A seventeen-year observation of the antimicrobial susceptibility of clinical *Campylobacter jejuni* and the molecular mechanisms of erythromycin-resistant isolates in Beijing, China

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SUMMARY

**Objectives:** To investigate the dynamic development of the antimicrobial resistance of *Campylobacter jejuni* isolated from human diarrhea in Beijing, China, between 1994 and 2010, and to further analyze the molecular mechanisms of erythromycin-resistant strains.

**Methods:** Susceptibility tests were performed on 203 non-duplicate clinical *C. jejuni* strains against eight common antibiotics using the standard agar dilution method. The molecular determinants were further studied in the erythromycin (ERY) non-susceptible strains. The analysis focused on the 23S rRNA gene, the *rplD* and *rplV* ribosomal genes, the *ermB* gene, and the regulatory region of the CmeABC efflux pump.

**Results:** The rates of resistance of *C. jejuni* to ciprofloxacin (CIP), nalidixic acid (NAL), doxycycline (DOX), tetracycline (TET), florfenicol (FFC), and chloramphenicol (CHL) increased significantly over the period studied (all *p* < 0.05). Similarly, the proportions of resistant patterns (CIP–NAL–DOX–TET, CIP–NAL–DOX–TET–FFC, and CIP–NAL–DOX–TET–CHL) increased remarkably. In this study, 4.4% (9/203) of *C. jejuni* strains were ERY non-susceptible. The A2075G mutation in the 23S rRNA was found in all of the resistant strains except cj8091, which harbored the *ermB* gene. Interestingly, the *ermB* gene was also detected in intermediate resistant isolates, and the earliest *ermB*-positive strain cj94473 was derived in 1994. Moreover, none of the ribosomal *rplD* or *rplV* genes harbored mutations that have been described to confer resistance to macrolides. Different mutations affecting the regulatory region of the CmeABC efflux pump were also found.

**Conclusions:** This is the first comprehensive study on the recent trend in antimicrobial resistance and the molecular mechanisms of macrolide resistance in clinical *C. jejuni* strains isolated in China. More stringent monitoring and regulation of human and animal antimicrobial use are warranted.

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1. Introduction

*Campylobacter jejuni*, a food-borne zoonotic pathogen, is considered one of the most common causes of bacterial gastroenteritis worldwide, especially in developed countries.1 Few cases of *C. jejuni* infection have been reported in developing countries, and these cases have been characterized by marked seasonality and localized outbreaks.2 The transmission of *C. jejuni* to humans occurs largely through the consumption of contaminated food and animal products, especially poultry.3 The common symptoms of campylobacteriosis are watery or bloody diarrhea, abdominal pain, fever, and nausea.4,5 Additionally, Guillain–Barré syndrome (GBS) is a severe sequela that mainly occurs after *C. jejuni* infection and is mediated by an autoimmune demyelinating polyneuropathy of the peripheral nervous system.4

Although *C. jejuni* infections are typically self-limiting and usually resolve within a few days without antibiotic therapy,
antibiotics are urgently required for routine prophylaxis in cases of acute diarrhea and immunocompromised or pregnant patients, particularly in developing countries. Macrolides and fluoroquinolones, such as erythromycin and ciprofloxacin, are recommended as the first and alternative choices, respectively, in the clinical treatment of campylobacteriosis. However, rapidly increasing frequencies of C. jejuni strains that are resistant to these antibiotics and isolated from various sources (e.g., humans, poultry, or food production) have been reported in numerous studies, which could compromise future treatment.

Hence, the aim of this study was to describe the dynamic development of the antimicrobial resistance of C. jejuni isolated from human diarrhea between 1994 and 2010 in Beijing, China. The molecular mechanisms of resistance to macrolides were further analyzed.

2. Materials and methods

2.1. Bacterial isolates and culture conditions

A total of 203 non-duplicate strains were isolated from stool samples of diarrhea patients (n = 203) between January 1994 and December 2010 in the Department of Infectious Diseases, Peking University First Hospital, Beijing, China. All patients included in the study were over 14 years of age. The isolates were grown on Skirrow’s medium (Columbia agar base supplemented with 5% sheep blood at 37 °C for 48 h in a microaerobic environment containing 5% O2, 10% CO2, and 85% N2). The identification of C. jejuni was performed using multiple PCR, as reported previously. All strains were preserved in Mueller–Hinton (MH) broth with 20% glycerol at −80 °C.

2.2. Antimicrobial susceptibility testing

The minimum inhibitory concentrations (MICs) of eight antibiotics in the C. jejuni isolates were measured using the standard agar dilution method, according to the Clinical and Laboratory Standards Institute guidelines. Mueller–Hinton agar plates were supplemented with 5% sheep blood and incubated for 48 h at 37 °C. C. jejuni ATCC 33560 was used as a quality control strain for susceptibility testing in the antimicrobial susceptibility determinations. The C. jejuni isolates were considered resistant to chloramphenicol (CHL), ciprofloxacin (CIP), nalidixic acid (NAL), and tetracycline (TET) at MICs of ≥32, ≥4, ≥64, and ≥16 μg/ml, respectively. For gentamicin (GEN), florfenicol (FFC), and doxycycline (DOX), the isolates with MICs ≥8 μg/ml were considered resistant. Additionally, the MIC breakpoints for erythromycin (ERY) were those defined by the CLSI, as follows: susceptible, ≤8 μg/ml; intermediate, 8–32 μg/ml; and resistant, ≥32 μg/ml. Strains with MICs ≥256 μg/ml were considered to have high-level resistance to ERY.

2.3. PCR amplification of genes and sequence analysis

Genomic DNA was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) following the manufacturer’s instructions. The 23S rRNA, the ribosomal protein coding genes rplD and rplV, the regulatory region of CmeABC (between CmeR and CmeA), and the ermB gene were detected in erythromycin non-susceptible (i.e., intermediate and resistant) C. jejuni strains (MIC ≥16 μg/ml) and susceptible strains. The genomic targets and primer sets used are listed in Table 1.

The PCR products were purified and then sequenced by the Biomed Corporation (China, Beijing), and the sequences were analyzed to identify mutations using the BLAST program of the GenBank sequence database.

2.4. Statistical analysis

The statistical analysis was performed using GraphPad Prism 5.0 software. The Chi-square test and Fisher’s exact two-tailed test were used to compare differences in the ratios of resistance between the different periods of time. The rates of resistance of the isolates and their 95% confidence intervals (95% CIs) were calculated overall. Differences were considered significant at p-values of <0.05.

3. Results

3.1. Changes in the antimicrobial susceptibility of C. jejuni

A total of 203 non-duplicate C. jejuni isolates obtained at Peking University First Hospital from 1994 to 2010 were analyzed in this study. By time period, the numbers of isolates obtained were 18 in 1994–1996, 25 in 1997–1999, 63 in 2000–2002, 27 in 2003–2005, 41 in 2006–2008, and 29 in 2009–2010. The frequencies of resistance of C. jejuni to eight common antimicrobial agents are summarized in Table 2. The data revealed a continuous increase in antimicrobial resistance to CIP, NAL, DOX, TET, FFC, and CHL during the period 1994–2010, and all of the changes were statistically significant (50–100%, p < 0.001; 50–100%, p < 0.0001; 66.7–100%, p < 0.0004; 72–100%, p < 0.0006; 12–62%, p < 0.005; 5.6–34.5%, p < 0.05, respectively). In contrast, the total proportions of isolates that were resistant to ERY and GEN were relatively low, and the changes in the resistance to these antibiotics remained nonsignificant (p = 0.5797 and 0.2621, respectively).

The proportions of the C. jejuni isolates that were resistant to at least four of the antimicrobial agents used in the study were also

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<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Sequence (5’→3’)</th>
<th>Annealing temperature (°C)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>23S rRNA</td>
<td>F1-campy-23S</td>
<td>AAGAGGATGTATAGCCTGTAACG</td>
<td>55</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>R1-campy-23S</td>
<td>AAGCATTTCAAGCGTCTC</td>
<td>44</td>
<td>15</td>
</tr>
<tr>
<td>rplD</td>
<td>L4 Fwd</td>
<td>GATGTTAAGGCTGACATACCA</td>
<td>50</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>L4 Rev</td>
<td>GCCAGATTTGAATACACG</td>
<td>58</td>
<td>This studyb</td>
</tr>
<tr>
<td>rplV</td>
<td>CJ20</td>
<td>TCCGTTTATATTTACGAA</td>
<td>55</td>
<td>17</td>
</tr>
<tr>
<td>C21</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ermB</td>
<td>Erm-F</td>
<td>CAGGTAAGGCAGTTTAAACG</td>
<td>58</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>Erm-R</td>
<td>CATCCTGATGACTGCCGGAAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IR region</td>
<td>CmejejuniF</td>
<td>TGGCAATTTTGATAGAAATAATC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CmejejuniR</td>
<td>TGGCAATTTTGATAGAAATAATC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CmeR-F</td>
<td>CATCGTTTATGGCGGTAAG</td>
<td>58</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>CmeR-R</td>
<td>CAGGTAAGGCAGTTTAAACG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ATTACACGTACGTCG</td>
<td>55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* IR region, a CmeR–CmeA intergenic upstream of the CmeA gene.

b The primers for the ermB gene were designed on the basis of GenBank accession number KC575115.1.
analyzed and are presented in Table 2. The rates of resistance to CIP–NAL–DOX–TET, FFC–CIP–NAL–DOX–TET, and CHL–FFC–CIP–NAL–DOX–TET increased significantly over the 17-year period (p < 0.0001, p = 0.0003, and p = 0.0004, respectively). In contrast, a trend towards an increase in the susceptibility of CHL–FFC–CIP–NAL–DOX–TET was not significant (p = 0.2194).

### 3.2. Mechanisms of macrolide resistance

To investigate the molecular basis of the ERY-resistant isolates, the characteristics of macrolide resistance associated with the genes was analyzed in four resistant isolates (cj8091, cj8036, cj2000, and cj8083), five intermediate isolates (cj94473, cj6033, cj2260, cj5082, and cj99117) and two susceptible isolates (cj6073 and cj99166). As illustrated in Table 3, multiple mutations were observed in the target genes, including 23S rRNA, rrlD, rrlP, and the regulatory region of CmeABC; furthermore, the presence of the recently reported macrolide resistance-related ribosomal RNA methylase gene ermB was noted.18

#### 3.2.1. Analysis of the 23S rRNA gene

The A2075G mutation in the 23S RNA gene, which is responsible for high-level resistance to ERY (MIC ≥256 µg/ml), was detected in all of the resistant strains except cj8091 (Table 3).

#### 3.2.2. Analysis of the L4 and L22 ribosomal protein genes

Regarding the L4 ribosomal protein gene, none of the high-level ERY-resistant strains harbored the described mutations Gly-to-Asp/Val (G57V/D) and/or Gly-to-Asp (G74D) that confer resistance to macrolides. However, three different mutations in the L4 ribosomal protein gene, including Val-to-Ala (V196A), Asn-to-Ser (N95S), and Ala-to-Val (A114V), were identified in these strains (Table 3). Regarding the L22 ribosomal protein gene, eight non-synonymous mutations and two deletions (delThr–Thr–Thr–Lys–Ala at positions 120–124 and delThr at position 130) were detected.

#### 3.2.3. cmeR–cmeA intergenic region analysis

The IR region was located either from −46 to −31 (consensus sequence: TGTAAATAATTATTACA) or from −50 to −35 (consensus sequence: TGTAAATAATTATTACA) upstream of the CmeA gene. Three resistant and five intermediate strains presented polymorphisms (Table 3) in these regions: six strains had a modified IR, either a T–A or C transition in the first half-site of the IR or an A–T or G–C transition in the second half-site of the IR, and two strains exhibited single deletions.

#### 3.2.4. Presence of the ermB gene

Four ermB-positive isolates, including two ERY-resistant strains (cj8091 and cj8036) and two ERY-intermediate resistant

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**Table 2**

Rates of antibiotic resistance in clinical Campylobacter jejuni isolates between 1994 and 2010

<table>
<thead>
<tr>
<th>Antibioticsa</th>
<th>Rates of resistant isolates (% (95% CI))</th>
<th>Total % (95% CI) (n = 203)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CIP</td>
<td>(50.0 (26.7–73.9)</td>
<td>(50.0 (26.7–73.9)</td>
</tr>
<tr>
<td>NAL</td>
<td>(50.0 (26.7–73.9)</td>
<td>(50.0 (26.7–73.9)</td>
</tr>
<tr>
<td>DOX</td>
<td>(82.3 (59.3–97.3)</td>
<td>(95.1 (83.5–99.4)</td>
</tr>
<tr>
<td>TET</td>
<td>(82.3 (59.3–97.3)</td>
<td>(92.7 (80.1–98.5)</td>
</tr>
<tr>
<td>GEN</td>
<td>(82.3 (59.3–97.3)</td>
<td>(100 (88.1–100)</td>
</tr>
<tr>
<td>CHL</td>
<td>(82.3 (59.3–97.3)</td>
<td>(100 (88.1–100)</td>
</tr>
<tr>
<td>FFC</td>
<td>(82.3 (59.3–97.3)</td>
<td>(100 (88.1–100)</td>
</tr>
<tr>
<td>ERY</td>
<td>(82.3 (59.3–97.3)</td>
<td>(100 (88.1–100)</td>
</tr>
<tr>
<td>CIP–NAL–DOX–TET</td>
<td>(33.3 (13.3–59.0)</td>
<td>(78.0 (62.4–89.4)</td>
</tr>
<tr>
<td>FFC–CIP–NAL–DOX–TET</td>
<td>(11.1 (1.4–34.7)</td>
<td>(26.8 (14.2–42.9)</td>
</tr>
<tr>
<td>CHL–FFC–CIP–NAL–DOX–TET</td>
<td>(0 (0–18.5)</td>
<td>(9.8 (2.7–23.1)</td>
</tr>
<tr>
<td>GEN–FFC–CIP–NAL–DOX–TET</td>
<td>(0 (0–18.5)</td>
<td>(9.8 (2.7–23.1)</td>
</tr>
</tbody>
</table>

**Table 3**

Characteristics of macrolide-resistant genes in Campylobacter jejuni

<table>
<thead>
<tr>
<th>Strain/Year</th>
<th>ERY MIC (µg/ml)</th>
<th>Mutation in 23S RNA gene</th>
<th>ermB</th>
<th>Ribosomal protein polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>cj8091/2008</td>
<td>512</td>
<td>wt</td>
<td>+</td>
<td>V196A</td>
</tr>
<tr>
<td>cj8036/2008</td>
<td>512</td>
<td>A2075G</td>
<td>+</td>
<td>V196A</td>
</tr>
<tr>
<td>cj2000/2000</td>
<td>256</td>
<td>A2075G</td>
<td>-</td>
<td>V196A</td>
</tr>
<tr>
<td>cj7083/2007</td>
<td>512</td>
<td>A2075G</td>
<td>-</td>
<td>V196A</td>
</tr>
<tr>
<td>cj94473/1994</td>
<td>16</td>
<td>wt</td>
<td>+</td>
<td>N95S V196A Q24R S109A</td>
</tr>
<tr>
<td>cj0333/2006</td>
<td>16</td>
<td>wt</td>
<td>+</td>
<td>V196A</td>
</tr>
<tr>
<td>cj2260/2002</td>
<td>16</td>
<td>wt</td>
<td>+</td>
<td>V196A</td>
</tr>
<tr>
<td>cj5082/2005</td>
<td>16</td>
<td>wt</td>
<td>+</td>
<td>A114V V196A</td>
</tr>
<tr>
<td>cj99117/1999</td>
<td>16</td>
<td>wt</td>
<td>+</td>
<td>A114V V196A</td>
</tr>
<tr>
<td>cj6073/2006</td>
<td>2</td>
<td>wt</td>
<td>+</td>
<td>V196A</td>
</tr>
<tr>
<td>cj99166/1999</td>
<td>0.5</td>
<td>wt</td>
<td>+</td>
<td>V196A</td>
</tr>
</tbody>
</table>

ERY, erythromycin; MIC, minimum inhibitory concentration; V, Val; A, Ala; N, Asn; S, Ser; I, Ile; G, Gly; T, Thr; E, Gln; R, Arg; K, Lys; wt, wild-type.

a Based on corresponding sequences of Campylobacter jejuni NCTC 1168 (GenBank accession number NC_002163).

b +/− indicates whether or not the target genes were detected.

c *, Nucleotide A deletion in the second half-site of the IR.
strains (cJ94473 and cJ6033), were detected in this study. To further identify the length of the ermB gene, the whole fragment was amplified using Cjerm-F (5′-GAGTGGTGTGATACAGTCATC-3′) and Cjerm-R (5′-GTAAAGTGTACTTCTAATTAC-3′) primers designed on the basis of GenBank accession number KC575115. 1. The ermB gene in these four isolates contained 738 base pairs.

4. Discussion

Campylobacter jejuni has been one of the leading causes of gastrointestinal illness in humans in recent years. Although a rapid increase in the proportion of antimicrobial agent-resistant C. jejuni isolated from diarrhea patients has been reported recently in other countries, similar increases in antibiotic resistance rates in clinical C. jejuni isolates have rarely been reported in China. In the present study, the proportion of quinolone-resistant isolates was found to have increased markedly over a period of 17 years and reached 93.7–100% after 2000. Previous studies in other developing countries, such as India, have reported similar results (97%; 2008–2010). Fluoroquinolones are first-line antibiotics for acute diarrhea that can easily be purchased without a prescription, and the overuse of fluoroquinolones is thought to be the main underlying cause of the trends described above. Additionally, the increase in level of fluoroquinolone-resistant isolates might be attributed to the widespread use of quinolones in the food animal industry, especially in poultry production in China.

Similar to quinolones, an increase in tetracycline (TET/DOX) resistance in C. jejuni was also found in the present study. These data are consistent with those from previous studies on tetracycline-resistant C. jejuni isolated from non-clinical samples in other regions. However, the tetracycline resistance rates of clinical isolates from diarrheal patients in several studies have been distinctly lower than those observed in the present study. In China, tetracyclines are also commonly used to treat and prevent bacterial diseases in poultry and have rarely been used to treat human campylobacteriosis over the past two decades. Hence, it is suspected that the food-borne transmission of tetracycline-resistant strains to humans might be the dominant cause of the high prevalence of resistance to this antibiotic in C. jejuni.

With phenicols (FFC/CHL) and aminoglycosides (GEN), the resistance rates of C. jejuni to these two classes of drugs were found to be relatively low, but they have been rising significantly. In general, phenicol and aminoglycoside resistance has been documented as being of low occurrence among C. jejuni strains. CHL and GEN are used minimally in the treatment of human diarrhea due to their adverse effects, and florfenicol has been added to animal feed as a growth promoter. Therefore, the high prevalence of resistance to these antibiotics in China might be due to their overuse in food-producing animals.

In this study, the majority of the C. jejuni strains had multiple resistance to combined quinolones and tetracyclines, and the prevalence of isolates that were resistant to the combination of these two antibiotics and phenicols increased strikingly from 1994 to 2010. Few reports have observed such high frequencies of multidrug resistance in clinical C. jejuni isolates. The multidrug resistance patterns and the high MICs of these antibiotics restrict the options for suitable antimicrobial agents for use against human campylobacteriosis, making empirical treatment more difficult. It is noteworthy that the ratios of resistant strains for almost all of the aforementioned antibiotics improved significantly after 2000 (see Table 2). Nevertheless, it was not possible to find direct evidence for the significantly increasing resistant strains in China after 2000 in the literature. It is of note that along with the economic development in China, animal husbandry has grown rapidly since the beginning of the 21st century, and a number of antibiotics, such as fluoroquinolones, tetracyclines, aminoglycosides, and phenicols, are added routinely to animal feed to treat and prevent bacterial diseases. This might be one of the reasons for the increased antibiotic-resistant strains in China. Further studies are necessary to explore the mechanisms of this prevalent trend.

In this study, the rate of ERY resistance seemed to be the lowest (4/203; 2%), and this result is similar to those of other studies. These data also confirm that macrolides remain the most effective antibiotics for human campylobacteriosis in China. Despite decades of ERY use, its resistance rate in Campylobacter strains has remained low. Nevertheless, an increase in ERY-resistant C. jejuni in poultry has been observed recently in China. Therefore, further investigation of the mechanisms underlying resistance to macrolides is needed and may provide a basis for monitoring the emergence of antibiotic resistance.

Target modification (e.g., point mutations in the 23S rRNA gene or in the ribosomal proteins) and the active efflux responsible for ERY-resistant strains have been identified in Campylobacter. The A2075G mutation in the 23S rRNA appeared to be the main contributor to high-level ERY resistance (MIC ≥256 μg/ml) in C. jejuni. In the present study, all of the strains resistant to ERY had MICs ≥256 μg/ml, and three of the four C. jejuni strains (cJ8036, cJ2000, and cJ7083) exhibited the A2075G mutation. The high-level ERY-resistant strain cJ8091 carried no A2075G mutation, but the RNA methylase gene ermB was detected. Similar reports have also been observed by Qin et al. The ermB gene represents a major mechanism for macrolide resistance in other bacteria; however, it was not identified in Campylobacter until recently. Qin et al. first reported the identification of a horizontally transmitted ermB gene that mediated high-level macrolide resistance in a single Campylobacter coli isolated from a farm animal. They further analyzed the characteristics of the 58 ermB-positive Campylobacter isolates, and the presence of both the 23S rRNA mutation and the ermB gene did not appear to exert an additive effect on resistance, indicating that macrolide-resistant isolates harboring both mechanisms do not differ significantly from ermB alone with regard to antibiotic resistance. Compared with cJ80891, the macrolide MIC of cJ8036, which harbors both the A2075G mutation and the ermB gene, did not exceed 512 μg/ml, the result of which is consistent with the above report. The detailed mechanisms by which ermB is involved in macrolide resistance in combination with the A2075G mutation warrant further investigation. Surprisingly, the ermB gene was also observed in two intermediate strains (cJ94473 and cJ6033) and the earliest ermB-positive strain cJ94473, which was derived in 1994. This ermB-positive strain was isolated far earlier than in previous reports in which most of the isolates harboring ermB with identical high-level MICs against macrolide were derived between 2011 and 2012. Notably, the ermB-positive isolates typically exhibited resistance to multiple classes of antibiotics irrespective of the location of ermB (i.e., on a plasmid or the chromosome). Although the authors are not aware of the location of ermB in the strains observed in the present study, multidrug resistance similar to that reported in previous studies was observed in this study (data not shown).

L4 and L22 are highly conserved proteins among bacteria due to their functions in ribosomes. Mutations in the large loop of the L4 protein (residues 55 to 77) and the L22 protein (residues 78 to 98) have been confirmed to be associated with macrolide resistance in various bacteria. In the present study, no variations were found in loop regions of the L4 or L22 proteins (Table 3). Most importantly, these mutations have also been identified in susceptible isolates in previous reports. In L22, amino acid deletions or insertions seemed to be more important than substitutions. In the present study, however, the delThr–Thr–Thr–Lys–Ala at positions 120–124 and delThr at position 130 were observed in L22 in both the susceptible and resistant strains. These insertions likely do not
affect the entry of macrolides to the ribosome. These observations suggest that the mutations in L4 and L22 were unlikely to directly contribute to ERY resistance in the isolates.

CmeK is a transcriptional repressor that modulates the expression of the efflux pump CmeABC operon by binding specifically with a CmeK–CmeA intergenic region upstream of the CmeA gene in C. jejuni. Single-nucleotide deletions and transitions in the IR region have been associated with the overproduction of the CmeABC and contribute to macrolide resistance. In this study, the T-48→C transition was found in two resistant (cj0306 and cj2000) and two intermediate strains (cj0633 and cj2260) in which mutations have also been observed previously. Therefore, the misregulation of the CmeABC operon can be expected in these strains. Moreover, the interplay between CmeABC and the mutations in the 235 rRNA may have resulted in high-level ERY resistance in the strains observed in the present study. Other mutations, such as Δ341A and A-37→G transitions in the second half-site of the IR, were detected in intermediate strains. To the authors’ knowledge, this is the first report of these mutations in clinical isolates. It is speculated that these mutations would theoretically prevent the binding of the half-sites and consequently deregulate the expression of the CmeABC operon. Recent studies have described mutations of CmeR, CmeS, CmeABC, and CmeK resistance genes such as CmeDEF that have also been deemed to be involved in the occurrence of macrolide resistance in C. jejuni. Therefore, the specific mechanisms of the contribution of efflux systems to ERY resistance remain unclear, and additional studies are needed. Overall, these results indicated that CmeABC might have contributed to the macrolide resistance in the C. jejuni strains investigated in this study.

In summary, this is the first comprehensive study on the recent trend of antimicrobial resistance and the molecular mechanisms of macrolide resistance in clinical C. jejuni strains isolated in China. This study suggests that further application of the aforementioned antibiotics should be restricted in human and veterinary practice due to the presence of high percentages of resistant strains resulting from these antibiotics.

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