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**Research Paper**

# Prevalence and Characterization of *Campylobacter jejuni* Isolated from Retail Chicken in Tianjin, China

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## ABSTRACT

*Campylobacter jejuni* is an important foodborne pathogen worldwide; however, there is a lack of information on the prevalence and antibiotic-resistant profile of *C. jejuni* in the People's Republic of China. We determined the prevalence and characteristics of *C. jejuni* on the retail level in Tianjin, one of the five national central cities in China. A total of 227 samples of chicken wings, legs, and breasts were collected from supermarkets and wet markets; 42 of these samples were confirmed to be positive for *Campylobacter* contamination. The contamination rates of *C. jejuni* and other *Campylobacter* species were 13.7% (31 of 227 samples) and 5.7% (13 of 227 samples), respectively. A group of 31 *C. jejuni* isolates was subjected to antimicrobial susceptibility testing. All (100%) the selected isolates were resistant to ciprofloxacin and nalidixic acid; 77.4% were resistant to tetracycline, 67.7% to doxycycline, 35.5% to gentamicin, 25.8% to clindamycin and florfenicol, 19.4% to chloramphenicol, and 12.9% to erythromycin and azithromycin. A remarkably high proportion (41.9%) of multidrug-resistant isolates was identified. Multilocus sequence typing was conducted to study the population structure of the *C. jejuni* strains and their relationship to human isolates. The correlation between antimicrobial resistance traits and certain sequence types (STs) or clonal complexes was determined as well. A great genetic diversity of poultry isolates was identified, with 11 STs belonging to 6 clonal complexes and 11 singleton STs. The novel STs accounted for 40.9% ($n = 9$) of the 22 STs. ST-21, ST-353, ST-354, ST-443, ST-607, and ST-828 complexes had been previously identified from human isolates. This study revealed an extensive level of antimicrobial resistance and genetic diversity in *C. jejuni* isolated from chicken products in Tianjin, highlighting the necessity of performing enforced interventions to reduce *Campylobacter* prevalence in China.

Key words: Antimicrobial resistance; *Campylobacter jejuni*; Food safety; Multilocus sequence typing; Poultry; Retail

*Campylobacter* species are the most prevalent foodborne pathogens in both developing and developed countries. *Campylobacter jejuni* is the leading species of *Campylobacter* bacteria and is acknowledged to be the most common cause of campylobacteriosis; infections may lead to a sequela known as Guillain-Barré syndrome *(49)*. The Centers for Disease Control and Prevention estimates that campylobacteriosis ranks as the second most common foodborne illness in the United States, affecting over 1.3 million people per year. In 2007, an outbreak of 36 Guillain-Barré syndrome cases was reported in a village in Changchun (Jilin Province, People's Republic of China), and *C. jejuni* was confirmed to be responsible for this outbreak *(55)*. The consumption or mishandling of contaminated raw or undercooked poultry products is believed to be the major vehicle of transmission of *Campylobacter*. As a microaerobic organism, *Campylobacter* may adhere to the skin surface of chicken and form a multispecies biofilm with other bacteria (e.g., *Pseudomonas aeruginosa*), protecting the encased *C. jejuni* cells from the aerobic stress and creating a potential risk to consumers *(4)*.

The ingestion of a low dose of *C. jejuni* (500 to 800 cells) seems to be sufficient to cause illness *(26)*. The infected populations suffered from abdominal cramping, fever, and mild to severe diarrhea. Generally, these symptoms are self-limited and disappear after approximately 1 week without any specific treatment. However, *Campylobacter* infections can lead to some complications in the immunocompromised patients, such as those suffering from reactive arthritis and Guillain-Barré syndrome *(41)*. For the relatively severe cases, antimicrobial chemotherapy is required. Macrolides and quinolones are commonly used as the first-line therapies, and tetracycline, doxycycline, chloramphenicol, and gentamicin are the alternative drugs *(15)*.

Antimicrobials are widely used to promote the growth of livestock and prevent the occurrence of disease *(2, 7)*. However, the abuse of these drugs in livestock can increase the antimicrobial resistance of *Campylobacter*, consequently

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causing their therapeutic efficacy on human campylobacteriosis to be ineffective *(16)*. Thus, the emergence of antimicrobial-resistant strains of *C. jejuni* has occurred.

Although *C. jejuni* has been reported to be the major causative agent of bacterial gastroenteritis, there are still limited data regarding the prevalence and characterization of *C. jejuni* in China, where poultry constitutes a great proportion of the nation's meat consumption. One recent study *(53)* reported that the positive rates of *Campylobacter* in chicken samples ranged from 32.7 to 44.1% during 2008 to 2014 and that 100% of the *C. jejuni* and *Campylobacter coli* isolates found were resistant to fluoroquinolone. Another study *(56)* conducted in central China showed a 17.2% *Campylobacter* contamination rate in chicken products and that 100% of the *C. jejuni* and *C. coli* isolates found were resistant to norfloxacin and ciprofloxacin.

Various phenotyping and genotyping methods have been developed for the epidemiological surveillance and population structure analysis of *C. jejuni*. Current methods of genotyping are mainly divided into two groups. One approach is based on comparing enzyme-digested DNA fragment patterns after electrophoresis, such as pulsed-field gel electrophoresis *(50),* amplified fragment length polymorphism, and flagellin gene typing by restriction fragment length polymorphism *(35)*. The other approach is based on the sequence divergence of specific genes, such as sequencing the short variable region of the *flaA* gene (flaSVR) *(31)* and multilocus sequence typing (MLST) *(13)*. MLST is performed by amplifying and sequencing several housekeeping genes, followed by analyzing the sequence variation among alleles from different bacterial isolates *(13, 33)*. MLST has been widely used for the characterization of pathogenic bacteria such as *Arcobacter (34), Bacillus (6), Campylobacter (13), Helicobacter (38), Mycoplasma (30), Staphylococcus (44),* and *Streptococcus (14)*.

Tianjin is one of the major economic municipalities in north China and one of the five national central cities of China, but the prevalence and characterization of *Campylobacter* in this area has never been reported. In this study, we investigated the prevalence and antibiotic resistance profiles of *C. jejuni* isolated from chicken products in Tianjin, and we analyzed the genetic diversity of *C. jejuni* using MLST. The relationship between the antimicrobial resistance and genetic diversity of *C. jejuni* was also assessed.

## MATERIALS AND METHODS

**Sample collection and preparation.** Chicken samples were collected from eight supermarkets and nine wet markets located in four representative districts of Tianjin from October 2015 to May 2016. These sampling sites were the most popular markets in each district. Sampling visits were conducted once per week. The chicken samples included chicken wing middle joints ($n = 59$), wing roots ($n = 57$), legs ($n = 56$), and breasts ($n = 55$). All the samples were randomly selected and transported on ice to the laboratory within 2 h. The sample analysis was performed immediately after arrival at the laboratory.

Each sample was placed in a sterile bag, and 30 to 60 mL of buffered peptone water (Landbridge, Beijing, China) was added to a ratio of 1 mL/2.5 g of sample. The bag was shaken for 3 to 5 min, and the rinse solution was collected for the isolation of *Campylobacter*.

**Bacterial isolation.** The conventional plating analysis and enrichment analysis were carried out separately for the isolation of *C. jejuni*. For the conventional plating analysis, we streaked 100 μL of the rinse solution onto modified charcoal cefoperazone deoxycholate agar (Oxoid, Hants, UK), and incubated the plates under microaerobic conditions (5% $O_2$, 10% $CO_2$, and 85% $N_2$; Thermo Forma 3131 incubator, Thermo Scientific, Waltham, MA) at 42°C for 48 h. The enrichment analysis was carried out as described by the International Organization for Standardization in ISO 10272-1 *(24)* with minor modifications. In brief, we added 1 mL of the rinse solution to 9 mL of Preston broth (Haibo, Qingdao, China). The mixture was preenriched at 37°C for 4 h under microaerobic conditions and then incubated for 48 h under microaerobic conditions at 42°C. Subsequently, 10 μL of the bacterial culture was streaked onto modified charcoal cefoperazone deoxycholate agar and incubated under microaerobic conditions at 42°C for another 48 h *(24)*. In both analysis methods, we reported the colonies with typical morphologies as *Campylobacter*; these were translucent, wet, and flat or slightly raised.

Presumptive colonies were collected from each plate and subcultivated onto Mueller-Hinton agar (BD, Franklin Lakes, NJ) supplemented with 5% sterile defibrinated sheep blood (MHB agar) until further characterization. The bacterial isolates were identified using a colony duplex PCR assay *(12)*. Confirmed isolates were further characterized using MLST analysis and antimicrobial susceptibility testing.

**DNA preparation and colony duplex PCR assay.** We performed a colony duplex PCR assay for the rapid identification of *C. jejuni*. Two sets of primers were used for the confirmation of *C. jejuni*; the primer sequences are shown in Table 1. The 16S rRNA was specific to the genus *Campylobacter,* and the *mapA* gene was specific to *C. jejuni (12, 40, 52)*. A single colony was picked from the MHB agar using a sterilized toothpick and then suspended in 10 μL of sterile water. Then, 1 μL of the mixture was directly transferred to a PCR tube as the DNA template. We conducted PCR in a 20-μL reaction mixture containing 2.5 μL of 10× PCR buffer, 0.5 μM 16S rRNA primers, 0.5 μM *mapA* primers, 0.25 mM each deoxynucleoside triphosphate (dNTP), and 1 U of *Taq* polymerase (Tiangen, Beijing, China). PCR was conducted using a Biometra TProfessional thermocycler (Biometra, Jena, Germany), and the reaction conditions were set as follows: 1 cycle at 95°C for 10 min; 35 cycles at 95°C for 30 s, at 59°C for 60 s, and at 72°C for 60 s; and a final extension at 72°C for 10 min *(12)*. The PCR products were stained with a 1% (v/v) solution of ethidium bromide and visualized using a GelDoc 2000 gel imaging system (Bio-Rad, Hercules, CA) after gel electrophoresis in 1% agarose. All the confirmed isolates were stored at −80°C in vials containing 12% glycerol and 85% sterile defibrinated sheep blood.

**MLST.** A total of 31 confirmed *C. jejuni* isolates were plated onto MHB agar plates. We collected a loopful of the colonies of each sample and washed them using sterile deionized water. Bacterial genomic DNA was extracted using a TIANamp bacteria DNA kit (TIANGEN Biotech, Beijing, China), according to the manufacturer's instructions. Seven housekeeping genes (*aspA, glnA, gltA, glyA, pgm, tkt,* and *uncA*) were amplified and sequenced using a method developed by Miller and coauthors *(33),* with some modifications (Table 2). Each 25-μL of amplification mixture was composed of 2.5 μL of 10× PCR buffer, 1 μM of the corresponding primer, 0.2 mM each dNTP, and 0.5 U of *Taq*

TABLE 1. *Primers used for duplex PCR assays*

| Primer | Primer sequence (5′ → 3′)[a] | Amplicon size (bp) |
|---|---|---|
| 16S rRNA | F: ATCTAATGGCTTAACCATTAAAC | 857 |
| | R: GGACGGTAACTAGTTTAGTATT | |
| mapA | F: CTATTTTATTTTTGAGTGCTTGTG | 589 |
| | R: GCTTTATTTGCCATTTGTTTTATTA | |

[a] F, forward; R, reverse.

polymerase. The purification of the PCR products and the Sanger sequencing were both conducted by GENEWIZ (Suzhou, China).

**Assignment of allele numbers, STs, and clonal complexes.** The alleles and STs were determined by querying the samples against the *Campylobacter* MLST database (http://pubmlst.org/campylobacter). Each isolate was designated by seven allele numbers, which constituted an ST. Genetically related STs were assigned to clonal complexes. Novel alleles and STs were submitted to the database Web site, and specific numbers were assigned. We used Mega 6 (Tempe, AZ) to construct the cladogram of *C. jejuni,* and we created a minimum spanning tree using BioNumerics v. 7.6 (Applied Maths, Sint-Martens-Latem, Belgium) to illustrate the relationship between human isolates and chicken isolates.

**Antimicrobial susceptibility test.** The antimicrobial resistance profiles of the *Campylobacter* isolates were determined using the agar dilution method, according to the Clinical and Laboratory Standard Institute guidelines *(9)*. Briefly, bacterial colonies collected from the MHB agar were suspended in 1 mL of Mueller-Hinton broth to yield a suspension equivalent of 0.5 McFarland standard that contained approximately 1 to 4 × $10^8$ CFU/mL within 30 min. Then, 2 μL of the suspension was streaked onto the MHB agar containing twofold dilutions of antimicrobials. The agar plates were incubated microaerobically at 42°C for 24 h. The results were expressed as MICs *(15)*. Isolates that exhibited resistance to more than two antimicrobials from different classes were defined as being multidrug resistant. We repeated the test at least in triplicate. *C. jejuni* ATCC 33560 was used as the quality control strain. The tested antimicrobials, interpretive criteria, and criteria for the quality control tests are all listed in Table 3.

**Statistical analysis.** We compared the prevalence of *Campylobacter* in different chicken products using chi-square test in Minitab v. 16.2.3 (Minitab, State College, PA). A value of $P < 0.05$ was considered statistically significant. The correlations between certain genotypes and antimicrobial resistance patterns were tested using the Spearman correlation coefficient in SPSS v. 13.0 (IBM, Armonk, NY). The level of significance was set at $P < 0.01$.

## RESULTS AND DISCUSSION

**Isolation and prevalence of *C. jejuni* from chicken meat.** In our study, 227 retail chicken products were collected in Tianjin, China, for the isolation and detection of *Campylobacter*. Campylobacters are fastidious bacteria with demanding growth conditions. Moreover, *Campylobacter* grows more slowly than other coexisting microorganisms, such as *Escherichia coli, Pseudomonas* spp., *Acinetobacter* spp., and *Proteus (8, 51)*. Thus, appropriate isolation approaches and careful handling are required for the detection of *Campylobacter* in meat samples. To eliminate false-negative results, a few studies *(8, 18, 51)* developed novel selective agars or combined direct plating methods with enrichment analysis. The direct plating method reduces the excessive proliferation of coexisting bacteria, and enrichment analysis provides a better recovery of the injured cells. The combination of these two methods could achieve a maximally accurate contamination rate.

In our current study, the 16S rRNA is specific to the genus *Campylobacter* and the *mapA* gene is specific to *C. jejuni* only. The sample that presented a positive 16S rRNA product but negative *mapA* PCR product was determined to be *Campylobacter* but not *C. jejuni*. As shown in Table 4, 42 of the 227 chicken samples were contaminated by *Campylobacter,* which yielded a positive rate of 18.5%. Among the *Campylobacter*-positive samples, *C. jejuni* (13.7%, 31 of 227 samples) was the leading *Campylobacter* species, as identified by duplex PCR. The other 13 (5.7%) isolates, with two samples contaminated by both *C. jejuni* and *C. coli,* were determined to be other *Campylobacter* species. The contamination rate of *Campylobacter* in our study was relatively lower than reported in other districts in China and in some other countries. According to an epidemiological investigation by the China National Centre for Food Safety Risk Assessment *(3),* 26.3% of the retail whole chicken carcasses in Beijing were contaminated with *Campylobacter*. Ma and coauthors *(28)* determined that the incidence of *Campylobacter* in retail broiler carcasses in Shanghai was 31.3%. The reported *Campylobacter* contamination rate in France was 76.0% *(17),* in the United Kingdom it was 60.9% *(27),* and in Louisiana it was 43.3% *(20).*

TABLE 2. *Oligonucleotide primers for MLST of* C. jejuni

| | Oligonucleotide primer set | | | | |
|---|---|---|---|---|---|
| | Forward (5′–3′) | | Reverse (5′–3′) | | |
| Locus | Primer | Sequence | Primer | Sequence | Amplicon size (bp) |
| *aspA* | aspAF | GAGAGAAAAGCWGAAGAATTTAAAGAT | aspAR | TTTTTTCATTWGCRSTAATACCATC | 676 |
| *glnA* | glnAF | TGATAGGMACTTGGCAYCATATYAC | glnAR | ARRCTCATATGMACATGCATACCA | 751 |
| *gltA* | gltAF | GARTGGCTTGCKGAAAAYAARCTTT | gltAR | TATAAACCCTATGYCCAAAGCCCAT | 706 |
| *glyA* | glyAF | ATTCAGGTTCTCAAGCTAATCAAGG | glyAR | GCTAAATCYGCATCTTTKCCRCTAAA | 716 |
| *pgm* | pgmF | CATTGCGTGTDGTTTTAGATGTVGC | pgmR | AATTTTCHGTBCCAGAATAGCGAAA | 720 |
| *tkt* | tktF | GCAAAYTCAGGMCAYCCAGGTGC | tktR | TTTTAATHAVHTCTTCRCCCAAAGGT | 730 |
| *uncA* | uncAF | GWCAAGGDGTTATYTGTATWTATGTTGC | uncAR | TTTAADAVYTCAACCATTCTTTGTCC | 700 |

TABLE 3. *Antimicrobial test ranges, MIC QC range, and breakpoints used for antimicrobial susceptibility testing*[a]

| Antimicrobial class | Antimicrobial agent | MIC QC range (μg/mL) | Test range (μg/mL) | MIC breakpoint (μg/mL) | | |
|---|---|---|---|---|---|---|
| | | | | S | I | R |
| Aminoglycoside | Gentamicin | 0.5–4 | 0.12–128 | ≤2 | 4 | ≥8 |
| Lincosamides | Clindamycin | 0.12–0.5 | 0.03–128 | ≤2 | 4 | ≥8 |
| Macrolides | Erythromycin | 1–8 | 0.03–128 | ≤8 | 16 | ≥32 |
| | Azithromycin | 0.03–0.12 | 0.015–128 | ≤2 | 4 | ≥8 |
| Quinolones | Ciprofloxacin | 0.06–0.5 | 0.015–128 | ≤1 | 2 | ≥4 |
| | Nalidixic acid | 4–16 | 4–128 | ≤16 | 32 | ≥64 |
| Tetracycline | Tetracycline | 0.25–1 | 0.06–128 | ≤4 | 8 | ≥16 |
| | Doxycycline | 0.5–2 | 0.06–128 | ≤2 | 4 | ≥8 |
| Phenicols | Chloramphenicol | 1–4 | 0.5–128 | ≤8 | 16 | ≥32 |
| | Florfenicol | 0.5–2 | 0.25–128 | ≤4 | 8 | ≥16 |

[a] QC, quality control; S, susceptible isolate; I, intermediate isolate; R, resistant isolate.

Previous studies *(17, 37)* indicated that different types of chicken products might have different *Campylobacter* contamination rates. For instance, the occurrence of *Campylobacter* was reported to be 31.0% in carcasses and 17.0% in carcass parts at the slaughterhouse level in Sichuan Province, China *(21)*. Another survey *(28)* investigated the incidence of *Campylobacter* in broiler carcasses and retail broiler meat, reporting rates of 34.1% (46 of 135 samples) and 31.3% (40 of 128 samples), respectively.

We collected the four chicken products used in the current study based on the dietary habits of consumers: chicken meat (breasts) and three chicken carcass parts (legs, wing middle joints, and wing roots). However, the differences among the of *Campylobacter* incidences in the different chicken products were not statistically significant ($P = 0.750$); the prevalence rates we found were 21.8% for breasts, 20.3% for wing middle joints, 17.5% for wing roots, and 14.3% for legs.

Packaging has been reported to be another factor related to *Campylobacter* contamination. Prepackaged chicken meat is more likely to be contaminated by *Campylobacter* because the packaging can preserve moisture on the surface of the chicken meat and increase the survival of *Campylobacter (22)*. In contrast, Burgess and colleagues *(5)* found that packaged raw meats were contaminated less frequently with *Campylobacter* than unpackaged raw meats. Therefore, our study also investigated the relationship between packaging and the *Campylobacter* contamination rate (Table 5). We collected 51 samples from supermarkets that were prepackaged and another 176 samples that were not prepackaged. The contamination rate of the prepackaged samples was 25.5%, which was slightly higher than that of the unpackaged samples, 16.5%. Nevertheless, no significant difference ($P = 0.144$) was observed between the two packaging conditions. In other words, in the current study the *Campylobacter* contamination rate of chicken products was not related to the packaging status.

**MLST.** MLST is widely recognized as a potent tool for subtyping bacteria due to its simple operation, its easy data processing, and the accessibility of the information by researchers around the world. In the current study, we performed MLST to analyze the genetic diversity of all the *C. jejuni* isolates. Overall, the MLST typing for the 31 *C. jejuni* isolates identified 22 STs belonging to 6 clonal complexes and singletons (Table 6). New alleles were discovered in the genes *aspA, glnA, gltA, uncA,* and *tkt*. Nine STs were determined to be novel ones and were assigned new ST numbers through the MLST database: ST-8240, ST-8255, ST-8256, ST-8257, ST-8258, ST-8259, ST-8260, ST-8261, and ST-8262. Of the nine novel STs, four were new combinations of the alleles that had already been detected. The other five STs were the combinations of novel alleles and previously described alleles. In addition, half the STs (11 of 22) were not assigned to any clonal complex. The high genetic diversity shown in the current study is consistent with many other previous studies *(13, 29, 33, 46)*. It was reported that poultry isolates are genetically diverse and have a broader distribution among the clonal complexes *(13, 25, 29),* probably because *C. jejuni* might endure variations only during its growth inside the host *(29, 42)*.

TABLE 4. *Prevalence of* Campylobacter *spp. isolated from different chicken products*

| Chicken part | No. of samples | No. (%) of positive samples | No. (%) of *C. jejuni* samples | No. (%) of other *Campylobacter* spp. samples |
|---|---|---|---|---|
| Legs | 56 | 8 (14.3) | 8 (14.3)[a] | 1 (1.8)[a] |
| Breasts | 55 | 12 (21.8) | 10 (18.2) | 2 (3.6) |
| Wing middle joints | 59 | 12 (20.3) | 8 (13.6)[a] | 5 (8.5)[a] |
| Wing roots | 57 | 10 (17.5) | 5 (8.8) | 5 (8.8) |
| Total | 227 | 42 (18.5) | 31 (13.7) | 13 (5.7) |

[a] One sample each of chicken leg and wing middle joint was contaminated by both *C. jejuni* and *C. coli*.

TABLE 5. *Positive rates of chicken products with different packaging status*

| Packaging status | No. of samples | No. (%) of positive samples |
|---|---|---|
| Prepackaged chicken | 51 | 13 (25.5) |
| Unpackaged chicken | 176 | 29 (16.5) |

In the current study, the most prevalent clonal complexes were ST-354 and ST-21. In a survey to identify the sequence types and antimicrobial susceptibility profiles of *C. jejuni* from north China, the most common clonal complex containing both human isolates and chicken isolates was the ST-21 complex, followed by the ST-353 complex. The minimum spanning tree created on the basis of collected data about *C. jejuni* strains screened from chicken and human stool showed that the ST-21, ST-353, ST-354, ST-443, ST-607, and ST-828 complexes, along with some singletons, were isolated from both humans and chickens (Fig. 1). These dominant clonal complexes were also observed in two other investigations *(53, 54)* characterizing *C. jejuni* in east China, indicating that they were potential reservoirs of human *C. jejuni* infection. A larger sample size may be required to study the population structure and epidemiology of *C. jejuni* in the future.

**Antimicrobial resistance.** In this study, we tested the MICs of 31 *C. jejuni* isolates from chicken products using six clinically important classes of antimicrobial agents: aminoglycoside (gentamicin [GEN]), lincosamide (clindamycin [CLI]), macrolide (erythromycin and azithromycin), quinolone (ciprofloxacin [CIP] and nalidixic acid [NAL]), tetracycline (tetracycline [TET] and doxycycline [DOX]), and phenicol (chloramphenicol [CHL] and florfenicol [FLO]). The MICs of the quality control strain *C. jejuni* ATCC 33560 were within the reference quality control range (Table 3). Based on the selected breakpoints, all (100%) *C. jejuni* isolates were resistant to the quinolone class, including ciprofloxacin and nalidixic acid (Fig. 2). This is consistent with the previous studies *(3, 7, 20, 28)* in China showing that almost all the *C. jejuni* bacteria were resistant to ciprofloxacin. The incidence of resistance to the tetracycline class was also relatively high (77.4% for tetracycline and 67.7% for doxycycline). The *C. jejuni* isolates analyzed in the current study showed less resistance to the aminoglycoside class (gentamicin, 35.5%), lincosamide class (clindamycin, 25.8%), and phenicol class (florfenicol, 25.8%; chloramphenicol, 19.4%). Most of the isolates were susceptible to the macrolide class; only 12.9% of the *C. jejuni* isolates were resistant to erythromycin and azithromycin simultaneously. Overall, the antimicrobial resistance rates in the current study were slightly higher than found in other geographical regions. For example, the rates of ciprofloxacin and tetracycline resistance were determined to be low in Australia (ciprofloxacin, 0%; tetracycline, 19.2%) *(32)* and Canada (ciprofloxacin, 6%) *(11)*.

We distinguished 11 antimicrobial resistance patterns in the current study, shown in Table 7. Of the 31 *C. jejuni* isolates, 26 (83.9%) were resistant to more than one class of antimicrobial agent. The most common pattern was CIP-NAL-TET-DOX (29%), followed by CIP-NAL (16.1%) and

TABLE 6. *Allele numbers, sequence types, and clonal complexes of* C. jejuni *isolates*[a]

| | | | MLST profile | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Clonal complex | ST | No. of isolates | *aspA* | *glnA* | *gltA* | *glyA* | *pgm* | *tkt* | *uncA* |
| ST-21 complex | 6500 | 1 | 2 | 1 | 52 | 3 | 23 | 100 | 5 |
| | **8261** | 1 | 2 | **609** | 12 | 3 | 2 | 3 | 5 |
| | 1811 | 1 | 2 | 4 | 12 | 3 | 2 | 1 | 5 |
| ST-353 complex | **8258** | 1 | 67 | 2 | 5 | 2 | 23 | 3 | 6 |
| ST-354 complex | 354 | 4 | 8 | 10 | 2 | 2 | 11 | 12 | 6 |
| | 2988 | 1 | 8 | 10 | 2 | 2 | 10 | 12 | 6 |
| | 4259 | 1 | 8 | 10 | 2 | 2 | 13 | 12 | 6 |
| ST-443 complex | 6522 | 1 | 24 | 17 | 389 | 10 | 23 | 3 | 12 |
| ST-607 complex | 2927 | 1 | 166 | 2 | 5 | 10 | 151 | 3 | 1 |
| ST-828 complex | 6267 | 2 | 33 | 42 | 30 | 82 | 189 | 35 | 17 |
| | 890 | 1 | 33 | 38 | 30 | 82 | 104 | 35 | 36 |
| Singletons | 2131 | 2 | 7 | 4 | 1 | 68 | 11 | 58 | 6 |
| | 4240 | 1 | 67 | 4 | 5 | 68 | 11 | 3 | 6 |
| | 4258 | 1 | 2 | 2 | 2 | 10 | 10 | 59 | 5 |
| | 8089 | 4 | 8 | 455 | 291 | 668 | 127 | 24 | 19 |
| | **8240** | 2 | 7 | 112 | 5 | 2 | 13 | **630** | 26 |
| | **8255** | 1 | 14 | 21 | **511** | 10 | 86 | 3 | 6 |
| | **8256** | 1 | 8 | 364 | 80 | 28 | 74 | 385 | **533** |
| | **8257** | 1 | 9 | 114 | 2 | 53 | 127 | 3 | 3 |
| | **8259** | 1 | **437** | **605** | 291 | 26 | 127 | 24 | 35 |
| | **8260** | 1 | 7 | 112 | 5 | 2 | 23 | 1 | 26 |
| | **8262** | 1 | 8 | 61 | 291 | 26 | 470 | 24 | 35 |

[a] Novel alleles and STs are in boldface.

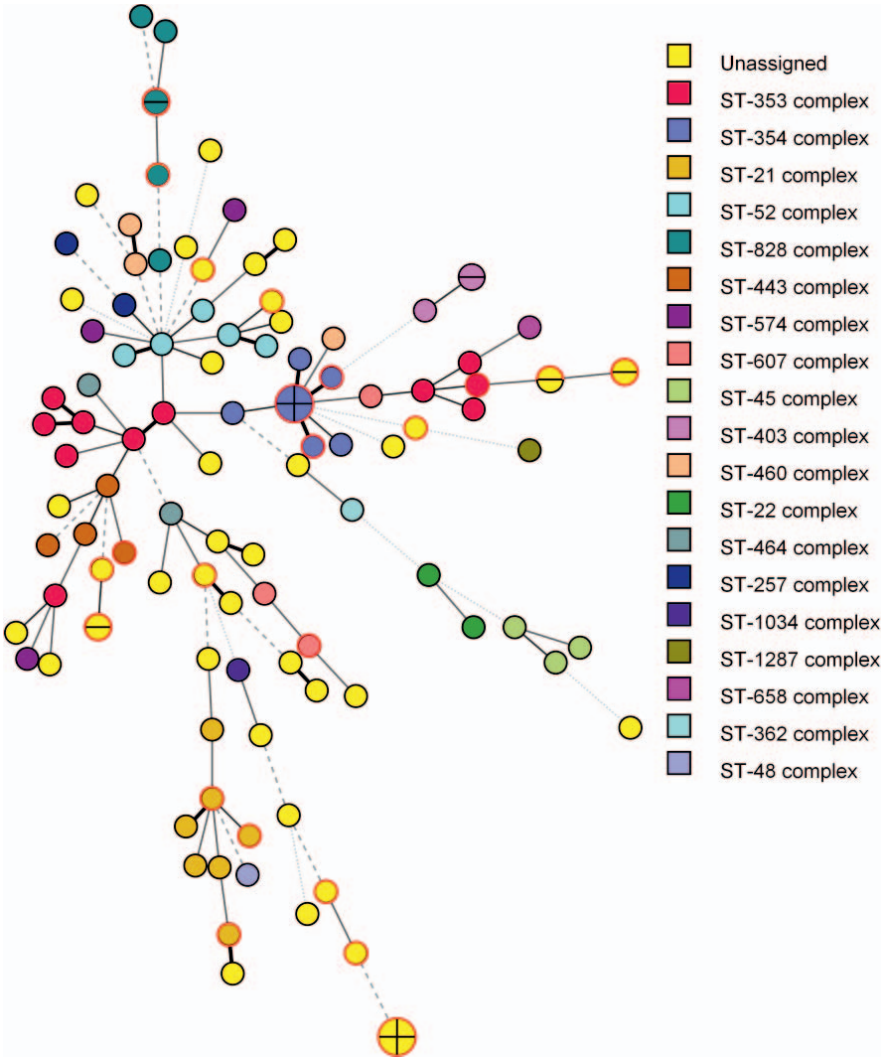


FIGURE 1. *Minimum spanning tree established on the 31 identified STs in this study and isolate STs of human and chicken origin collected in China from the MLST database. Each circle represents an ST. The size of circle is positively correlated to the isolate numbers with the same ST. The branch styles represent the numbers of identical alleles between connected STs (black thick solid line, six alleles; black thin solid line, five alleles; gray thick solid line, four alleles; dashed line, three alleles; and dotted line, two alleles or fewer). The color inside the circle stands for the clonal complex. Chicken and human clinical isolates are shown with red and black frames, respectively.*

CIP-NAL-TET-DOX-CHL-FLO-GEN-CLI (12.9%). This result is partially consistent with other studies *(17, 23, 43, 48, 52),* in which CIP-TET was reported to be the primary antimicrobial resistance pattern in countries such as Japan, Korea, Iran, Poland, and France. Surprisingly, the multidrug resistance rate was 41.9% in the current study, which is dramatically lower than in most other investigations in China but higher than that in some European countries. For example, the prevalence of multidrug resistance in *C. jejuni* isolates was determined to be 39.2% in Beijing, 71.7% in Shanghai, 90.0% in Shangdong Province, and 91.3% in Sichuan Province *(3, 7, 21, 28)*. In contrast, multidrug resistance of *C. jejuni* was identified in 17.8% of the isolates tested in the United Kingdom *(27),* 11.9% in a central province of Spain *(39),* and 0.2% in Poland *(48)*. Interestingly, in our study the macrolide-resistant isolates were all multidrug resistant, and there was one isolate (ST-8259) that was resistant to all the tested classes of antimicrobials. Moreover, 87.1% of the isolates showed high levels of resistance, with MIC $\geq$ 128 μg/mL for one or more antimicrobial agents, including gentamicin, clindamycin, erythromycin, azithromycin, ciprofloxacin, nalidixic acid, and tetracycline (see Supplemental Table S1).

Antimicrobial agents have been widely applied for the prophylaxis of disease and as a growth promoter in livestock husbandry. The misuse and overuse of antimicrobials are accelerating the emergence and spread of antimicrobial-

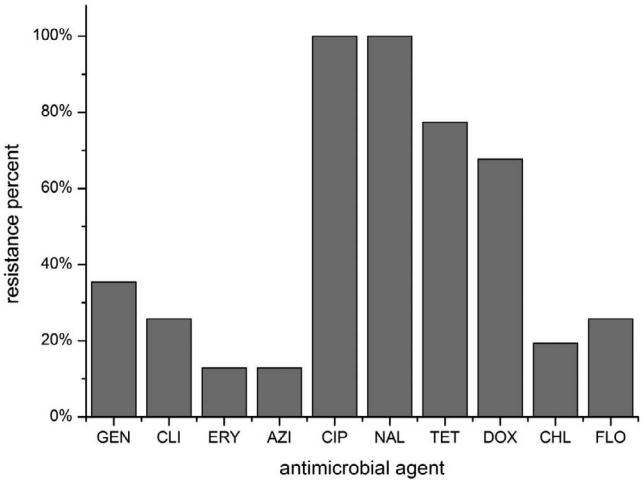


FIGURE 2. *Antimicrobial resistance of* C. jejuni. *GEN, gentamicin; CLI, clindamycin; ERY, erythromycin; AZI, azithromycin; CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline; DOX, doxycycline; CHL, chloramphenicol; FLO, florfenicol.*

TABLE 7. *Antimicrobial resistant profiles of* C. jejuni *isolates*[a]

| Antimicrobial resistance profile | No. of antimicrobial classes | No. of isolates | Percentage of isolates |
|---|---|---|---|
| CIP-NAL | 1 | 5 | 16.1 |
| CIP-NAL-TET | 2 | 3 | 9.7 |
| CIP-NAL-GEN | 2 | 1 | 3.2 |
| CIP-NAL-TET-DOX | 2 | 9 | 29 |
| CIP-NAL-TET-DOX-FLO | 3 | 2 | 6.5 |
| CIP-NAL-TET-DOX-GEN | 3 | 2 | 6.5 |
| CIP-NAL-TET-DOX-CHL-FLO | 3 | 1 | 3.2 |
| CIP-NAL-GEN-CLI-ERY-AZI | 4 | 1 | 3.2 |
| CIP-NAL-TET-DOX-CHL-FLO-GEN-CLI | 5 | 4 | 12.9 |
| CIP-NAL-TET-DOX-GEN-CLI-ERY-AZI | 5 | 2 | 6.5 |
| CIP-NAL-TET-DOX-CHL-FLO-GEN-CLI-ERY-AZI | 6 | 1 | 3.2 |
| Total | 6 | 31 | 100 |

[a] $n = 31$. CIP, ciprofloxacin; NAL, nalidixic acid; TET, tetracycline; GEN, gentamicin; DOX, doxycycline; FLO, florfenicol; CHL, chloramphenicol; CLI, clindamycin; ERY, erythromycin; AZI, azithromycin.

resistant bacteria. In our current study, we found a high prevalence of antimicrobial-resistant *C. jejuni* isolated from chicken products in Tianjin, which highlights the importance of developing mitigation strategies. These results provide the Chinese food administration a better understanding of which antibiotics should be limited in animal husbandry as well as emphasizing the importance of performing regular tests to detect *Campylobacter* bacteria in retail chicken products.

**Relationship between MLST and antimicrobial resistance.** The cladogram in Figure 3 depicts the connection between MLST and the corresponding antimicrobial resistance profiles. Generally, isolates with the same ST have the same resistance profile. For example, four ST-354 isolates were all resistant to ciprofloxacin, nalidixic acid, tetracycline, and doxycycline (coefficient of 0.602; $P < 0.01$), and four ST-8089 isolates had the same antimicrobial resistance pattern (coefficient of 1.000; $P < 0.01$). Interestingly, although some recovered isolates were attributed to the identical clonal complex, they were distant in the phylogenetic tree and presented different antimicrobial resistance profiles. For example, ST-8261, ST-1811, and ST-6500 were all assigned to the ST-21 complex. ST-8261 and ST-1811 are close to each other in the phylogenetic tree, and both were resistant to ciprofloxacin, nalidixic acid, tetracycline, and doxycycline, whereas ST-6500 is slightly

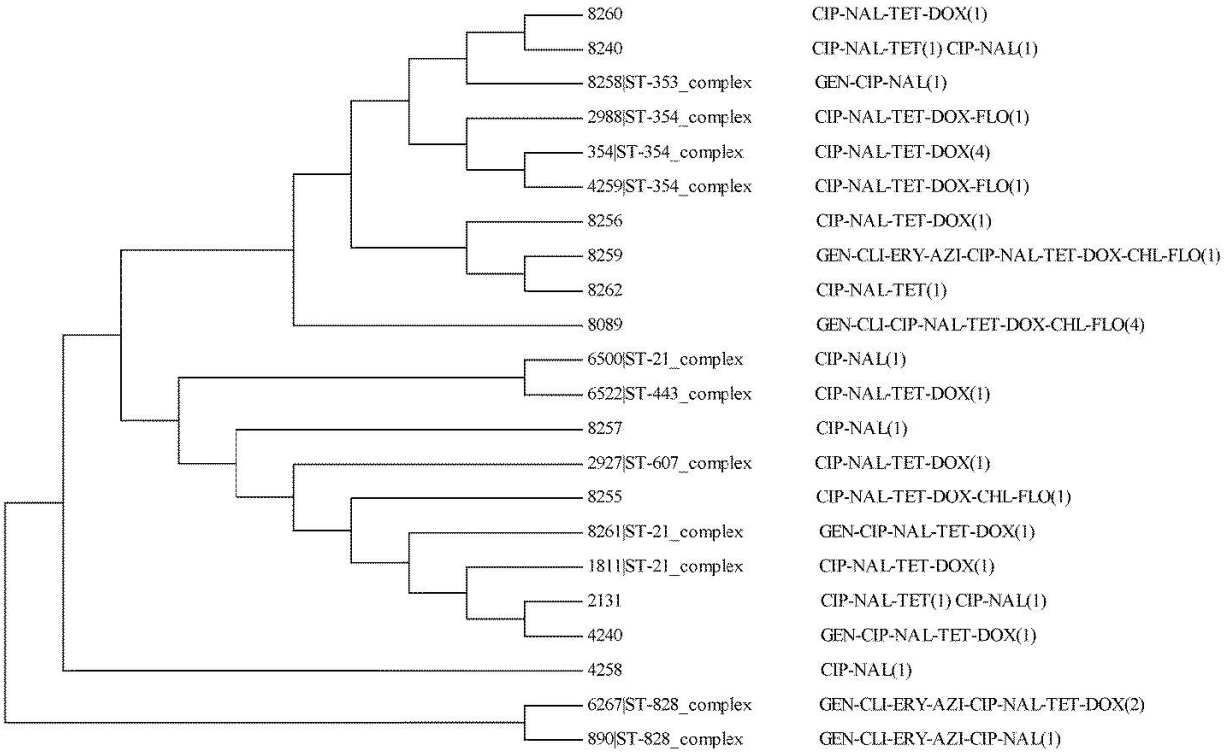


FIGURE 3. *Phylogenetic tree of* C. jejuni *isolates with the relative clonal complex and resistance profile. Values in the parentheses represent the number of the isolates with an identical resistance profile.*

distant from them and was resistant to only ciprofloxacin and nalidixic acid. Another intriguing discovery was that ST-8259, which carries two novel alleles (Table 6), showed high resistance to all the antimicrobials used in the study (Table S1).

Previous studies indicated that the ST-354 complex and ST-607 complex were significantly associated with ciprofloxacin resistance *(10)* and that the ST-21 complex was frequently resistant to ciprofloxacin (65.7%) and tetracycline (40%) *(19)*. These findings are consistent with our observation in the current study.

The mechanism of the generation and development of antimicrobial-resistant bacteria is complex. The major factor might be the use of antimicrobials that constitute a selective environment for the treated bacteria. In addition, spontaneous mutation and the acquisition of resistance determinants from other bacteria also play critical roles in the development and transmission of antimicrobial resistance *(1, 47)*. Molecular typing could be a powerful tool to track down the origin of antimicrobial-resistant isolates by analyzing the relationship between a specific clonal complex and the correlated antimicrobial resistance patterns *(36, 45, 46)*.

In conclusion, the current study provides a comprehensive understanding of *Campylobacter* contamination in retail chicken products in Tianjin. In addition, we identified a high level of antibiotic resistance in the *C. jejuni* isolates and certain clonal complexes that were related to human disease. Therefore, further study is needed to find the most efficient control measures to adopt. Because poultry products are the major reservoir of the bacteria and are the most commonly implicated foods for *Campylobacter* infections in human, further mitigation and intervention strategies should be developed to reduce the prevalence of this microaerophilic bacterium in China.

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## SUPPLEMENTAL MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/0362-028X.JFP-16-561.s1.

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