6.45% | 6.45% | 3.23% | 6.45% | 9.68% | 6.45% | 0.00% | 6.45% |
| Balck Swan (1)  | 100.00% | 100.00% | 100.00% | 100.00% | 0.00% | 100.00% | 100.00% | 0.00% | 0.00% | 100.00% | 0.00% |
| Silver Pheasant (1) | 100.00% | 100.00% | 100.00% | 100.00% | 0.00% | 100.00% | 100.00% | 0.00% | 0.00% | 100.00% | 0.00% |
| Mallard (6)     | 50.00% | 50.00% | 83.33% | 50.00% | 16.67% | 16.67% | 50.00% | 33.33% | 33.33% | 33.33% | 50.00% |
| Mandarin duck (1) | 100.00% | 100.00% | 100.00% | 100.00% | 0.00% | 100.00% | 100.00% | 100.00% | 0.00% | 0.00% | 100.00% |
| Rock Pigeon (2) | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% | 100.00% |
| Total (57)      | 29.82% | 36.84% | 29.82% | 28.07% | 7.02% | 14.04% | 28.07% | 17.54% | 15.79% | 14.04% | 26.32% |

In parentheses, (A), (B/A), A represent the number of isolates from each bird, B represent the number of isolates from each bird resistance to each antibiotic. AZI, azithromycin; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; STR, streptomycin; CHL, chloramphenicol; FLO, florfenicol; TET, tetracycline; TEL, telithromycin; CLI, clindamycin; ERY, erythromycin.
| Location (Number) | Site (Number) | Aminoglycosides | Tetracyclines | Lincomides | Macrolides Ketolides | Quinolones | Chloramphenicolos |
|------------------|--------------|-----------------|---------------|------------|----------------------|-------------|-------------------|
|                  |              | GEN             | STR           | TET        | CLI                  | ERY         | AZI               | TEL             | NAL          | CIP          | CHL          | FLO          |
|                  | BNU (11)     | 72.73% (8/11)   | 72.73% (8/11) | 36.36% (4/11) | 54.55% (6/11) | 9.09% (1/11) | 27.27% (3/11) | 63.64% (7/11) | 18.18% (2/11) | 27.27% (3/11) | 36.36% (4/11) | 36.36% (4/11) |
| Urban (16)       |              |                 |               |            |                      |             |                  |                 |              |              |              |              |
|                  | BP (1)      | 100.00% (1/1)   | 100.00% (1/1) | 100.00% (1/1) | 0.00% (0/1)       | 0.00% (0/1) | 100.00% (1/1) | 0.00% (0/1)   | 100.00% (1/1) | 0.00% (0/1)   | 100.00% (1/1) | 0.00% (0/1)   |
|                  | THP (4)     | 25.00% (1/4)    | 25.00% (1/4)  | 25.00% (1/4) | 0.00% (0/4)       | 0.00% (0/4) | 100.00% (1/4) | 0.00% (0/4)   | 8.00% (1/4)    | 0.00% (0/4)   | 100.00% (1/4) | 8.00% (1/4)   |
|                  | Subtotal (16)| 62.50% (10/16)  | 62.50% (10/16)| 37.50% (9/16)| 6.25% (1/16)     | 6.25% (1/16)| 50.00% (8/16) | 6.25% (1/16)  | 18.75% (3/16)  | 18.75% (3/16) | 50.00% (8/16) | 6.25% (1/16) |
|                  | BWRC (4)    | 50.00% (2/4)    | 50.00% (2/4)  | 50.00% (2/4) | 0.00% (0/4)       | 0.00% (0/4) | 25.00% (1/4)  | 0.00% (0/4)   | 8.00% (1/4)    | 8.00% (1/4)   | 25.00% (1/4) | 8.00% (1/4)   |
|                  | Subtotal (41)| 100.00% (8/41) | 100.00% (8/41)| 100.00% (8/41)| 0.00% (0/81) | 0.00% (0/81) | 25.00% (2/81) | 0.00% (0/81)  | 8.00% (2/81)   | 8.00% (2/81)  | 25.00% (2/81) | 8.00% (2/81)  |
|                  | CP (25)     | 4.00% (1/25)    | 4.00% (1/25)  | 4.00% (1/25) | 0.00% (0/25)     | 0.00% (0/25)| 25.00% (6/25) | 0.00% (0/25)  | 8.00% (2/25)   | 8.00% (2/25)  | 25.00% (6/25) | 8.00% (2/25)  |
|                  | Subtotal (41)| 50.00% (8/41)  | 50.00% (8/41) | 50.00% (8/41) | 0.00% (0/81)     | 0.00% (0/81)| 25.00% (2/81) | 0.00% (0/81)  | 8.00% (2/81)   | 8.00% (2/81)  | 25.00% (2/81) | 8.00% (2/81)  |
|                  |               |                |               |            |                      |             |                  |                 |              |              |              |              |
| Beijing (57)     | BWP (1)     | 100.00% (1/1)   | 100.00% (1/1) | 100.00% (1/1) | 0.00% (0/1)       | 0.00% (0/1) | 100.00% (1/1) | 0.00% (0/1)   | 100.00% (1/1) | 0.00% (0/1)   | 100.00% (1/1) | 0.00% (0/1)   |
|                  | MP (7)      | 0.00% (0/7)     | 14.29% (1/7)  | 28.57% (2/7) | 28.57% (2/7)     | 0.00% (0/7) | 0.00% (0/7)   | 14.29% (1/7)  | 14.29% (1/7)   | 14.29% (1/7)  | 14.29% (1/7) | 14.29% (1/7)  |
|                  | NWP (4)     | 75.00% (3/4)    | 75.00% (3/4)  | 75.00% (3/4) | 75.00% (3/4)     | 75.00% (3/4)| 75.00% (3/4) | 75.00% (3/4)  | 75.00% (3/4)   | 75.00% (3/4)  | 75.00% (3/4) | 75.00% (3/4)  |
|                  | Subtotal (41)| 17.07% (7/41)  | 26.83% (11/41)| 19.51% (8/41)| 7.32% (3/41)     | 9.76% (3/41)| 17.07% (7/41) | 14.63% (6/41) | 17.07% (7/41)  | 14.63% (6/41) | 17.07% (7/41) | 14.63% (6/41) |
|                  | Beijing (57)| 29.62% (17/57) | 36.84% (17/57)| 28.07% (16/57)| 7.02% (4/57)     | 14.04% (5/7)| 28.07% (16/57)| 15.79% (9/57) | 15.79% (9/57)  | 15.79% (9/57) | 15.79% (9/57) | 15.79% (9/57) |

1. In parentheses, (A), (B/A). A represent the number of isolates from each site, B represent the number of isolates from each site resistance to each antibiotic. 2. AZI, azithromycin; NAL, nalidixic acid; CIP, ciprofloxacin; GEN, gentamicin; STR, streptomycin; CHL, chloramphenicol; FLO, florfenicol; TET, tetracycline; TEL, telithromycin; CLI, clindamycin; ERY, erythromycin. 3. BNU, Beijing Normal University; BP, Beihai Park; THP, Temple of Heaven Park; CR, Cuihu Park; BWRC, Beijing Wildlife rescue center; BWP, Beijing Wildlife Park; MP, Milu Park; NWP, Niukouyu Wetland Park; BRRC, Beijing Raptor rescue center; WDL, Wild Duck Lake; HL, Hongluxu Lake; XR, Xiyu Reservoir.
that encodes an adhesin that involves in colonization for all C. jejuni isolates. The results showed that all isolates contained flaA genes, cadF genes, and cdt gene cluster. Further analysis indicated that the truncated cdt gene clusters (approximately 1400 bp) only existed in the isolates from crows, daurian jackdaws and silver pheasants. In particular, the truncated cdt gene clusters were first detected in C. jejuni isolates from silver pheasant. C. jejuni isolates from Corvidae are more likely to carry truncated cdt gene clusters, other species belong to family Corvids and Phasianidae may also have deletions within the cdt Gene Cluster and still need a further survey. These results above indicated that bacteria in the family of Anatidae and Columbidae might have complete cdt gene clusters, but further research is still needed for two families species (Table 7).

**DISCUSSION**

*Campylobacter jejuni* carried by wild birds has been identified to be potentially pathogenic to humans and other animals (Kapperud et al., 2003; Gardner et al., 2012). The majority of studies have focused on food sources that may cause human campylobacteriosis, while studies on wild birds are rare, especially in China. In order to get a better understanding of the distribution and transmission of C. jejuni in wild birds, we analyzed the prevalence, genetic diversity, antimicrobial resistance and virulence genes of C. jejuni in Beijing, China. In the present study, we found that 10.96% of fecal samples collected from 33 species carried with C. jejuni. Previous studies demonstrated that the prevalence of C. jejuni among different countries and wild birds species varied from 1.4 to 72.7% (Ramonaite et al., 2015). Among these species we found that the highest prevalence of C. jejuni was daurian jackdaw 51.67%, followed by crow 24.19%, silver pheasant 14.29%, black swan 12.5%, mallard 8.57%, rock pigeon 7.69%, and mandarin duck 6.25%. C. jejuni isolated from different wild birds with different prevalence which in agreement with other studies that the prevalence of *Campylobacter* spp. in different taxonomic families wild birds were diverse (Waldenstrom et al., 2007; French et al., 2009).

It is believed that the variation of C. jejuni prevalence in wild bird species is due to ecological factors including feeding habits, habitat preferences, and migration patterns (Waldenstrom et al., 2002; Colles et al., 2009; Waldenstrom et al., 2010; Colles et al., 2011). Located in urban, BNU had the highest prevalence of C. jejuni. Although the proportion of human disease attributable to environmental sources is relatively low, we could not ignore the effect on humans (Wilson et al., 2008; Sheppard et al., 2009; Colles et al., 2011). Most importantly, to our knowledge, it is the first detection of C. jejuni carried by black swans in China. These data strengthen the hypothesis that the high prevalence of C. jejuni in wild birds might provide evidence of wild birds being a natural reservoir of C. jejuni (Oravcova et al., 2014).

**TABLE 7** Prevalence of CDT isolates from different species.

| Family     | Species                                  | CDT character (%) |
|------------|------------------------------------------|-------------------|
| Corvidae   | Daurian Jackdaw (Corvus dauurica)        | Truncated 100%    |
| Phasianidae| Silver Pheasant (Lophura nycthemera)     | Truncated 100%    |
| Anatidae   | Mandarin duck (Aix galericulata)         | WT 100%           |
|            | Black Swan (Cygnus atratus)              | WT 100%           |
| Columbidae | Rock Pigeon (Columba livia)              | WT 100%           |

1 In parentheses, the positive number of isolates from each species of bird.
Multilocus sequence typing was the golden standard to use for comparison between isolates from different sources because of its high reproducibility and accessible to comparability amongst laboratories worldwide (Dingle et al., 2001). Previous studies have shown that human patients and other animals have the same predominant STs suggesting that these animals are important reservoirs of human domestically acquired infections (Olkkola et al., 2016). From our results, 36 different STs belonging to 4 CCs and unassigned, among which 20 were novel. Of the 20 novel STs, 25% were from one or more new allelic sequences, and a total of 6 novel alleles were found (aspaA484, aspaA485, glnA673, glyA754, tkt171, tkt719). No new allele sequences were found for another 75% new STs, and these STs resulted from novel combinations of alleles already existed in the PubMLST database. These results indicated that mutation frequency in the MLST alleles is substantially lower than the recombination frequency, which in agreement with previous research (Schouls et al., 2003). ST448, ST951, and ST52 these isolates from daurian jackdaw and black swan, however other researches indicated that all of these three strains isolated from other animals, poultry, and humans (Griekspoor et al., 2010; Olkkola et al., 2016). So wild birds as a potential source of known and novel multilocus sequence types of *C. jejuni* may have the potential to transmit to other animals, poultry, and humans.

The antibiotic resistance profile of *C. jejuni* isolates from these animals was determined using 11 antibiotics. The results of antimicrobial susceptibility testing in this study indicated that the isolates were in general resistant to the tested antibiotics at rates ranging from 7.02 to 36.84%. The high rate of resistance to streptomycin and gentamicin was seen among *C. jejuni* isolates from these birds. All Campylobacter isolates were also investigated for the presence of the *aphA*3 gene associated with the resistance to Aminoglycosides. The most common form of resistance to Aminoglycosides related antibiotics involves the synthesis of 3′-aminoglycoside phosphotransferases [APH(3′)](Gibreel et al., 2004a). The *aphA*-3 marker was detected in all but one resistant strains (1/17), which is consistent with previous research results.

From the different locations and sites, the antibiotic resistance profile performed differently. In urban areas, the isolates from wild birds have high antibiotic resistance, which might be due to contaminated environment water. The reasonable interpretation for this difference may be human activities, such as antibiotic abuse, are more active in urban areas than in the suburbs, resulting in birds in different habitats getting different resistant bacteria from the environment. However, the relationship between high antibiotic resistance and contaminated environment water needs further study. As previous study indicated that *C. jejuni* isolates detected in crows and pigeons as potential infection sources to humans (Ramonaite et al., 2015), it was worth noting that four strains from wild birds (crow, mallard, and rock pigeon) are resistant to all 11 antibiotics which may be an important indicator of public health safety.

All wild birds isolates had flaA genes, *cadF* genes, and *cdt* gene clusters, which in agreement with the previous study (Sen et al., 2018). The results suggest that these three genes are conserved amongst different sources. As before, *C. jejuni* isolates from crows had a truncated gene cluster of about 1400 bp (Sen et al., 2018), *C. jejuni* isolates from daurian jackdaw also had a truncated gene cluster (Kovanen et al., 2019). However, the most unexpected results were isolates from silver pheasant which also have a truncated gene cluster of about 1400 bp. To our knowledge, it was the first detection of the isolates from silver pheasant also have a truncated gene cluster of about 1400 bp. The CDT toxin is a tripartite protein formed by the expression of three tandem genes, *cdtA*, *cdtB*, and *cdtC* where *cdtB* encodes the active component of the toxin, while *cdtA* and *cdtC* are responsible for binding and internalization of the toxin (Pickett et al., 1996). Some research confirmed that *C. jejuni* 81-176 *cdtB*~−~ strains were significantly attenuated in HeLa cytotoxicity assays, while still holding some toxigenicity. However, *C. jejuni* NCTC 11168 *cdtB*~−~ strains produced no detectable cytotoxicity in HeLa cell (Purdy et al., 2000). So, if these isolates with a truncated *cdt* gene cluster still retaining the toxigenicity required further verification.

**CONCLUSION**

Wild birds as a reservoir of potentially pathogenic *C. jejuni* strains and can be a vector of disease transmission. However, further studies are needed to link the high occurrence of *Campylobacter* in wild birds to human campylobacteriosis cases and transmission to other animals.

**EXPERIMENTAL PROCEDURES**

**Samples Collection and *Campylobacter* Isolation**

Five hundred and twenty fecal samples were collected from 33 species in 12 sites, Beijing, China, between January 2018 and April 2018 (Figure 1 and Table 1). Samples were collected in sterile tubes and stored at 4 to 7°C for 2 to 6 h before culturing in Lab (Sen et al., 2018). Fecal samples were inoculated onto Modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) containing Cefoperazone, Rifampicin, and Amphotericin B (Qingdao Hope Bio-Technology, Co., Ltd.), with incubation at 37°C under microaerophilic conditions (CampyGen; Oxoid Limited, Hampshire, United Kingdom) for 48 to 168 h. Then picked white to translucent colonies subculture onto mCCDA for further characterization.

**Campylobacter Identification**

All positive samples were used to extract DNA by adding 100 μL 0.25% SDS and boiling in a heater block at 95°C for 10 min, followed by centrifugation at 12,000 g for 5 min. Template DNA was stored at –20°C until used for PCR and at least 1 year without any degradation (Sen et al., 2018). Then *Campylobacter* spp. identified by a qPCR method based on the 16S rRNA gene and primers used as previously described (Lund and Madsen, 2006) (Table 8). The qPCR conditions as follows: 1 cycle at 95°C for 10 min, followed by 40 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 30 s, with a final cycle of 72°C for 2 min.
5 min. For determining the presence of *Campylobacter jejuni*, *Campylobacter coli*, *Campylobacter lari*, and *Campylobacter upsaliensis*, a multiplex PCR method was performed with the isolates using the lipid A gene (*lpxA*) as previously described (Klena et al., 2004), PCR conditions were as follows: 35 cycles of denaturation at 94°C for 1 min, annealing at 50°C for 1 min, extension at 72°C for 1 min and a final extension step at 72°C for 5 min. The presence of *flaA*, *cadF* and CDT gene cluster in 57 isolates were detected by PCR using the primer sets described in Table 8 (Nachamkin et al., 1993; Konkel et al., 1999; Bang et al., 2003). The thermocycling conditions which can be found in the respective references in Table 8. The PCR products were detected on 1% Agarose gels and verified by sequencing.

**Multiocus Sequence Typing for *Campylobacter jejuni***

Multilocus sequence typing was performed by amplifying and sequencing seven housekeeping genes loci, *aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt*, and *uncA*, and primers used for *C. jejuni* are listed in Table 9 (Dingle et al., 2001). The PCR reaction conditions were as follows: initial denaturation at 94°C for 5 min; followed by 35 cycles of 94°C for 2 min, 50°C for 1 min and 72°C for 1 min; last denaturation at 72°C for 7 min. The PCR products were detected on 1% Agarose gels and verified by sequencing, Allele numbers and STs were assigned using the *Campylobacter* MLST database.

**Antimicrobial Susceptibility Testing for *Campylobacter jejuni***

Antimicrobial resistance analysis was performed on 57 *Campylobacter* isolates. All isolates were cultured overnight before testing. The *C. jejuni* strains were tested against phenotypic resistance to 11 antimicrobial agents (erythromycin, azithromycin, nalidixic acid, ciprofloxacin, gentamicin, streptomycin, chloramphenicol, florfenicol, tetracycline, telithromycin, and clindamycin) (Zhongchuan biology technology Company, Qingdao, China) by the agar dilution method according the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2015). Mueller-Hinton agar (Oxoid) with dilutions ranging from 64 to 0.5 µg/mL for erythromycin, azithromycin, nalidixic acid, ciprofloxacin, gentamicin, streptomycin, chloramphenicol, florfenicol, tetracycline, telithromycin, and clindamycin was prepared. Subcultured colonies were harvested and suspended in sterile water to a standardized cell density (0.5 McFarland), and 2 µL of approximately 10⁶ CFU of bacteria was pipetted into each well (after diluting in PBS). The plates were incubated under a microaerophilic atmosphere at 42°C for 24 h. The MIC values were defined as the lowest concentration that produces complete inhibition of *C. jejuni* growth. For quality control, the reference strain *C. jejuni* ATCC 33560 was included. The *C. jejuni* isolates were considered resistant to chloramphenicol (CHL), erythromycin (ERY), ciprofloxacin (CIP), nalidixic acid (NAL) tetracycline (TET), streptomycin (STR), and telithromycin (TEL) at MICs of ≥32, ≥32, ≥4, ≥64, ≥16, ≥16, and ≥16 µg/mL, respectively. For gentamicin (GEN), florfenicol (FLO), clindamycin (CLI) and azithromycin (AZI), the isolates with MICs ≥8 µg/mL were considered resistant.

**Determination of Mechanisms of Antimicrobial Resistance**

All strains tested for the antibiotic resistance were examined for the presence of molecular background of the appearing resistance. For the determination of macrolide resistance, the 23S rRNA genes mutations were detected by the use of PCR for *C. jejuni* (Zhou et al., 2016). For the determination of fluoroquinolone resistance, the gyrA mutations were detected by the use of the Mismatch Amplification Mutation Assay – PCR (MAMA-PCR) suitable for *C. jejuni* (Wieczorek, 2011). The presence of tet(O) gene associated with the resistance to...
tetracyclines was also detected (Wieczorek, 2011). The presence of aphA-3 gene associated with the resistance to aminoglycosides was also detected (Gibreel et al., 2004a) primers used for determination of Mechanisms of Antimicrobial Resistance are listed in Table 10.

The 23S rRNA genes PCR reaction conditions were as follows: initial denaturation at 94°C for 2 min; followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; last denaturation at 72°C for 7 min. The tet(O) gene PCR reaction conditions were as follows: initial denaturation at 94°C for 2 min; followed by 35 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 30 s; last denaturation at 72°C for 7 min. The gyrA gene PCR reaction conditions were as follows: initial denaturation at 94°C for 2 min; followed by 35 cycles of 94°C for 30 s, 54°C for 30 s and 72°C for 30 s; last denaturation at 72°C for 7 min. The tet(O) gene PCR reaction conditions were as follows: initial denaturation at 94°C for 2 min; followed by 35 cycles of 94°C for 30 s, 52°C for 30 s and 72°C for 30 s; last denaturation at 72°C for 7 min. The primers used for these three genes are listed in Table 10. The PCR products were detected on 1% Agarose gels and verified by sequencing, and the sequences were analyzed to identify mutations using the BLAST program of the GenBank sequence database.

### Phylogenetic Analysis

The MLST profiles were clustered with the Bionumerics software, version 7.6 by using a categorical coefficient and a graphing method called the minimum spanning tree as described before (Schouls et al., 2004). The minimum spanning tree showing the relatedness of 57 C. jejuni strains and other C. jejuni strains from the PubMLST database, which was based on the STs. Each ST is represented by a circle that is proportional to the number of isolate species comprising that ST. Circles (STs) are linked by lines indicating allelic variation. The different color of each ST indicates the animal host from which each isolate was recovered (red-human, green -wild bird, blue-chicken, yellow-dog, sky blue-monkey). Thick and short lines connect single-locus variants, thin and longer lines connect double-locus variants and dashed lines represent three or more allele differences. For MLST, a maximum neighbor difference of 2 was used to create complexes. Background shading highlights clonal complexes.

### Accession Number(s)

Multilocus sequence typing sequences of the bird isolates that represented novel STs were deposited in the PubMLST database (see footnote 1) to assign new sequence types and allelic profiles.

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**TABLE 9 | Oligonucleotide primers for Campylobacter MLST.**

| Locus | Function | Forward | Reverse | Amplification size (bp) |
|-------|----------|---------|---------|------------------------|
| asp   | Amplification | asp-A9, 5′-AGT ACT AAT GAT GCT TAT CC-3′ | asp-A10, 5′-ATT TCA TCA ATT TGT TCT TGG-3′ | 899 |
|       | Sequencing  | asp-S3, 5′-CCA ACT GAA TGC TGT ACC-3′ | asp-S6, 5′-TTT ATT TCC GGT AAT ACC ATC-3′ | 1262 |
| gln   | Amplification | gln-A1, 5′-TAG AGA CTT GGG ATC ATA TTA CC-3′ | gln-A2, 5′-TGG GAC GAG CTT CTA CTG GC-3′ | 1012 |
|       | Sequencing  | gln-S1, 5′-GCT CAA TTT TTC GAG GGC-3′ | gln-S4, 5′-GCA TAC CAT TGC CAT TAT CTC G-3′ | 816 |
| gft   | Amplification | gft-A1, 5′-GGG GTC TAC AGC TAG TTG-3′ | gft-A2, 5′-CCG AAT AAA GGT GTC TTG GAC G-3′ | 1150 |
|       | Sequencing  | gft-S3, 5′-CTT ATA TTG ATG GAG AAA ATG G-3′ | gft-S8, 5′-TGC TAT ACA GGC ATA AGG G-3′ | 1120 |
| gly   | Amplification | gly-A1, 5′-GAG TTA GAG CTT TAA GAT GAC-3′ | gly-A2, 5′-AAA CTC GTC GAA GAT GC-3′ | 1120 |
|       | Sequencing  | gly-S5, 5′-GCT AAT CCA GGT GTT TAT AT-3′ | gly-S4, 5′-AGG TGA TTA TCC GTC TCA G-3′ | 1120 |
| pgm   | Amplification | pgm-A7, 5′-TAC TAA TAA TAT CTT ATG AGG-3′ | pgm-A8, 5′-CAC AAC ATT TTG CAT TTG TTC TCC-3′ | 1120 |
|       | Sequencing  | pgm-S6, 5′-GTT TTA AGA TGA TGT GGC TCA-3′ | pgm-S2, 5′-TCC AGA ATA GCG AAA TAA G-3′ | 1120 |
| tkt   | Amplification | tkt-A3, 5′-GCA AAG TCA GGA CAC CCA G-3′ | tkt-A6, 5′-GCA TGA TTA ATG GCT OC-3′ | 1120 |
|       | Sequencing  | tkt-S5, 5′-GCT TAG ACG ATA TTT TAA GTG-3′ | tkt-S6, 5′-AAG CCT GCT TGT TCT TTG G-3′ | 1120 |
| unc   | Amplification | unc-A7, 5′-ATG GAC TTA AGA ATA TTA TGG C-3′ | unc-A8, 5′-ATA AAT TCC ATC TTC AAA TCA C-3′ | 1120 |
|       | Sequencing  | unc-S3, 5′-AAA GTA CAG TGG CAC AAG TGG-3′ | unc-S4, 5′-TGC CTC ATC TAA ACT ACT AGC-3′ | 1120 |

**TABLE 10 | Campylobacter specific primers in this study.**

| Primer | Sequence (5′-3′) | Gene | References |
|--------|------------------|------|------------|
| F1-campy-23S | 5′-AAAGAGGTGATAG GGTGTGACG-3′ | 23S rRNA | Zhou et al., 2016 |
| R1-campy-23S | 5′-AAAGATTTCC AACGTTTGC-3′ | tet(O) | Wieczorek, 2011 |
| DMT 1 | 5′-GGGCTTTTTT TATGTCG-3′ | gyrA | Wieczorek, 2011 |
| DMT 2 | 5′-GTTAAAGGTGCGGGTAT AACATT-3′ | CampyMAMAgyrA1 | CampyMAMAgyrA1 | 5′-TTTTTAGCAA AGATCTGTGAT-3′ |
|       | 5′-CAGTATAAGGATC GCACGC-3′ | GZgyrA4 | CampyMAMAgyrA5 | 5′-CAGAAGCACA TAAACGTCA-3′ |
|       | 5′-AGGACCAACCTAGG GTCGACAGG-3′ | ApfA-3 F | ApfA-3 | 5′-CGGCTTGACAC CCTG-3′ |
|       | 5′-GAGCCTGATCC CGAGAATGTC-3′ | ApfA-3 R | ApfA-3 | 5′-CGGCTTGACAC CCTG-3′ |
Statistical Analysis
Statistical analysis of the prevalence of positive rate in the C. jejuni isolates was performed using the chi-squared test with SPSS version 16.0. A value of \( p < 0.05 \) was considered to be statistically significant.

DATA AVAILABILITY STATEMENT
The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

AUTHOR CONTRIBUTIONS
JD, HH, JL, and CW designed the project. JD and JH performed the main experiments. JD, JL, CW, and ML wrote and revised the manuscript. BJW, BW, HC, JJ, and KS conducted part of the experiments.

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