Review

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

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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

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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 selection of FQ-resistant *Campylobacter*, which is commonly present in the intestinal tract of birds (McDermott et al., 2002; 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 (Gibreel 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 Mittal, 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 12% 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, and most *C. coli* isolates were resistant to FQ, tetracycline, and trimethoprim (Thomrongsuwannakij *et al.*, 2017). In China, 41.9 to 97.6% of retail chicken isolates exhibited MDR to three or more classes of antimicrobials (Wang *et al.*, 2016; Li *et al.*, 2017; Ma *et al.*, 2017). Usually, the overall MDR rate in *C. coli* tends to be higher than in *C. jejuni* (Li *et al.*, 2017; Ma *et al.*, 2017; Szczepanska *et al.*, 2017). In the USA, the most common MDR pattern was to ciprofloxacin, nalidixic acid, and tetracycline. Except for FQs and tetracycline, the *Campylobacter* isolates examined are generally susceptible to other antimicrobials, such macrolide, florfenicol, gentamicin and telithromycin (Benoit *et al.*, 2014; Ricotta *et al.*, 2014). In our previous study, we observed ∼30% of *C. jejuni* and 50% of *C. coli* isolates were resistant to both FQs and tetracycline, respectively, but the MDR rate in *C. jejuni* and in *C. coli* only account for 0.3 and 4.3%, respectively (Tang *et al.*, 2017c).

Mechanisms of antibiotic resistance in *Campylobacter*

Campylobacter has developed various mechanisms to counteract the selection pressure from antimicrobial agents. These mechanisms include (i) restricting the access of antibiotics to their targets, which involves reducing membrane permeability and increasing extrusion of antibiotics by efflux pumps; (ii) modification or protection of antibiotic targets; and (iii) modification or inactivation of antibiotics. These mechanisms may act together in the resistance to different classes of antibiotic. In this section, mechanisms involved in *Campylobacter* resistance to FQ, macrolides and florfenicol will be discussed due to their clinical significance or importance for animal production.

FQ resistance mechanisms

The quinolones are a class of broad spectrum antimicrobials that are potent against both gram-negative and gram-positive bacteria (Andersson and MacGowan, 2003). According to their spectrum of activity, quinolones have been classified into four generations. The majority of quinolones currently used for clinical therapies are FQs, which are derived from the quinolones by a fluorine substitution at the C-6 or C-7 position, thereby increasing their activity against gram-negative bacteria (Andersson and MacGowan, 2003). Once inside bacterial cells, FQ antimicrobials exert their antibacterial effect by interacting with DNA gyrase and topoisomerase IV, resulting in double-stand DNA breaks and cell death (Jacoby, 2005). Two main mechanisms of resistance to FQs are currently recognized in *Campylobacter* bacteria, including mutations that change the antibiotic's target and that reduce antibiotic intracellular accumulation. In other gram-negative bacteria, target protection mediated by the Qnr protein was also involved in FQ resistance (Martin-Gutierrez *et al.*, 2017), but this mechanism has not been reported in *Campylobacter*.

In gram-negative bacteria, gyrase is the main target of FQ antibiotics, whereas, in gram-positive bacteria, topoisomerase IV is more susceptible to the action of FQ (Jacoby, 2005). Both enzymes consisting of two pairs of subunits, named GyrA and GyrB (DNA gyrase), and ParC and ParE (topoisomerase IV) (Payot *et al.*, 2006). Although most bacteria have both enzymes, *Campylobacter* lacks the *parC* and *parE* 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) $\geq 16 \, \mu g \, ml^{-1}$] in *Campylobacter*, while other mutations are associated with a low to medium level of resistance (MIC = 1–8 $\mu g \, ml^{-1}$) (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 FQ^R 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 $\geq$ 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 FQ^R *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 relithromycin, 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 staphylococci and streptococci), gram-positive bacilli, gram-negative cocci, and some gram-negative bacilli, 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 *Campylboacter*, 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 (*rrn* 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 rRNA 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 (MLS$_B$ phenotype). To date, 43 *erm* (erythromycin ribosome methylase) genes have been reported (http://faculty.washington.edu/marilynr/), but only *erm(B)* has been detected in *Campylobacter*, including *C. jejuni* and *C. coli* in China and Europe (Qin *et al.*, 2014; Deng *et al.*, 2015; Florez-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)*, *aadE* and *aad9*] in a MDRGI that was inserted in the chromosome at a different location when compared with that in *C. coli*

(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 MDRGIs 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 (Cagliero 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 (Syriopoulou 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 peptidyl transferase 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 PhLOPS$_A$ 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 Peptoclostridium difficile 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 conjugative 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 PhLOPS$_A$ (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 floR gene, encoding a MDR efflux pump, mediates resistance to chloramphenicol and florfenicol (Arcangioli et al., 1999). It was first been discovered in Salmonella typhimurium DT104 (Arcangioli et al., 1999) and had also been detected in C. coli (Frye et al., 2011). floR encodes a protein of 404 amino acids, which functions as efflux transporter. Interestingly, pp-flo, florSt, flo, and floR, are closely related even though they were assigned different names in the literature (Kim and Aoki,

1996; Arcangioli *et al.*, 1999; Bolton *et al.*, 1999). Functionally, they all confer resistance to both chloramphenicol and florfenicol. Sequence alignment showed 96–100% identity in their nucleotide sequences and 88–100% identity in the amino acid sequences. The *fexA* and *fexB* genes, coding for phenicol specific efflux pumps, also confer resistance to florfenicol. They have been found in *Staphylococcus*, *Bacillus*, and *Enterococcus*, but not in *Campylobacter* (Dai *et al.*, 2010; Liu *et al.*, 2012; Gomez-Sanz *et al.*, 2013).

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 GC015 has been functionally characterized and was shown to confer a ≥32-fold increase in the MICs of ampicillin, piperacillin, and carbenicillin 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 bla_{OXA-61} led to overexpression of bla_{OXA-61} 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 bla_{OXA-61} 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 teracycline-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 (Mingeot-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 poults. Due to the nephro- and ototoxicity, 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*-acytyltransfearse, confer resistance to aminoglycosides containing purpurosamine ring including gentamicin, and was the first gentamicin resistant gene found in *C. jejuni* isolates (Lee *et al.*, 2002). The gene, *aph(2″)-If* 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'-Ie)/aph(2″-Ia)* (also named *aacA/aphD*), later it was confirmed as a monofunctional aminoglycoside kinase and named as *aph(2″)-If* (Toth *et al.*, 2013). A recent study from China found that *aph(2″)-If* 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, *aph(2″)-Ig*, which

share 28% amino acid identity with *aph(2″)-If*, was detected on a 55 kb conjugative MDR plasmid that shared 95% nucleotide sequence identity with a pTet plasmid in *Campylobacter* (Chen *et al.*, 2013). A recent study identified nine variants of gentamicin resistance genes in *Campylobacter* isolates from the NARMS program, including *aph(2″)-Ib, -Ic, -Ig, -If, -If1, -If3 and -Ih, aac(6′)Ie/ aph(2″)-Ia* and *aac(6′)-Ie/aph(2″)-If2* (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 *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 *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 *Campylobacter*, CmeABC and CmeDEF are the functionally characterized RND-type of efflux systems. However, CmeDEF only contribute moderately to intrinsic resistance, while CmeABC plays a significant role in both intrinsic and acquired resistance of *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 *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 *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 *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 *C. jejuni* (∼35%) than in *C. coli* (∼3%), and the proportion of *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 *C. jejuni* strains carrying RE-CmeABC. The findings on Re-CmeABC also explains why florfenicol resistance is highly prevalent, and more so in *C. jejuni* than in *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 *cmeABC* is subject to regulation. CmeR, a transcriptional repressor of *cmeABC*, directly interacts with the *cmeABC* promotor region and represses the transcription of this operon (Guo *et al.*, 2008; Routh *et al.*, 2009). Insertional mutagenesis of *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 cmeABC (Cagliero *et al.*, 2007; Guo *et al.*, 2008). CosR, a response regulator in *C. jejuni*, modulates the oxidative stress response and also plays a role in the repression of *cmeABC* expression (Hwang *et al.*, 2012; Grinnage-Pulley *et al.*, 2016). *cosR* is an essential gene in *Campylobacter*, but knockdown of *cosR* expression by use of antisense peptide nucleic acid increased the transcriptional levels of *cmeABC* (Hwang *et al.*, 2012). CosR directly binds to the promoter region of *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 *cmeABC* by inhibiting the function of CmeR (Lin *et al.*, 2005b; Gu *et al.*, 2007). This induced expression of CmeABC facilitates *Campylobacter* adaptation to the intestinal environment as it plays a key role in *Campylobacter* resistance to bile (Lin *et al.*, 2003). Additionally, Salicylate, a nonsteroidal anti-inflammatory compound, is also shown to induce *cmeABC* expression by inhibiting binding of CmeR to the promoter of *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, *Campylobacter* continues to pose a significant threat to food safety and public health. As a foodborne pathogen, *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 *erm(B)* and *cfr(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. 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.

Acknowledgment

References

Aarestrup FM and Engberg J (2001). Antimicrobial resistance of thermophilic *Campylobacter*. *Veterinary Research* **32**: 311–321.

Akiba M, Lin J, Barton YW and Zhang Q (2006). Interaction of CmeABC and CmeDEF in conferring antimicrobial resistance and maintaining cell viability in *Campylobacter jejuni*. *The Journal of Antimicrobial Chemotherapy* **57**: 52–60.

Alfredson DA and Korolik V (2005). Isolation and expression of a novel molecular class D beta-lactamase, OXA-61, from *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy* **49**: 2515–2518.

Allos BM (2001). *Campylobacter jejuni* infections: update on emerging issues and trends. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **32**: 1201–1206.

Andersson MI and MacGowan AP (2003). Development of the quinolones. *The Journal of Antimicrobial Chemotherapy* **51**(Suppl. 1): 1–11.

Arcangioli MA, Leroy-Setrin S, Martel JL and Chaslus-Dancla E (1999). A new chloramphenicol and florfenicol resistance gene flanked by two integron structures in *Salmonella typhimurium* DT104. *FEMS Microbiology Letters* **174**: 327–332.

Bachoual R, Ouabdesselam S, Mory F, Lascols C, Soussy CJ and Tankovic J (2001). Single or double mutational alterations of gyrA associated with fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Microbial Drug Resistance* **7**: 257–261.

Bardon J, Kolar M, Cekanova L, Hejnar P and Koukalova D (2009). Prevalence of *Campylobacter jejuni* and its resistance to antibiotics in poultry in the Czech Republic. *Zoonoses and Public Health* **56**: 111–116.

Benoit SR, Lopez B, Arvelo W, Henao O, Parsons MB, Reyes L, Moir JC and Lindblade K (2014). Burden of laboratory-confirmed *Campylobacter* infections in Guatemala 2008–2012: results from a facility-based surveillance system. *Journal of Epidemiology and Global Health* **4**: 51–59.

Bolton LF, Kelley LC, Lee MD, Fedorka-Cray PJ and Maurer JJ (1999). Detection of multidrug-resistant *Salmonella enterica* serotype typhimurium DT104 based on a gene which confers cross-resistance to florfenicol and chloramphenicol. *Journal of Clinical Microbiology* **37**: 1348–1351.

Cagliero C, Mouline C, Payot S and Cloeckaert A (2005). Involvement of the CmeABC efflux pump in the macrolide resistance of *Campylobacter coli*. *The Journal of Antimicrobial Chemotherapy* **56**: 948–950.

Cagliero C, Mouline C, Cloeckaert A and Payot S (2006). Synergy between efflux pump CmeABC and modifications in ribosomal proteins L4 and L22 in conferring macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Antimicrobial Agents and Chemotherapy* **50**: 3893–3896.

Cagliero C, Maurel MC, Cloeckaert A and Payot S (2007). Regulation of the expression of the CmeABC efflux pump in *Campylobacter jejuni*: identification of a point mutation abolishing the binding of the CmeR repressor in an in vitro-selected multidrug-resistant mutant. *FEMS Microbiology Letters* **267**: 89–94.

Caldwell DB, Wang Y and Lin J (2008). Development, stability, and molecular mechanisms of macrolide resistance in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy* **52**: 3947–3954.

Candela T, Marvaud JC, Nguyen TK and Lambert T (2017). A *cfr*-like gene *cfr*(C) conferring linezolid resistance is common in *Clostridium difficile*. *International Journal of Antimicrobial Agents* **50**: 496–500.

CDC (2009). *Campylobacter jejuni* infection associated with unpasteurized milk and cheese – Kansas, 2007. *MMWR. Morbidity and Mortality Weekly Report* **57**: 1377–1379.

CDC (2013). Antibiotic Resistance Threats in the United States. http://www.cdc.gov/drugresistance/threat-report-2013/.

Cha W, Mosci R, Wengert SL, Singh P, Newton DW, Salimnia H, Lephart P, Khalife W, Mansfield LS, Rudrik JT and Manning SD (2016). Antimicrobial susceptibility profiles of human *Campylobacter jejuni* isolates and association with phylogenetic lineages. *Frontiers in Microbiology* **7**: 589.

Chen X, Naren GW, Wu CM, Wang Y, Dai L, Xia LN, Luo PJ, Zhang Q and Shen JZ (2010). Prevalence and antimicrobial resistance of *Campylobacter* isolates in broilers from China. *Veterinary Microbiology* **144**: 133–139.

Chen Y, Mukherjee S, Hoffmann M, Kotewicz ML, Young S, Abbott J, Luo Y, Davidson MK, Allard M, McDermott P and Zhao S (2013). Whole-genome sequencing of gentamicin-resistant *Campylobacter coli* isolated from U.S. retail meats reveals novel plasmid-mediated aminoglycoside resistance genes. *Antimicrobial Agents and Chemotherapy* **57**: 5398–5405.

Chittum HS and Champney WS (1994). Ribosomal protein gene sequence changes in erythromycin-resistant mutants of *Escherichia coli*. *Journal of Bacteriology* **176**: 6192–6198.

Chopra I (2001). Glycylcyclines: third-generation tetracycline antibiotics. *Current Opinion in Pharmacology* **1**: 464–469.

Chopra I and Roberts M (2001). Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiology and Molecular Biology Reviews: MMBR* **65**: 232–260.

Connell SR, Trieber CA, Dinos GP, Einfeldt E, Taylor DE and Nierhaus KH (2003). Mechanism of Tet(O)-mediated tetracycline resistance. *The EMBO Journal* **22**: 945–953.

Corcoran D, Quinn T, Cotter L and Fanning S (2006). An investigation of the molecular mechanisms contributing to high-level erythromycin resistance in *Campylobacter*. *International Journal of Antimicrobial Agents* **27**: 40–45.

Dai L, Wu CM, Wang MG, Wang Y, Wang Y, Huang SY, Xia LN, Li BB and Shen JZ (2010). First report of the multidrug resistance gene cfr and the phenicol resistance gene fexA in a Bacillus strain from swine feces. *Antimicrobial Agents and Chemotherapy* **54**: 3953–3955.

Dai L, Muraoka WT, Wu Z, Sahin O and Zhang Q (2015). A single nucleotide change in mutY increases the emergence of antibiotic-resistant *Campylobacter jejuni* mutants. *The Journal of Antimicrobial Chemotherapy* **70**: 2739–2748.

Deng F, Wang Y, Zhang Y and Shen Z (2015). Characterization of the genetic environment of the ribosomal RNA methylase gene erm(B) in *Campylobacter jejuni*. *The Journal of Antimicrobial Chemotherapy* **70**: 613–615.

Deshpande LM, Ashcraft DS, Kahn HP, Pankey G, Jones RN, Farrell DJ and Mendes RE (2015). Detection of a new cfr-like gene, cfr(B), in *Enterococcus faecium* isolates recovered from human specimens in the United States as part of the SENTRY Antimicrobial Surveillance Program. *Antimicrobial Agents and Chemotherapy* **59**: 6256–6261.

Doktor SZ, Shortridge VD, Beyer JM and Flamm RK (2004). Epidemiology of macrolide and/or lincosamide resistant *Streptococcus pneumoniae* clinical isolates with ribosomal mutations. *Diagnostic Microbiology and Infectious Disease* **49**: 47–52.

Elviss NC, Williams LK, Jorgensen F, Chisholm SA, Lawson AJ, Swift C, Owen RJ, Griggs DJ, Johnson MM, Humphrey TJ and Piddock LJ (2009). Amoxicillin therapy of poultry flocks: effect upon the selection of amoxicillin-resistant commensal *Campylobacter* spp. *The Journal of Antimicrobial Chemotherapy* **64**: 702–711.

Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T and Mouton RP (1991). Quinolone resistance in *campylobacter* isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. *The Journal of Antimicrobial Chemotherapy* **27**: 199–208.

Engberg J, Aarestrup FM, Taylor DE, Gerner-Smidt P and Nachamkin I (2001). Quinolone and macrolide resistance in *Campylobacter jejuni* and *C. coli*: resistance mechanisms and trends in human isolates. *Emerging Infectious Diseases* **7**: 24–34.

Falagas ME, Grammatikos AP and Michalopoulos A (2008). Potential of old-generation antibiotics to address current need for new antibiotics. *Expert Review of Anti-infective Therapy* **6**: 593–600.

Florez-Cuadrado D, Ugarte-Ruiz M, Quesada A, Palomo G, Dominguez L and Porrero MC (2016). Description of an erm (B)-carrying *Campylobacter coli* isolate in Europe. *The Journal of Antimicrobial Chemotherapy* **71**: 841–843.

French G (2001). Linezolid. *International Journal of Clinical Practice* **55**: 59–63.

Friedman SM, Lu T and Drlica K (2001). Mutation in the DNA gyrase A gene of *Escherichia coli* that expands the quinolone resistance-determining region. *Antimicrobial Agents and Chemotherapy* **45**: 2378–2380.

Frye JG, Lindsey RL, Meinersmann RJ, Berrang ME, Jackson CR, Englen MD, Turpin JB and Fedorka-Cray PJ (2011). Related antimicrobial resistance genes detected in different bacterial species co-isolated from swine fecal samples. *Foodborne Pathogens and Disease* **8**: 663–679.

Gallay A, Prouzet-Mauleon V, Kempf I, Lehours P, Labadi L, Camou C, Denis M, de Valk H, Desenclos JC and Megraud F (2007). *Campylobacter* antimicrobial drug resistance among humans, broiler chickens, and pigs, France. *Emerging Infectious Diseases* **13**: 259–266.

Ge B, McDermott PF, White DG and Meng J (2005). Role of efflux pumps and topoisomerase mutations in fluoroquinolone resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Antimicrobial Agents and Chemotherapy* **49**: 3347–3354.

Gibreel A and Taylor DE (2006). Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *The Journal of Antimicrobial Chemotherapy* **58**: 243–255.

Gibreel A, Kos VN, Keelan M, Trieber CA, Levesque S, Michaud S and Taylor DE (2005). Macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*: molecular mechanism and stability of the resistance phenotype. *Antimicrobial Agents and Chemotherapy* **49**: 2753–2759.

Gibreel A, Wetsch NM and Taylor DE (2007). Contribution of the CmeABC efflux pump to macrolide and tetracycline resistance in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy* **51**: 3212–3216.

Gomez-Sanz E, Kadlec K, Fessler AT, Zarazaga M, Torres C and Schwarz S (2013). A novel fexA variant from a canine *Staphylococcus pseudintermedius* isolate that does not confer florfenicol resistance. *Antimicrobial Agents and Chemotherapy* **57**: 5763–5766.

Gootz TD and Martin BA (1991). Characterization of high-level quinolone resistance in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy* **35**: 840–845.

Griggs DJ, Peake L, Johnson MM, Ghori S, Mott A and Piddock LJ (2009). Beta-lactamase-mediated beta-lactam resistance in *Campylobacter* species: prevalence of Cj0299 (bla OXA-61) and evidence for a novel beta-Lactamase in *C. jejuni*. *Antimicrobial Agents and Chemotherapy* **53**: 3357–3364.

Grinnage-Pulley T and Zhang Q (2015). Genetic basis and functional consequences of differential expression of the CmeABC efflux pump in *Campylobacter jejuni* isolates. *PloS ONE* **10**: e0131534.

Grinnage-Pulley T, Mu Y, Dai L and Zhang Q (2016). Dual repression of the Multidrug Efflux Pump CmeABC by CosR and CmeR in *Campylobacter jejuni*. *Frontiers in Microbiology* **7**: 1097.

Gu R, Su CC, Shi F, Li M, McDermott G, Zhang Q and Yu EW (2007). Crystal structure of the transcriptional regulator CmeR from *Campylobacter jejuni*. *Journal of Molecular Biology* **372**: 583–593.

Guo B, Wang Y, Shi F, Barton YW, Plummer P, Reynolds DL, Nettleton D, Grinnage-Pulley T, Lin J and Zhang Q (2008). Cmer functions as a pleiotropic regulator and is required for optimal colonization of *Campylobacter jejuni* in vivo. *Journal of Bacteriology* **190**: 1879–1890.

Guo B, Lin J, Reynolds DL and Zhang Q (2010). Contribution of the multidrug efflux transporter CmeABC to antibiotic resistance in different *Campylobacter* species. *Foodborne Pathogens and Disease* **7**: 77–83.

Hannula M and Hanninen ML (2008). Effect of putative efflux pump inhibitors and inducers on the antimicrobial susceptibility of *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Medical Microbiology* **57**: 851–855.

Hansen LH and Vester B (2015). A cfr-like gene from *Clostridium difficile* confers multiple antibiotic resistance by the same mechanism as the cfr gene. *Antimicrobial Agents and Chemotherapy* **59**: 5841–5843.

Hoge CW, Gambel JM, Srijan A, Pitarangsi C and Echeverria P (1998). Trends in antibiotic resistance among diarrheal pathogens isolated in Thailand over 15 years. *Clinical infectious diseases* **26**: 341–345.

Humphrey TJ, Jorgensen F, Frost JA, Wadda H, Domingue G, Elviss NC, Griggs DJ and Piddock LJ (2005). Prevalence and subtypes of ciprofloxacin-resistant *Campylobacter* spp. in commercial poultry flocks before, during, and after treatment with fluoroquinolones. *Antimicrobial Agents and Chemotherapy* **49**: 690–698.

Hwang S, Zhang Q, Ryu S and Jeon B (2012). Transcriptional regulation of the CmeABC multidrug efflux pump and the KatA catalase by CosR in *Campylobacter jejuni*. *Journal of Bacteriology* **194**: 6883–6891.

Jacoby GA (2005). Mechanisms of resistance to quinolones. *Clinical Infectious Diseases* **41**(Suppl. 2): S120–S126.

Jensen LB and Aarestrup FM (2001). Macrolide resistance in *Campylobacter coli* of animal origin in Denmark. *Antimicrobial Agents and Chemotherapy* **45**: 371–372.

Kashoma IP, Kassem II, Kumar A, Kessy BM, Gebreyes W, Kazwala RR and Rajashekara G (2015). Antimicrobial resistance and genotypic diversity of *Campylobacter* isolated from pigs, dairy, and beef cattle in Tanzania. *Frontiers in Microbiology* **6**: 1240.

Kashoma IP, Kassem II, John J, Kessy BM, Gebreyes W, Kazwala RR and Rajashekara G (2016). Prevalence and antimicrobial resistance

of *Campylobacter* isolated from dressed beef carcasses and Raw milk in Tanzania. *Microbial Drug Resistance* **22**: 40–52.

Kehrenberg C, Schwarz S, Jacobsen L, Hansen LH and Vester B (2005). A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503. *Molecular Microbiology* **57**: 1064–1073.

Kim E and Aoki T (1996). Sequence analysis of the florfenicol resistance gene encoded in the transferable R-plasmid of a fish pathogen, *Pasteurella piscicida*. *Microbiology and Immunology* **40**: 665–669.

Komba EV, Mdegela RH, Msoffe PL, Nielsen LN and Ingmer H (2015). Prevalence, antimicrobial resistance and risk factors for thermophilic *Campylobacter* infections in symptomatic and asymptomatic humans in Tanzania. *Zoonoses and Public Health* **62**: 557–568.

Leclercq R (2002). Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clinical Infectious Diseases: An Official Publication of the Infectious Diseases Society of America* **34**: 482–492.

Lee MD, Sanchez S, Zimmer M, Idris U, Berrang ME and McDermott PF (2002). Class 1 integron-associated tobramycin-gentamicin resistance in *Campylobacter jejuni* isolated from the broiler chicken house environment. *Antimicrobial Agents and Chemotherapy* **46**: 3660–3664.

Lehtopolku M, Kotilainen P, Haanpera-Heikkinen M, Nakari UM, Hanninen ML, Huovinen P, Siitonen A, Eerola E, Jalava J and Hakanen AJ (2011). Ribosomal mutations as the main cause of macrolide resistance in *Campylobacter jejuni* and *Campylobacter coli*. *Antimicrobial Agents and Chemotherapy* **55**: 5939–5941.

Li B, Ma L, Li Y, Jia H, Wei J, Shao D, Liu K, Shi Y, Qiu Y and Ma Z (2016). Antimicrobial resistance of *Campylobacter* species isolated from Broilers in Live Bird Markets in Shanghai, China. *Foodborne Pathogens and Disease* **14**: 96–102.

Li B, Ma L, Li Y, Jia H, Wei J, Shao D, Liu K, Shi Y, Qiu Y and Ma Z (2017). Antimicrobial resistance of *Campylobacter* species isolated from Broilers in Live Bird Markets in Shanghai, China. *Foodborne Pathogens and Disease* **14**: 96–102.

Li D, Wu C, Wang Y, Fan R, Schwarz S and Zhang S (2015). Identification of multiresistance gene *cfr* in methicillin-resistant *Staphylococcus aureus* from pigs: plasmid location and integration into a staphylococcal cassette chromosome mec complex. *Antimicrobial Agents and Chemotherapy* **59**: 3641–3644.

Li XZ, Mehrotra M, Ghimire S and Adewoye L (2007). Beta-Lactam resistance and beta-lactamases in bacteria of animal origin. *Veterinary Microbiology* **121**: 197–214.

Lin J, Michel LO and Zhang Q (2002). CmeABC functions as a multidrug efflux system in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy* **46**: 2124–2131.

Lin J, Sahin O, Michel LO and Zhang Q (2003). Critical role of multidrug efflux pump CmeABC in bile resistance and *in vivo* colonization of *Campylobacter jejuni*. *Infection and Immunity* **71**: 4250–4259.

Lin J, Akiba M, Sahin O and Zhang Q (2005a). Cmer functions as a transcriptional repressor for the multidrug efflux pump CmeABC in *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy* **49**: 1067–1075.

Lin J, Cagliero C, Guo B, Barton YW, Maurel MC, Payot S and Zhang Q (2005b). Bile salts modulate expression of the CmeABC multidrug efflux pump in *Campylobacter jejuni*. *Journal of Bacteriology* **187**: 7417–7424.

Lin J, Yan M, Sahin O, Pereira S, Chang YJ and Zhang Q (2007). Effect of macrolide usage on emergence of erythromycin-resistant *Campylobacter* isolates in chickens. *Antimicrobial Agents and Chemotherapy* **51**: 1678–1686.

Liu H, Wang Y, Wu C, Schwarz S, Shen Z, Jeon B, Ding S, Zhang Q and Shen J (2012). A novel phenicol exporter gene, fexB, found in enterococci of animal origin. *The Journal of Antimicrobial Chemotherapy* **67**: 322–325.

Liu Y, Wang Y, Schwarz S, Li Y, Shen Z, Zhang Q, Wu C and Shen J (2013). Transferable multiresistance plasmids carrying *cfr* in

Enterococcus spp. from swine and farm environment. *Antimicrobial Agents and Chemotherapy* **57**: 42–48.

Liu Y, Wang Y, Dai L, Wu C and Shen J (2014). First report of multiresistance gene *cfr* in *Enterococcus* species *casseliflavus* and *gallinarum* of swine origin. *Veterinary Microbiology* **170**: 352–357.

Long KS, Poehlsgaard J, Kehrenberg C, Schwarz S and Vester B (2006). The Cfr rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. *Antimicrobial Agents and Chemotherapy* **50**: 2500–2505.

Luangtongkum T, Jeon B, Han J, Plummer P, Logue CM and Zhang Q (2009). Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence. *Future Microbiology* **4**: 189–200.

Lucey B, Cryan B, O'Halloran F, Wall PG, Buckley T and Fanning S (2002). Trends in antimicrobial susceptibility among isolates of *Campylobacter* species in Ireland and the emergence of resistance to ciprofloxacin. *The Veterinary Record* **151**: 317–320.

Luo N, Sahin O, Lin J, Michel LO and Zhang Q (2003). *In vivo* selection of *Campylobacter* isolates with high levels of fluoroquinolone resistance associated with gyrA mutations and the function of the CmeABC efflux pump. *Antimicrobial Agents and Chemotherapy* **47**: 390–394.

Ma H, Su Y, Ma L, Ma L, Li P, Du X, Golz G, Wang S and Lu X (2017). Prevalence and characterization of *Campylobacter jejuni* isolated from Retail Chicken in Tianjin, China. *Journal of Food Protection* **80**: 1032–1040.

Ma L, Shen Z, Naren G, Li H, Xia X, Wu C, Shen J, Zhang Q and Wang Y (2014a). Identification of a novel G2073A mutation in 23S rRNA in amphenicol-selected mutants of *Campylobacter jejuni*. *PloS ONE* **9**: e94503.

Ma L, Wang Y, Shen J, Zhang Q and Wu C (2014b). Tracking *Campylobacter* contamination along a broiler chicken production chain from the farm level to retail in China. *International Journal of Food Microbiology* **181**: 77–84.

Malbruny B, Nagai K, Coquemont M, Bozdogan B, Andrasevic AT, Hupkova H, Leclercq R and Appelbaum PC (2002). Resistance to macrolides in clinical isolates of *Streptococcus pyogenes* due to ribosomal mutations. *The Journal of Antimicrobial Chemotherapy* **49**: 935–939.

Martin-Gutierrez G, Rodriguez-Martinez JM, Pascual A, Rodriguez-Beltran J and Blazquez J (2017). Plasmidic qnr genes confer clinical resistance to ciprofloxacin under urinary tract physiological conditions. *Antimicrobial Agents and Chemotherapy* **61**: e02615–16.

Mavri A and Smole Mozina S (2013). Effects of efflux-pump inducers and genetic variation of the multidrug transporter *cmeB* in biocide resistance of *Campylobacter jejuni* and *Campylobacter coli*. *Journal of Medical Microbiology* **62**: 400–411.

McDermott PF, Bodeis SM, English LL, White DG, Walker RD, Zhao S, Simjee S and Wagner DD (2002). Ciprofloxacin resistance in *Campylobacter jejuni* evolves rapidly in chickens treated with fluoroquinolones. *The Journal of Infectious Diseases* **185**: 837–840.

Mingeot-Leclercq MP, Glupczynski Y and Tulkens PM (1999). Aminoglycosides: activity and resistance. *Antimicrobial Agents and Chemotherapy* **43**: 727–737.

Nachamkin I, Ung H and Li M (2002). Increasing fluoroquinolone resistance in *Campylobacter jejuni*, Pennsylvania, USA,1982–2001. *Emerging Infectious Diseases* **8**: 1501–1503.

Nguyen TN, Hotzel H, El-Adawy H, Tran HT, Le MT, Tomaso H, Neubauer H and Hafez HM (2016). Genotyping and antibiotic resistance of thermophilic *Campylobacter* isolated from chicken and pig meat in Vietnam. *Gut Pathogens* **8**: 19.

Nirdnoy W, Mason CJ and Guerry P (2005). Mosaic structure of a multiple-drug-resistant, conjugative plasmid from *Campylobacter jejuni*. *Antimicrobial Agents and Chemotherapy* **49**: 2454–2459.

Niwa H, Chuma T, Okamoto K and Itoh K (2001). Rapid detection of mutations associated with resistance to erythromycin in *Campylobacter jejuni/coli* by PCR and line probe assay. *International Journal of Antimicrobial Agents* **18**: 359–364.

Ohno H, Wachino J, Saito R, Jin W, Yamada K, Kimura K and Arakawa Y (2016). A highly macrolide-resistant *Campylobacter jejuni*

strain with rare A2074T mutations in 23S rRNA genes. *Antimicrobial Agents and Chemotherapy* **60**: 2580–2581.

Padungton P and Kaneene JB (2003). *Campylobacter* spp in human, chickens, pigs and their antimicrobial resistance. *The Journal of Veterinary Medical Science* **65**: 161–170.

Papavasileiou E, Voyatzi A, Papavasileiou K, Makri A, Andrianopoulou I and Chatzipanagiotou S (2007). Antimicrobial susceptibilities of *Campylobacter jejuni* isolates from hospitalized children in Athens, Greece, collected during 2004–2005. *European Journal of Epidemiology* **22**: 77–78.

Parkhill J, Wren BW, Mungall K, Ketley JM, Churcher C, Basham D, Chillingworth T, Davies RM, Feltwell T, Holroyd S, Jagels K, Karlyshev AV, Moule S, Pallen MJ, Penn CW, Quail MA, Rajandream MA, Rutherford KM, van Vliet AH, Whitehead S and Barrell BG (2000). The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences. *Nature* **403**: 665–668.

Payot S, Cloeckaert A and Chaslus-Dancla E (2002). Selection and characterization of fluoroquinolone-resistant mutants of *Campylobacter jejuni* using enrofloxacin. *Microbial Drug Resistance* **8**: 335–343.

Payot S, Bolla JM, Corcoran D, Fanning S, Megraud F and Zhang Q (2006). Mechanisms of fluoroquinolone and macrolide resistance in *Campylobacter* spp. *Microbes and infection/Institut Pasteur* **8**: 1967–1971.

Pezzotti G, Serafin A, Luzzi I, Mioni R, Milan M and Perin R (2003). Occurrence and resistance to antibiotics of *Campylobacter jejuni* and *Campylobacter coli* in animals and meat in northeastern Italy. *International Journal of Food Microbiology* **82**: 281–287.

Piddock LJ, Ricci V, Pumbwe L, Everett MJ and Griggs DJ (2003). Fluoroquinolone resistance in *Campylobacter* species from man and animals: detection of mutations in topoisomerase genes. *The Journal of Antimicrobial Chemotherapy* **51**: 19–26.

Qin S, Wang Y, Zhang Q, Chen X, Shen Z, Deng F, Wu C and Shen J (2012). Identification of a novel genomic island conferring resistance to multiple aminoglycoside antibiotics in *Campylobacter coli*. *Antimicrobial Agents and Chemotherapy* **56**: 5332–5339.

Qin S, Wang Y, Zhang Q, Zhang M, Deng F, Shen Z, Wu C, Wang S, Zhang J and Shen J (2014). Report of ribosomal RNA methylase gene erm(B) in multidrug-resistant *Campylobacter coli*. *The Journal of Antimicrobial Chemotherapy* **69**: 964–968.

Ricotta EE, Palmer A, Wymore K, Clogher P, Oosmanally N, Robinson T, Lathrop S, Karr J, Hatch J, Dunn J, Ryan P and Blythe D (2014). Epidemiology and antimicrobial resistance of international travel-associated *Campylobacter* infections in the United States, 2005–2011. *American Journal of Public Health* **104**: e108–e114.

Routh MD, Su CC, Zhang Q and Yu EW (2009). Structures of AcrR and CmeR: insight into the mechanisms of transcriptional repression and multi-drug recognition in the TetR family of regulators. *Biochimica et biophysica acta* **1794**: 844–851.

Ruiz J, Goni P, Marco F, Gallardo F, Mirelis B, Jimenez De Anta T and Vila J (1998). Increased resistance to quinolones in *Campylobacter jejuni*: a genetic analysis of *gyrA* gene mutations in quinolone-resistant clinical isolates. *Microbiology and Immunology* **42**: 223–226.

Ruiz-Palacios GM (2007). The health burden of *Campylobacter* infection and the impact of antimicrobial resistance: playing chicken. *Clinical Infectious Diseases* **44**: 701–703.

Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL and Griffin PM (2011). Foodborne illness acquired in the United States – major pathogens. *Emerging Infectious Diseases* **17**: 7–15.

Schwarz S, Werckenthin C and Kehrenberg C (2000). Identification of a plasmid-borne chloramphenicol-florfenicol resistance gene in *Staphylococcus sciuri*. *Antimicrobial Agents and Chemotherapy* **44**: 2530–2533.

Schwarz S, Kehrenberg C, Doublet B and Cloeckaert A (2004). Molecular basis of bacterial resistance to chloramphenicol and florfenicol. *FEMS Microbiology Reviews* **28**: 519–542.

Shen Z, Pu XY and Zhang Q (2011). Salicylate functions as an efflux pump inducer and promotes the emergence of fluoroquinolone-resistant *Campylobacter jejuni* mutants. *Applied and Environmental Microbiology* **77**: 7128–7133.

Shobo CO, Bester LA, Baijnath S, Somboro AM, Peer AK and Essack SY (2016). Antibiotic resistance profiles of *Campylobacter* species in the South Africa private health care sector. *Journal of Infection in Developing Countries* **10**: 1214–1221.

Sierra-Arguello YM, Perdoncini G, Morgan RB, Salle CT, Moraes HL, Gomes MJ and do Nascimento VP (2016). Fluoroquinolone and macrolide resistance in *Campylobacter jejuni* isolated from broiler slaughterhouses in southern Brazil. *Avian Pathology: Journal of the W.V.P.A* **45**: 66–72.

Singh S and Mittal A (2016). Transmembrane domain lengths serve as signatures of organismal complexity and viral transport mechanisms. *Scientific Reports* **6**: 22352.

Stanley K and Jones K (2003). Cattle and sheep farms as reservoirs of *Campylobacter*. *Journal of Applied Microbiology* **94** (Suppl.): 104S–113S.

Su CC, Yin L, Kumar N, Dai L, Radhakrishnan A, Bolla JR, Lei HT, Chou TH, Delmar JA, Rajashankar KR, Zhang Q, Shin YK and Yu EW (2017). Structures and transport dynamics of a *Campylobacter jejuni* multidrug efflux pump. *Nature Communications* **8**: 171.

Syriopoulou VP, Harding AL, Goldmann DA and Smith AL (1981). *In vitro* antibacterial activity of fluorinated analogs of chloramphenicol and thiamphenicol. *Antimicrobial Agents and Chemotherapy* **19**: 294–297.

Szczepanska B, Andrzejewska M, Spica D and Klawe JJ (2017). Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. *BMC Microbiology* **17**: 80.

Tait-Kamradt A, Davies T, Appelbaum PC, Depardieu F, Courvalin P, Petitpas J, Wondrack L, Walker A, Jacobs MR and Sutcliffe J (2000). Two new mechanisms of macrolide resistance in clinical strains of *Streptococcus pneumoniae* from Eastern Europe and North America. *Antimicrobial Agents and Chemotherapy* **44**: 3395–3401.

Tang Y, Dai L, Sahin O, Wu Z, Liu M and Zhang Q (2017a). Emergence of a plasmid-borne multidrug resistance gene *cfr*(C) in foodborne pathogen *Campylobacter*. *The Journal of Antimicrobial Chemotherapy* **72**: 1581–1588.

Tang Y, Dai L, Sahin O, Wu Z, Liu M and Zhang Q (2017b). Emergence of a plasmid-borne multidrug resistance gene *cfr*(C) in foodborne pathogen *Campylobacter*. *The Journal of Antimicrobial Chemotherapy* **72**: 1581–1588.

Tang Y, Sahin O, Pavlovic N, LeJeune J, Carlson J, Wu Z, Dai L and Zhang Q (2017c). Rising fluoroquinolone resistance in *Campylobacter* isolated from feedlot cattle in the United States. *Scientific Reports* **7**: 494.

Tenson T, Lovmar M and Ehrenberg M (2003). The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *Journal of Molecular Biology* **330**: 1005–1014.

Thomrongsuwannakij T, Blackall PJ and Chansiripornchai N (2017). A study on *Campylobacter jejuni* and *Campylobacter coli* through commercial broiler production chains in Thailand: antimicrobial resistance, the characterization of DNA gyrase subunit A mutation, and genetic diversity by flagellin A gene restriction fragment length polymorphism. *Avian Diseases* **61**: 186–197.

Toth M, Frase H, Antunes NT and Vakulenko SB (2013). Novel aminoglycoside 2″-phosphotransferase identified in a gram-negative pathogen. *Antimicrobial Agents and Chemotherapy* **57**: 452–457.

USDA (2011). Salmonella, Listeria, and Camplobacter on U.S. Dairy Operation, 1996–2007. *USDA-APHIS-VS, CEAH Fort Collins, CO #578.0311*.

Vacher S, Menard A, Bernard E and Megraud F (2003). PCR-restriction fragment length polymorphism analysis for detection of point mutations associated with macrolide resistance in *Campylobacter* spp. *Antimicrobial Agents and Chemotherapy* **47**: 1125–1128.

Vacher S, Menard A, Bernard E, Santos A and Megraud F (2005). Detection of mutations associated with macrolide resistance in thermophilic *Campylobacter* spp. by real-time PCR. *Microbial Drug Resistance* **11**: 40–47.

van Boven M, Veldman KT, de Jong MC and Mevius DJ (2003). Rapid selection of quinolone resistance in *Campylobacter jejuni* but not in *Escherichia coli* in individually housed broilers. *The Journal of Antimicrobial Chemotherapy* **52**: 719–723.

Wang Y, Huang WM and Taylor DE (1993). Cloning and nucleotide sequence of the *Campylobacter jejuni* gyrA gene and characterization of quinolone resistance mutations. *Antimicrobial Agents and Chemotherapy* **37**: 457–463.

Wang Y, He T, Schwarz S, Zhou D, Shen Z, Wu C, Wang Y, Ma L, Zhang Q and Shen J (2012a). Detection of the staphylococcal multiresistance gene *cfr* in *Escherichia coli* of domestic-animal origin. *The Journal of Antimicrobial Chemotherapy* **67**: 1094–1098.

Wang Y, Wang Y, Schwarz S, Shen Z, Zhou N, Lin J, Wu C and Shen J (2012b). Detection of the staphylococcal multiresistance gene *cfr* in *Macrococcus caseolyticus* and *Jeotgalicoccus pinnipedialis*. *The Journal of Antimicrobial Chemotherapy* **67**: 1824–1827.

Wang Y, Li D, Song L, Liu Y, He T, Liu H, Wu C, Schwarz S and Shen J (2013). First report of the multiresistance gene *cfr* in *Streptococcus suis*. *Antimicrobial Agents and Chemotherapy* **57**: 4061–4063.

Wang Y, Dong Y, Deng F, Liu D, Yao H, Zhang Q, Shen J, Liu Z, Gao Y, Wu C and Shen Z (2016). Species shift and multidrug resistance of *Campylobacter* from chicken and swine, China, 2008–14. *The Journal of Antimicrobial Chemotherapy* **71**: 666–669.

WHO (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics *Tacconelli-Magrini 25 Feb 2017*. http://www.who.int/medicines/publications/global-priority-list-antibiotic-resistant-bacteria/en/.

Wozniak-Biel A, Bugla-Ploskonska G, Kielsznia A, Korzekwa K, Tobiasz A, Korzeniowska-Kowal A and Wieliczko A (2017). High prevalence of resistance to fluoroquinolones and tetracycline *Campylobacter* spp. Isolated from Poultry in Poland. *Microbial Drug Resistance*.

Yan M, Sahin O, Lin J and Zhang Q (2006). Role of the CmeABC efflux pump in the emergence of fluoroquinolone-resistant *Campylobacter* under selection pressure. *The Journal of Antimicrobial Chemotherapy* **58**: 1154–1159.

Yao H, Shen Z, Wang Y, Deng F, Liu D, Naren G, Dai L, Su CC, Wang B, Wang S, Wu C, Yu EW, Zhang Q and Shen J (2016). Emergence of a potent multidrug efflux pump variant that enhances *Campylobacter* resistance to multiple antibiotics. *mBio* **7**: e01543-16.

Yao H, Liu D, Wang Y, Zhang Q and Shen Z (2017). High prevalence and predominance of the *aph(2″)-If* gene conferring aminoglycoside resistance in *Campylobacter*. *Antimicrobial Agents and Chemotherapy* **61**: e00112-17.

Zeng X, Brown S, Gillespie B and Lin J (2014). A single nucleotide in the promoter region modulates the expression of the beta-lactamase OXA-61 in *Campylobacter jejuni*. *The Journal of Antimicrobial Chemotherapy* **69**: 1215–1223.

Zhang Q, Lin J and Pereira S (2003). Fluoroquinolone-resistant *Campylobacter* in animal reservoirs: dynamics of development, resistance mechanisms and ecological fitness. *Animal Health Research Reviews* **4**: 63–71.

Zhang R, Sun B, Wang Y, Lei L, Schwarz S and Wu C (2015). Characterization of a cfr-carrying plasmid from porcine *Escherichia coli* that closely resembles plasmid pEA3 from the plant pathogen *Erwinia amylovora*. *Antimicrobial Agents and Chemotherapy* **60**: 658–661.

Zhao S, Young SR, Tong E, Abbott JW, Womack N, Friedman SL and McDermott PF (2010). Antimicrobial resistance of *Campylobacter* isolates from retail meat in the United States between 2002 and 2007. *Applied and Environmental Microbiology* **76**: 7949–7956.

Zhao S, Mukherjee S, Chen Y, Li C, Young S, Warren M, Abbott J, Friedman S, Kabera C, Karlsson M and McDermott PF (2015). Novel gentamicin resistance genes in *Campylobacter* isolated from humans and retail meats in the USA. *The Journal of Antimicrobial Chemotherapy* **70**: 1314–1321.

Zhou J, Zhang M, Yang W, Fang Y, Wang G and Hou F (2016). A seventeen-year observation of the antimicrobial susceptibility of clinical *Campylobacter jejuni* and the molecular mechanisms of erythromycin-resistant isolates in Beijing, China. *International Journal of Infectious Diseases: IJID: Official Publication of the International Society for Infectious Diseases* **42**: 28–33.