Review

Antibiotic resistance trends and mechanisms in the foodborne pathogen, *Campylobacter*

Yizhi Tang1, Liangxing Fang1,2, Changyun Xu1 and Qijing Zhang1*

1 Departments of Veterinary Microbiology and Preventive Medicine, Ames, IA, USA
2 National Risk Assessment Laboratory for Antimicrobial Resistance in Bacteria of Animal Origin, South China Agricultural University, Guangzhou, China

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Abstract

*Campylobacter* is a major foodborne pathogen and is commonly present in food producing animals. This pathogenic organism is highly adaptable and has become increasingly resistant to various antibiotics. Recently, both the Centers for Disease Control and Prevention and the World Health Organization have designated antibiotic-resistant *Campylobacter* as a serious threat to public health. For the past decade, multiple mechanisms conferring resistance to clinically important antibiotics have been described in *Campylobacter*, and new resistance mechanisms constantly emerge in the pathogen. Some of the recent examples include the *erm(B)* gene conferring macrolide resistance, the *cfr(C)* genes mediating resistance to florfenicol and other antimicrobials, and a functionally enhanced variant of the multidrug resistance efflux pump, CmeABC. The continued emergence of new resistance mechanisms illustrates the extraordinary adaptability of *Campylobacter* to antibiotic selection pressure and demonstrate the need for innovative strategies to control antibiotic-resistant *Campylobacter*. In this review, we will briefly summarize the trends of antibiotic resistance in *Campylobacter* and discuss the mechanisms of resistance to antibiotics used for animal production and important for clinical therapy in humans. A special emphasis will be given to the newly discovered antibiotic resistance.

Keywords: *Campylobacter*, antibiotic resistance, multidrug efflux pump, food safety.

Introduction

*Campylobacter* species, particularly *Campylobacter jejuni* and *Campylobacter coli*, are a major cause of foodborne bacterial gastroenteritis in humans (Ruiz-Palacios, 2007). As estimated by the Centers for Disease Control and Prevention (CDC), *Campylobacter* is responsible for 1.3 million cases of foodborne illnesses annually in the USA (Scallan et al., 2011). It was also estimated that *Campylobacter* spp. are responsible for 400–500 million cases of diarrhea each year worldwide (Ruiz-Palacios, 2007). Transmission of *Campylobacter* to human beings occurs mainly through contaminated food of animal origin, particularly raw or undercooked poultry meat, unpasteurized milk, and dairy products (Allos, 2001; Stanley and Jones, 2003; CDC, 2009).

Although the majority of *Campylobacter* infections are self-limited, do not require antimicrobial treatment, and usually resolve within a few days without antibiotic treatment, severe or prolonged infection may occur, particularly in the young, elderly, and individuals with compromised immunity (Allos, 2001). In these circumstances, fluoroquinolone (FQ) and macrolide antibiotics are the drugs of choice for treatment (Allos, 2001; Engberg et al., 2001). Intravenous administration of aminoglycosides are only used for the treatment of serious bacteremia and other systemic infections due to *Campylobacter* (Aarestrup and Engberg, 2001). Beta-lactam is not recommended for treatment of campylobacteriosis, but oral beta-lactam, such as co-amoxiclav, might be an appropriate agent when *Campylobacter* isolates are resistant to both FQ and macrolides (Elviss et al., 2009; Griggs et al., 2009).

As a foodborne pathogen, *Campylobacter* is prevalent in the intestinal tracts of food producing animals and is frequently exposed to antibiotics used for animal production. In response
to the selection pressure from antibiotics used for animal agriculture and human medicine, *Campylobacter* has evolved various mechanisms for resistance to clinically important antibiotics. Both the CDC and the World Health Organization have recently listed drug-resistant *Campylobacter* as a serious antibiotic resistance threat (CDC, 2013; WHO, 2017). Because of the importance of *Campylobacter* in food safety and public health, many studies have been performed to understand the epidemiology and mechanisms of antibiotic resistance in this organism. This review will summarize the current knowledge of antibiotic resistance in *Campylobacter*, with an emphasis on clinically important and newly discovered antibiotic-resistance mechanisms.

**Trends of antibiotic resistance in *Campylobacter***

FQ antimicrobials were first introduced for clinical therapy and animal production in the 1980s, and FQ-resistant *Campylobacter* was initially reported in the late 1980s in Europe (Engberg et al., 2001). Since then, a drastic increase in the incidence of FQ-resistant *Campylobacter* has been reported in different countries worldwide (Padungton and Kaneene, 2003; Luangtongkum et al., 2009; Nguyen et al., 2016; Sierra-Arguello et al., 2016; Wozniak-Biel et al., 2017). Several studies have also linked the use of FQs with the emergence and spread of FQ-resistant *Campylobacter* (Endtz et al., 1991; van Boven et al., 2003; Humphrey et al., 2005). In the USA, the introduction of sarafloxacin and enrofloxacin in the mid-1990s, for use in poultry, was linked to the rise of FQ-resistant *Campylobacter* (Nachamkin et al., 2002). Although FQs were used for the control of respiratory disease and were not intended for control of *Campylobacter* in poultry, the unintended consequence of this usage is the use of FQs with the emergence and spread of FQ-resistant *Campylobacter*, which is commonly present in the intestinal tract of birds (McDermott et al., 2003; Luo et al., 2003; Zhang et al., 2003). One study indicated that before 1992 FQ-resistant *C. jejuni* was rarely observed in the USA, whereas from 1992 to 2001, FQ-resistant *C. jejuni* of human origin increased from 1.3 to 40.5% (Nachamkin et al., 2002). A similar rising trend in FQ resistance among *Campylobacter* isolates was also reported in other countries. For example, ciprofloxacin resistance among *Campylobacter* species from humans increased from zero before 1991 to 84% in 1995 in Thailand (Hoge et al., 1998). A study across 17 years showed that the rates of ciprofloxacin resistance of clinical *C. jejuni* isolated in China increased from 50% to 93.1% between 1994 and 2010 (Zhou et al., 2016). A recent study from China found that almost 100% of the *C. jejuni* and *C. coli* isolates from chicken and swine were resistant to FQs (Wang et al., 2016). In Spain, FQ resistance among clinical *Campylobacter* isolates was not observed in 1987; however, in 1991 the frequency of FQ-resistant *Campylobacter* strains had increased remarkably to 30% (Endtz et al., 1991). Additionally, a steady increase in FQ-resistance among *Campylobacter* isolates has also been observed in many European countries (Lucey et al., 2002; Pezzotti et al., 2003; Gallay et al., 2007; Nguyen et al., 2016).

Compared with FQ resistance, macrolide resistance is much less prevalent in *Campylobacter*. However, increased but varied prevalence of macrolide-resistant *C. jejuni* and *C. coli* has been reported in both developed and developing countries (Wang et al., 2016). In most developed countries, macrolide resistance is <10% (Engberg et al., 2001; Cha et al., 2016), significantly lower than FQ resistance. In the USA, the NARMS (National Antimicrobial Resistance Monitoring System) 2014 report indicated that erythromycin resistance in the *C. jejuni* isolates from both human and chicken sources was <2%, which is lower than in *C. coli* (around 10%). Studies conducted by the National Animal Health Monitoring System (NAHMS) Dairy 2002 and Dairy 2007 reported that 0.4% of the cattle *Campylobacter* isolates were resistant to erythromycin (USDA, 2011). Similar findings also were observed in European countries, where macrolides resistance among *Campylobacter* isolates from human and *C. jejuni* isolates from chicken and cattle has been low and stable (Gibrel and Taylor, 2006; Papavasileiou et al., 2007; Bardon et al., 2009). However, in the case of *Campylobacter* isolates of animal origin from some developing countries, high prevalence of macrolide resistance, especially in *C. coli* from poultry and swine, has been reported in multiple studies (Li et al., 2016; Shobo et al., 2016; Singh and Mitral, 2016; Wang et al., 2016). This may be related to the use of macrolide agents for prevention and control of animal diseases. Interestingly, many studies have found that macrolide-resistant *C. coli* is much more prevalent than macrolide-resistant *C. jejuni* (Li et al., 2016; Shobo et al., 2016; Wang et al., 2016). For example, a recent report from China indicated that <10% of *C. jejuni* isolated from human, chicken and swine hosts were resistant to macrolides, while up to 73.2% of *C. coli* isolates were resistant to the antibiotics (Wang et al., 2016). The exact reason for the much higher prevalence of macrolide resistance in *C. coli* is unknown, but it might be possible that *C. coli* is intrinsically more capable of acquiring macrolide resistance.

The overall prevalence of phenicol resistance in *Campylobacter* has been low (<2%), but high prevalence was reported in some geographic areas. Zhou et al. (2016) analyzed 203 *Campylobacter* isolates from stool samples of diarrhea patients collected between 1994 and 2010 in China, and found the overall rate of florfenicol resistance was 31.5%, lowest at 1.2% in 1997–1999 and highest at 62% in 2009–2010. Ma et al. (2014b) profiled 259 *Campylobacter* isolates derived from a broiler chicken production chain and found the prevalence of florfenicol resistance in *C. jejuni* (37.7%) was significantly higher than that in *C. coli* (7.8%). In another study analyzing antibiotic resistance from broiler chickens, the florfenicol resistance rate of *C. jejuni* (79.8%) was found to be much higher than that of *C. coli* (6.4%) (Li et al., 2017). In the USA, NARMS analyzed 2258 *C. jejuni*, 925 *C. coli*, and 7 *Campylobacter lari* isolates from retail meat collected between 2002 and 2007, and found no resistance to florfenicol (Zhao et al., 2010). In a NARMS 2014 report, all 114 *Campylobacter* isolates tested were susceptible to florfenicol, and no genes associated with florfenicol resistance were detected. Similarly, no chloramphenicol or florfenicol resistance in *C. jejuni* isolates was detected in NAHMS Dairy 2002 and 2007 studies (USDA, 2011). However, the most recent study on *Campylobacter* isolates from feedlot cattle across five different states revealed 10% of the *C. coli* isolates were
resistant to florfenicol (Tang et al., 2017a) indicating the emergence of florfenicol resistance in bovine Campylobacter.

The prevalence rate of gentamicin-resistant Campylobacter was low in most countries (Kashoma et al., 2015, 2016; Nguyen et al., 2016). According to the NARMS surveillance data, the gentamicin resistance rate in Campylobacter was stable and low before 2007 in the USA, especially in C. jejuni. Between 2007 and 2011, gentamicin resistance increased sharply in C. coli from human and chicken sources, rising from 0 to 12% in human isolates and from 0.7 to 18% among retail chicken isolates. In China, several reports revealed a much higher gentamicin resistance rate in Campylobacter, especially for these strains isolated from chicken and swine, and in some studies the resistance rate reached above 90% (Chen et al., 2010; Yao et al., 2017).

Multidrug resistance (MDR) was defined as being resistant to three or more antimicrobial classes, and the most common drugs Campylobacter is resistant to include FQ, macrolides, tetracycline, florfenicol, trimethoprim-sulfamethoxazole (Li et al., 2017; Ma et al., 2017; Szczepanska et al., 2017). A recent study from Thailand revealed that 100% of C. jejuni and 98.9% of C. coli isolates from commercial broiler production chains were MDR, respectively, but the MDR rate in C. coli tends to be higher than in C. jejuni (Li et al., 2017; Ma et al., 2017). Usually, the overall MDR rate in C. coli examined to three or more classes of antimicrobials (Wang et al., 2016; Li et al., 2017; Ma et al., 2017). In China, 41.9 to 97.6% of retail chicken isolates exhibited MDR to three or more classes of antimicrobials (Andersson and MacGowan, 2003; Payot et al., 2006). Although most bacteria have both enzymes, Campylobacter lacks the parC and parE (topoisomerase IV) genes and thus they are not the targets of FQ antimicrobials in Campylobacter (Bachoual et al., 2001; Payot et al., 2002; Piddock et al., 2003). Additionally, no mutations in gyrB have been associated with FQ resistance in Campylobacter (Bachoual et al., 2001). Therefore, mutations linked to FQ resistance in C. jejuni and C. coli mainly occur in GyrA. Specifically, resistance to FQs involves amino acid substitutions in a region of the GyrA termed the ‘quinolone-resistance-determining region’. This region is located within the DNA-binding domain on the surface of DNA gyrase and corresponding amino acids spans from position 51 to position 106 (E. coli numbering), with common mutations at amino acid positions 83 and 87 (position 86 and 90 in Campylobacter) (Friedman et al., 2001). The most frequent mutation observed in FQ-resistant Campylobacter isolates is Thr-86-Ile, followed by Asp-90-Asn, Thr-86-Lys, Thr-86-Ala, Thr-86-Val, Asp-90-Tyr, and Ala-70-Thr (Wang et al., 1993; Engberg et al., 2001; Luo et al., 2003). The Thr-86-Ile mutation confers a high level of FQ resistance [ciprofloxacin minimum inhibitory concentration (MIC) ≥ 16 µg ml⁻¹] in Campylobacter, while other mutations are associated with a low to medium level of resistance (MIC = 1–8 µg ml⁻¹) (Luo et al., 2003; Payot et al., 2006; Yan et al., 2006). Double mutations including Thr-86-Ile/Pro-104-Ser and Thr-86-Ile/Asp-90-Asn have also been linked to FQ resistance in Campylobacter (Payot et al., 2006). Additionally, acquisition of high-level FQ resistance in Campylobacter does not require stepwise accumulation of point mutations in gyrA. Instead, a single point mutation in gyrA can lead to clinically relevant levels of resistance to FQ antimicrobials (Gootz and Martin, 1991; Wang et al., 1993; Ruiz et al., 1998; Luo et al., 2003; Yan et al., 2006).

The CmeABC efflux pump contributes significantly to both intrinsic and acquired resistance of C. jejuni to FQ antimicrobials by reducing the accumulation of FQs in Campylobacter cells
(Ge et al., 2005). In wild type 81–176, inactivation of CmeB led to a 8-fold reduction in the MIC of ciprofloxacin, suggesting that CmeABC contributes to the intrinsic resistance of Campylobacter to FQs (Lin et al., 2002). Even in the presence of resistance-conferring GyrA mutations, insertional mutagenesis of CmeABC led to drastic reduction in ciprofloxacin MIC in FQR isolates, indicating the importance of CmeABC in FQ resistance (Luo et al., 2003). Overexpression of CmeABC, either by inactivating its repressor CmeR or mutating the promoter region of cmeABC, increased the resistance to FQs in Campylobacter (Lin et al., 2005a; Yao et al., 2016). The recently identified CmeABC variant (RE-CmeABC) showed a much higher efficiency in the efflux function and conferred an exceedingly high-level resistance (ciprofloxacin MIC ≥ 256 μg ml⁻¹) to FQs in the presence of GyrA mutations (Yao et al., 2016). The RE-CmeABC appears to be increasingly prevalent in China, where FQs have been widely used for animal production practices (Yao et al., 2016). By reducing the intracellular concentration of antibiotics, CmeABC facilitates and promotes the emergence of FQR Campylobacter under selection pressure because GyrA mutations alone are not sufficient to survive the killing effect of ciprofloxacin (Yan et al., 2006). In the absence of a functional CmeABC, many spontaneous gyrA mutants would not be able to emerge under antibiotic selection (Yan et al., 2006).

**Macrolide resistance mechanisms**

Macrolide antibiotics, such as erythromycin, azithromycin, clarithromycin, and rifamycin, are a class of natural products that consist of a large macrocyclic lactone ring, which are usually 14-, 15-, or 16-membered (Tenson et al., 2003). Macrolides inhibit protein synthesis by binding to the ribosome that includes 23S rRNA and ribosomal proteins. Macrolides are usually used for the treatment of gram-positive cocci (mainly *Streptococcus* and *Staphylococcus*) and is also used for the treatment of gram-negative cocci, such as Campylobacter and Helicobacter (Leclercq, 2002). For clinical therapy of campylobacteriosis, macrolides such as erythromycin are often considered the drug of choice. Three mechanisms have been reported for macrolide resistance in bacteria, which include (i) modification of target sites by mutation or methylation, (ii) active efflux of antibiotics from bacterial cells, and (iii) antibiotic inactivation. In Campylobacter, the first two mechanisms have been documented, but macrolide inactivation by the action of esterases or phosphotransferases has not been reported.

In Campylobacter, positions 2074 and 2075 of the 23S rRNA correspond to positions 2058 and 2059 in E. coli, respectively. These two nucleotides interact directly with macrolide antibiotics and mutations in the two sites impair the binding of macrolides to 23S rRNA (Tenson et al., 2003). To date, four types of point mutations at 23S rRNA have been linked to macrolide resistance in Campylobacter, including A2074C, A2074G, A2074T, and A2075G. Among these point mutations, A2075G has been observed most frequently (Jensen and Aarestrup, 2001; Vacher et al., 2003, 2005). C. jejuni and C. coli have three copies of 23S rRNA (erm operon). In most clinical strains that are highly resistant to erythromycin (MIC > 128 μg ml⁻¹), all three copies of the rrn operons were mutated (Jensen and Aarestrup, 2001; Niwa et al., 2001; Gibreel et al., 2005). When the A2074T mutation occurred only in some of the rrn operons, it only conferred a low level resistance to macrolide (Vacher et al., 2005). However, when the A2074T mutations happened in all three copies of 23S rRNA genes, the mutant strains were highly resistant to macrolide (Ohno et al., 2016).

Modification of the ribosomal protein L4 and L22 has also been found conferring macrolide resistance in Campylobacter. L4 and L22 were encoded by the rplD and rplV genes, respectively, and both were considered as a portion of the peptide exit tunnel of the 50S ribosome. Amino acids spanning positions 63–74 are reported to be the most important target regions of the L4 protein (Corcoran et al., 2006). Mutation in this region had been reported in several bacteria with high levels of erythromycin resistance (Chittum and Champney, 1994; Tait-Kamradt et al., 2000; Malbruny et al., 2002). In Campylobacter, the G74D modification alone was found to confer low to medium resistance to macrolides (Cagliero et al., 2006). Outside the 63–74 amino acid region of L4, several other amino acid substitutions were associated with macrolide resistance in both Campylobacter and Streptococcus (Doktor et al., 2004; Corcoran et al., 2006). The L22 modifications, including insertion, mutation, and deletion are also involved in macrolide resistance in Campylobacter. Corcoran et al. (2006) identified a unique A103V substitution in the L22 protein, which was linked to high level erythromycin resistance in both *C. jejuni* and *C. coli*. Three to four amino acid insertions at position 86 or 98 of the L22 protein were also observed in macrolide-resistant isolates (Caldwell et al., 2008; Lehtopolku et al., 2011).

Recently, a new mechanism of macrolide resistance in Campylobacter has emerged (Qin et al., 2014), which is mediated by the *erm(B)* gene that encodes a RNA methyltransferase. This enzyme adds a methyl group to the A2058 (*E. coli* numbering) position located within a conserved region of domain V of the 23S rRNA. Methylation at this site gives rise to cross-resistance to macrolide, lincosamide, and streptogramin B (MLSb phenotype). To date, 43 *erm* (erythromycin ribosome methylase) genes have been reported (http://faculty.washington.edu/marilynr/), but only *ermB* has been detected in Campylobacter, including *C. jejuni* and *C. coli* in China and Europe (Qin et al., 2014; Deng et al., 2015; Flores-Cuadrado et al., 2016). In the first report of *erm(B)* in *C. coli*, it was identified in a 12,035 bp genomic segment on the chromosome and was found to confer a high-level resistance to erythromycin (MIC 512 μg ml⁻¹). This segment contained 17 open reading frames (ORFs), 8 of which were antibiotic resistance determinants, including *erm(B)*, *tet(O)*, and 6 genes encoding aminoglycoside-modifying enzymes (Qin et al., 2014). Thus, the genomic segment was named as the MDR genomic island (MDRGI). This MDRGI can be transferred between *C. jejuni* and *C. coli* via natural transformation (Qin et al., 2014). The *erm(B)* gene was also later identified in *C. jejuni*, where it was associated with several antimicrobial resistance genes (*tet(O), aadA2* and *aadA9*) in a MDRGI that was inserted in the chromosome at a different location when compared with that in *C. coli*. 

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(Deng et al., 2015). In Europe, the identified erm(B) in C. coli was also located in a MDRGI, but the MDGRI contents were different from those found in China (Florez-Cuadrado et al., 2016). Interestingly, the erm(B)-carrying MDGRIIs have different G + C contents from the rest of the chromosome, which suggests that Campylobacter acquired erm(B) from other bacterial organisms via horizontal gene transfer (Florez-Cuadrado et al., 2016). The emergence of erm(B) in Campylobacter is alarming because it alone confers a high-level resistance to macrolide antibiotics and is horizontally transferable. Thus, the spread of erm(B)-positive Campylobacter should be continuously monitored.

The multidrug efflux pump CmeABC also contributes significantly to macrolide resistance in Campylobacter. This was first demonstrated by an insertional mutation of the cmeB gene in wild-type 81–176, which resulted in a 4-fold decrease in the MIC of erythromycin (Lin et al., 2002). Additionally, overexpression of CmeABC by mutating the CmeR repressor led to 4-fold increase in the resistance to erythromycin (Lin et al., 2005a). Even in the highly resistant strains (harboring the resistance-conferring mutations in the 23S rRNA), inactivating the cmeB gene led to a drastic reduction in the MIC of erythromycin (Caglero et al., 2005; Lin et al., 2007) suggesting that the point mutations in 23S rRNA and CmeABC function synergistically in conferring high-level macrolide resistance.

Florfenicol resistance mechanisms

Florfenicol is a fluorinated derivative of thiamphenicol and has only been used in veterinary medicine since its introduction in the mid-1990s (Syriopoulos et al., 1981). In the USA, florfenicol is currently indicated for the treatment of bovine respiratory disease and bovine interdigital phlegmon. Florfenicol has a broad antibacterial spectrum against both gram-positive and gram-negative organisms, and shows a good tissue penetration property due to its lipophilicity (Schwarz et al., 2004). Once in a bacterial cell, florfenicol binds to the peptidyltransferase center to prevent the peptide chain elongation, resulting in inhibition of protein synthesis and bacterial death. Over the years, bacteria have developed mechanisms to counteract the selection pressure from florfenicol, including (i) modification or protection of the antibiotic targets and (ii) decrease of intracellular concentration by reducing the permeability and increasing efflux.

Functioning as an rRNA methyltransferase, Cfr adds a methyl group at position A2503 of 23S rRNA and plays an important role in bacterial resistance to florfenicol (Kehrenberg et al., 2005). Given that position A2503 of 23S rRNA is located at the peptidyltransferase center, which is the target of a number of antimicrobial agents, modification of this position affects binding of multiple classes of antibiotics. Indeed, antimicrobial susceptibility testing revealed that Staphylococcus aureus and E. coli strains expressing the cfr gene showed resistance to five chemically distinct classes of antimicrobials, including phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A (known as the PhilOPS₃ phenotype) (Long et al., 2006). The Cfr-mediated resistance to oxazolidinones is especially alarming as this class of antibiotics is considered the last resort for the treatment of MDR gram positives (French, 2001). The cfr gene was first discovered on a 16.5-kb plasmid from S. sciuri isolate of bovine origin in 2000 (Schwarz et al., 2000). Since its first discovery, cfr has been detected in a number of Gram-positive and Gram-negative bacteria (Schwarz et al., 2000; Dai et al., 2010; Wang et al., 2012a, b; Liu et al., 2013). The cfr gene is often carried by transferable plasmids with additional antibiotic resistance genes, facilitating its dissemination and emergence in different bacterial species and under various selective conditions (Wang et al., 2013; Liu et al., 2014; Li et al., 2015; Zhang et al., 2015). Later, a cfr-like variant, named cfr(B), was discovered in a mobile genetic element in both Peptostreptococcus difficilis and Enterococcus faecium of human origin (Deshpande et al., 2015; Hansen and Vester, 2015). Cfr(B) shares 74.9% amino acid (aa) sequence identity with the original Cfr and confers the same MDR phenotype (Deshpande et al., 2015).

Although florfenicol is not used for treating Campylobacter infection, its use in animal production imposes a selection pressure on Campylobacter. Recently, a novel cfr-like gene, named Cfr (C), was identified in Campylobacter coli and Clostridium difficile (Candela et al., 2017; Tang et al., 2017a). In Campylobacter, cfr (C) was located on a conjugative plasmid of ∼48 kb (Tang et al., 2017b) and encodes a 379 aa protein that only shows 55.1 and 54.9% aa identity to the original Cfr from Staphylococcus sciuri (Schwarz et al., 2000) and the recently reported Cfr(B) from E. faecium, respectively (Deshpande et al., 2015). Cloning of cfr(C) into C. jejuni NCTC11168 and conjugal transfer of the cfr(C)-containing plasmid confirmed its role in conferring resistance to phenicols, lincosamides, pleuromutilins, and oxazolidinones, which resulted in 8- to 256-fold increase in their MICs in both C. jejuni and C. coli. These findings established cfr(C) as a novel MDR gene and represent the first report of a cfr-like gene in a foodborne pathogen. In Clostridium difficile, cfr(C) is located on a putative 24 kb-transposon and also confers resistance to PhLOPS₃ (Candela et al., 2017). In addition to Cfr, mutation in the antibiotic target also confers resistance to florfenicol. For example, a G2073A mutation in all three copies of the 23S rRNA was shown to mediate resistance to chloramphenicol and florfenicol in C. jejuni (Ma et al., 2014a).

The typical CmeABC in C. jejuni NCTC 11168 had limited effect on florfenicol resistance (Tang et al., 2017b). However, the recently identified ‘super’ efflux pump variant, RE-CmeABC, is much more potent in conferring resistance to florfenicol and other antibiotics (Yao et al., 2016). The RE-CmeABC was discovered from MDR C. jejuni isolates, and transfer of this efflux mechanism to different C. jejuni strains resulted in a >32-fold increase in the MIC of florfenicol, suggesting its powerful role in the extrusion of florfenicol.

The fleR gene, encoding a MDR efflux pump, mediates resistance to chloramphenicol and florfenicol (Arcangiolì et al., 1999). It was first been discovered in Salmonella typhimurium DT104 (Arcangiolì et al., 1999) and had also been detected in C. coli (Frye et al., 2011). fleR encodes a protein of 404 amino acids, which functions as efflux transporter. Interestingly, pp-floR, floSr, floS, and floR, are closely related even though they were assigned different names in the literature (Kim and Aoki,
Beta-lactam resistance mechanisms

Beta-lactam antibiotics, such as penicillin, inhibit the growth of bacteria by disrupting peptidoglycan cross-linking during bacterial cell wall biosynthesis. Although beta-lactam antibiotics are not commonly prescribed for the treatment of Campylobacter infection, recent studies have proposed that oral beta-lactam, such as co-amoxiclav, could be an appropriate choice when Campylobacter is resistant to both FQ and macrolides. In Campylobacter, two mechanisms of beta-lactam resistance have been documented. One is the production of beta-lactamase OXA-61 and the other one is the multidrug efflux pump.

Several studies have reported that the majority of Campylobacter isolates were ampicillin resistant, and resistance was more common among C. coli isolates than among C. jejuni isolates (Li et al., 2007; Griggs et al., 2009; Komba et al., 2015). The genome sequence of C. jejuni NCTC 11168 revealed the presence of a putative chromosomally encoded class D beta-lactamase (Cj0299) (Parkhill et al., 2000). The corresponding gene in a clinical human isolate GCO15 has been functionally characterized and was shown to confer a ≥32-fold increase in the MICs of ampicillin, piperacillin, and carbencillin in C. jejuni (Alfredson and Korolik, 2005). The expression level of the gene can also modulate the susceptibility of Campylobacter to beta-lactams. For example, a single nucleotide mutation (G→T transversion) in the promoter region of blac_OXA-41 led to overexpression of blac_OXA-41 and consequently ≥256-fold increase in beta-lactam resistance in C. jejuni (Zeng et al., 2014). A mutator phenotype resulting from a single amino acid change (G199W) in MutY increased the mutation frequency of the G→T transversion in the blac_OXA-41 promoter region and consequently elevated the spontaneous ampicillin resistance mutation frequency in C. jejuni (Dai et al., 2015). In addition to OXA-61, other uncharacterized beta-lactamase genes may exist in Campylobacter (Griggs et al., 2009). CmeABC also plays an important role in intrinsic resistance to beta-lactam antibiotics as mutation of CmeB resulted in 32-fold reduction in ampicillin MIC (Lin et al., 2002).

Tetracycline resistance mechanisms

Tetracyclines are an important class of antibiotics widely used in both human and animal medicine (Chopra, 2001). This class of antibiotics prevent bacterial growth by inhibiting protein synthesis with interaction of the antibiotics to the ribosomal 30S subunit (Chopra, 2001). The most important mechanism of resistance to tetracyclines results from acquisition of genetically mobile tetracycline resistance (tet) genes, which encode proteins that either extrude tetracyclines, or confer ribosomal protection (Chopra and Roberts, 2001). In Campylobacter spp., two mechanisms of tetracycline resistance were reported, including (i) ribosomal protection protein tet(O) and (ii) endogenous efflux mediated by CmeABC. tet(O) is the only tetracycline-resistance gene identified in Campylobacter so far. The Tet(O) protein binds to the tetracycline molecule and promote its release from the target site on the ribosome (Connell et al., 2003). The Tet(O) gene may be located on plasmids or the chromosome. The G + C content (40%) of tet(O) is higher than that of Campylobacter genomes (~30%), suggesting that Campylobacter might have obtained the gene from other bacteria by horizontal gene transfer. The multidrug efflux pump, CmeABC, has been shown to contribute to both intrinsic and acquired resistance to tetracycline (Lin et al., 2002; Gibreel et al., 2007). In the CmeB mutant stain of 81–176 (harboring tet(O)), the MIC of tetracycline was decreased by 8-fold (Lin et al., 2002).

Aminoglycoside resistance mechanisms

The aminoglycoside antibiotics is a class of broad spectrum antibacterial agents used for the treatment of both Gram-positive and Gram-negative organisms. Aminoglycoside antibiotics exert their antibacterial activity by binding the 30S ribosomal subunit, thus disturbing the biosynthesis of proteins (Mingoz-Leclercq et al., 1999). Gentamicin is an important aminoglycoside and is used in human beings for treatment of severe infection, including the systemic infection caused by Campylobacter. Gentamicin is also approved for the prevention of bacterial infection-associated death in young food animals, including day-old chicks and 1- to 3- day-old turkey pouls. Due to the nephro- and otoxicity, the consumption of gentamicin has significantly decreased. However, the increasing antimicrobial resistance to newer agents has prompted physicians to reevaluate the use of these old antibiotic compounds (Falagas et al., 2008).

Several mechanisms of gentamicin resistance in Campylobacter have been reported. aacA4 encodes an aminoglycoside 6′-N-acetyltransferase, confer resistance to aminoglycosides containing purpuroamine ring including gentamicin, and was the first gentamicin resistant gene found in C. jejuni isolates (Lee et al., 2002). The gene, apb(2′)-Ia was identified on a MDR conjugative plasmid from a clinical strain of C. jejuni, which was isolated from a US soldier deployed to Thailand (Nirdnoy et al., 2005). Although this gene was initially considered as a bifunctional enzyme and annotated as aac(6′-Ive)/aph(2′-Ia) (also named aacA/aphD), later it was confirmed as a monofunctional aminoglycoside kinase and named as apb(2′)-Ia (Toth et al., 2013). A recent study from China found that apb(2′)-Ia was chromosomally encoded and has become the predominant gentamicin resistance determinant in Campylobacter isolates of chicken and swine origin (Yao et al., 2017). A genomic island containing multiple genes encoding aminoglycoside inactivating enzymes has been detected on transmissible plasmids in C. jejuni as well as in the chromosome of C. coli (Nirdnoy et al., 2005; Qin et al., 2012). Another gentamicin resistant gene, apb(2′)-Ig, which
share 28% amino acid identity with \( \text{aph}(2\″)-\text{I}_f \), was detected on a 55 kb conjugative MDR plasmid that shared 95% nucleotide sequence identity with a pTet plasmid in \textit{Campylobacter} (Chen et al., 2013). A recent study identified nine variants of gentamicin resistance genes in \textit{Campylobacter} isolates from the NARMS program, including \( \text{aph}(2\″)-\text{I}_b, -\text{I}_s, -\text{I}_g, -\text{I}_f, -\text{I}_f, -\text{I}_f, -\text{I}_f \), and \( \text{aad}(6) \text{lec}/ \text{aph}(2\″)-\text{I}_s \) and \( \text{aad}(6) \text{lec}/ \text{aph}(2\″)-\text{I}_f \) (Zhao et al., 2015). These recent findings clearly indicate a rising trend of aminoglycoside resistance and the continuous emergence of new gentamicin resistance mechanisms in \textit{Campylobacter}.

**MDR mechanisms**

Different from specific resistance mechanisms conferred by target or antibiotic modification, the multidrug efflux pump confers a broad spectrum of resistance to structurally unrelated antimicrobials. In \textit{Campylobacter}, two MDR mechanisms have been described including Cfr (described above) and multidrug efflux transporters, among which the RND type of transporters are the most significant for antibiotic resistance. In \textit{Campylobacter}, CmeABC and CmeDEF are the functionally characterized RND-type of efflux systems. However, CmeDEF only contributes moderately to intrinsic resistance, while CmeABC plays a significant role in both intrinsic and acquired resistance of \textit{Campylobacter} to different antibiotics (Lin et al., 2002; Akiba et al., 2006; Gibreel et al., 2007). CmeABC is a tripartite multidrug efflux pumps and consists a periplasmic fusion protein (CmeA), an inner membrane efflux transporter (CmeB) and an outer membrane protein (CmeC) (Lin et al., 2002). The three proteins function together to form an intact efflux system that extrudes antibiotics and toxic compounds. CmeB forms a trimeric structure in the bacterial membrane. A recent study using X-ray crystallography and single-molecule fluorescence resonance energy transfer imaging revealed that the CmeB transporter undergoes conformational transitions uncoordinated and independent of each other, suggesting a novel transport mechanism where CmeB protomers function independently within the trimer (Su et al., 2017). The function of CmeABC in antibiotic resistance has been demonstrated in many published studies (Lin et al., 2002; Hannula and Hanninen, 2008; Guo et al., 2010; Mavri and Smole Mozina, 2013). In addition to conferring resistance to antibiotics, CmeABC also plays a significant role in bile resistance and thus is essential for \textit{Campylobacter} colonization in the intestinal tract (Lin et al., 2003).

The recent discovery of Re-CmeABC further demonstrates the key role of CmeABC in conferring MDR (Yao et al., 2016). The CmeB of Re-CmeABC is unique and shares only ~80% amino acid identity with the CmeB in NCTC 11168 and other strains. This efflux variant is much more powerful than the typical CmeABC in the extrusion of antibiotics. For example, transforming \textit{C. jejuni} NCTC 11168 with Re-CmeABC showed 32-, 16-, 8-, 4-, and 4-fold increases in the MICs of florfenicol, chloramphenicol, ciprofloxacin, erythromycin, and tetracycline, respectively, compared with the recipient strain NCTC 11168 that has a typical CmeABC (Yao et al., 2016). Notably, Re-CmeABC confers exceedingly high-level resistance to FQs, resulting in a ciprofloxacin MIC ≥ 256 µg ml\(^{-1}\) in FQ-resistant \textit{C. jejuni} isolates. Re-CmeABC also contributes to enhanced emergence of FQ-resistant mutants under antibiotic selection, and drug accumulation assays confirmed the enhanced efflux function of Re-CmeABC (Yao et al., 2016). Interestingly, Re-CmeABC was found to be much more prevalent in \textit{C. jejuni} (~35%) than in \textit{C. coli} (~3%), and the proportion of \textit{C. jejuni} harboring Re-CmeABC appeared to be on the rise in China (Yao et al., 2016). This trend is probably driven by the extensive use of antibiotics for animal production in China and suggests a fitness advantage for \textit{C. jejuni} strains carrying Re-CmeABC. The findings on Re-CmeABC also explains why florfenicol resistance is highly prevalent, and more so in \textit{C. jejuni} than in \textit{C. coli} in China (described above). Additionally, homologs of Re-CmeABC are found in the GenBank database and are deposited by investigators from different countries, suggesting that Re-CmeABC is not unique to China. The exact mechanism for the enhanced function of Re-CmeABC is unknown, but structural modeling suggested that sequence variations in the drug-binding pocket of CmeB may enhance its interaction with antibiotics and consequently increase its efflux function (Yao et al., 2016).

The expression of \textit{cmeABC} is subject to regulation. CmeR, a transcriptional repressor of \textit{cmeABC}, directly interacts with the \textit{cmeABC} promotor region and represses the transcription of this operon (Guo et al., 2008; Routh et al., 2009). Insertional mutagenesis of \textit{cmeR} or point mutation in the binding sites of CmeR abolish the binding of CmeR to the promoter, releasing the repression and enhancing the expression of \textit{cmeABC} (Cagliero et al., 2007; Guo et al., 2008). CosR, a response regulator in \textit{C. jejuni}, modulates the oxidative stress response and also plays a role in the repression of \textit{cmeABC} expression (Hwang et al., 2012; Grinnage-Pulley et al., 2016). \textit{cosR} is an essential gene in \textit{Campylobacter}, but knockdown of \textit{cosR} expression by use of antisense peptide nucleic acid increased the transcriptional levels of \textit{cmeABC} (Hwang et al., 2012). \textit{cosR} directly binds to the promoter region of \textit{cmeABC}, but the binding site is independent of the one bound by CmeR (Grinnage-Pulley and Zhang, 2015). The fact that CmeABC is regulated by multiple mechanisms indicates that it may respond to multiple signals in the host or environment. For example, bile acids, which are present in the intestinal tract of animals, strongly induce the expression of \textit{cmeABC} by inhibiting the function of CmeR (Lin et al., 2005b; Gu et al., 2007). This induced expression of CmeABC facilitates \textit{Campylobacter} adaptation to the intestinal environment as it plays a key role in \textit{Campylobacter} resistance to bile (Lin et al., 2003). Additionally, Salicylate, a nonsteroidal anti-inflammatory compound, is also shown to induce \textit{cmeABC} expression by inhibiting binding of CmeR to the promoter of \textit{cmeABC} (Shen et al., 2011). These examples illustrate the essential functions of CmeABC beyond antibiotic resistance.

**Concluding remarks**

As a leading cause of bacterial foodborne illness worldwide, \textit{Campylobacter} continues to pose a significant threat to food safety and public health. As a foodborne pathogen, \textit{Campylobacter} is
exposed to antibiotics used for both animal agriculture and human medicine and has shown an amazing ability to adapt to antibiotic selection pressure. To acquire antibiotic resistance, Campylobacter may mutate the targets of antibiotics, such as the case with FQ and macrolide resistance, or acquire new antibiotic resistance determinants from other bacterial organisms by horizontal gene transfer, such as the case with \( \text{erm}(B) \) and \( \text{gyr}(C) \). Interestingly, Campylobacter tends to acquire foreign antibiotic resistance genes from Gram-positive organisms instead of other Gram-negative bacteria. The exact reason and how this happens are unclear and remain to be investigated. Notably, a highly potent variant of the CmeABC efflux pump (Re-CmeABC) has emerged in \( C. \text{jejuni} \), which confers enhanced resistance to multiple classes of antibiotics, providing a powerful mechanism for Campylobacter to adapt to antibiotic treatments. To survive and adapt in various environments, Campylobacter constantly evolves, and it would not be surprising that new antibiotic resistance mechanisms continue to emerge in this foodborne organism. These emerging mechanisms threaten the usefulness of clinically important antibiotics used for treating human campylobacteriosis. Thus, innovative strategies are needed to mitigate the development and spread of antimicrobial resistant Campylobacter, which should be the focus of future research efforts.

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