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Antibiotic resistance in *Campylobacter*: emergence, transmission and persistence

Taradon Luangtongkum[1], **Byeonghwa Jeon**[2], **Jing Han**[3], **Paul Plummer**[4], **Catherine M Logue**[5], and **Qijing Zhang**[6,†]

1 *Department of Veterinary Microbiology & Preventive Medicine, 1116 Veterinary Medicine Complex, Iowa State University, Ames, IA 500111, USA, ltaradon@iastate.edu and, Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Henry Dunant Road, Bangkok 10330, Thailand, taradon.l@chula.ac.th*

2 *Department of Veterinary Microbiology & Preventive Medicine, 1116 Veterinary Medicine Complex, Iowa State University, Ames, IA 500111, USA, jeonbh@iastate.edu*

3 *Department of Veterinary Microbiology & Preventive Medicine, 1116 Veterinary Medicine Complex, Iowa State University, Ames, IA 500111, USA, hanjing@iastate.edu*

4 *Department of Veterinary Microbiology & Preventive Medicine, 1116 Veterinary Medicine Complex, Iowa State University, Ames, IA 500111, USA, pplummer@iastate.edu*

5 *Department of Veterinary & Microbiological Sciences, North Dakota State University, Fargo, ND 581022, USA, catherine.logue@ndsu.edu*

6 *Department of Veterinary Microbiology & Preventive Medicine, 1116 Veterinary Medicine Complex, Iowa State University, Ames, IA 500111, USA, Tel.: +1 515 294 2038; Fax: +1 515 294 8500; zhang123@iastate.edu*

Abstract

Campylobacter is a leading foodborne bacterial pathogen, which causes gastroenteritis in humans. This pathogenic organism is increasingly resistant to antibiotics, especially fluoroquinolones and macrolides, which are the most frequently used antimicrobials for the treatment of campylobacteriosis when clinical therapy is warranted. As a zoonotic pathogen, *Campylobacter* has a broad animal reservoir and infects humans via contaminated food, water or milk. Antibiotic usage in both animal agriculture and human medicine, can influence the development of antibiotic-resistant *Campylobacter*. This review will describe the trend in fluoroquinolone and macrolide resistance in *Campylobacter*, summarize the mechanisms underlying the resistance to various antibiotics and discuss the unique features associated with the emergence, transmission and persistence of antibiotic-resistant *Campylobacter*. Special attention will be given to recent findings and emphasis will be placed on *Campylobacter* resistance to fluoroquinolones and macrolides. A future perspective on antibiotic resistance and potential approaches for the control of antibiotic-resistant *Campylobacter*, will also be discussed.

†Author for correspondence: Department of Veterinary Microbiology & Preventive Medicine, 1116 Veterinary Medicine Complex, Iowa State University, Ames, IA 50011, USA, Tel.: +1 515 294 2038, Fax: +1 515 294 8500, E-mail: zhang123@iastate.edu.

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Keywords

antibiotic resistance; *Campylobacter*; ecological fitness; fluoroquinolone; food safety; macrolide; public health

Thermophilic *Campylobacter* species, particularly *Campylobacter jejuni,* have been recognized as a major cause of acute bacterial gastroenteritis in humans since the late 1970s and it is estimated that *Campylobacter* sp. are responsible for 400–500 million cases of diarrhea each year, worldwide [1]. As an enteric organism, *Campylobacter* is carried in the intestinal tracts of a wide range of domestic animals and poultry as well as wild animals and birds [2, 3]. Transmission of *Campylobacter* to humans occurs mainly through the consumption of contaminated foods of animal origin, especially undercooked poultry meat, unpasteurized milk and dairy products, as well as by the ingestion of other foods that are cross-contaminated by raw poultry meat during food preparation [3,4]. Although most *Campylobacter* infections are mild, self-limiting and usually resolve within a few days without antibiotic treatment, severe or prolonged infections can occur, particularly in the young, elderly and in individuals with compromised immunity. In these circumstances, therapeutic intervention is usually warranted [4,5]. For clinical therapy of campylobacteriosis, erythromycin (a macrolide) is considered the drug of choice, but fluoroquinolone (FQ) antimicrobials (e.g., ciprofloxacin) are also frequently used owing to their broad spectrum of activity against enteric pathogens [4–6]. Alternative drugs include tetracyclines and gentamicin, which are used in cases of systemic infection with *Campylobacter* [5]. However, *Campylobacter* is increasingly resistant to the clinically important antibiotics and this rising resistance is a concern for public health. Development and transmission of antibiotic-resistant *Campylobacter* is complicated by the fact that *Campylobacter* is a zoonotic pathogen and is therefore exposed to antibiotics used in both animal production and human medicine. Thus, an ecological approach is required to understand the emergence, transmission and persistence of antibiotic-resistant *Campylobacter.*

Prevalence of antibiotic resistance in *Campylobacter*

A rapid increase in the proportion of *Campylobacter* strains resistant to antimicrobial agents, particularly to FQs, has been reported in many countries worldwide [6–8]. Prior to 1992, FQ-resistant *Campylobacter* was rarely observed in the USA and Canada, but several recent reports have indicated that approximately 19–47% of *Campylobacter* strains isolated from humans were resistant to ciprofloxacin [9–11]. A steady increase in FQ resistance among *Campylobacter* isolates has also been observed in many European countries and 17–99% of *Campylobacter* strains isolated from humans and animals in this region were resistant to FQs, with the highest resistance levels reported in Spain [5,8,12–18]. FQ-resistant *Campylobacter* has also become prevalent in Africa and Asia. In both continents, FQ resistance among clinical *Campylobacter* isolates was not detected before 1991, however, since 1993 the frequency of FQ-resistant *Campylobacter* strains has increased remarkably and the FQ-resistance rates have reached more than 80% in Thailand and Hong Kong [19–22]. Although FQ resistance in *Campylobacter* isolates was also observed in Australia and New Zealand, the rate of FQ-resistant *Campylobacter* isolates in this region is significantly lower than that in other regions [8,23,24].

A trend for increased macrolide resistance in *Campylobacter* has been observed in some countries [25]. Generally, the prevalence of erythromycin resistance among *Campylobacter* strains (including both *C. jejuni* and *Campylobacter coli*) isolated from humans, broilers and cattle in the USA and Canada has been reported at 10% or lower [25–30]. In contrast, more than 40% of *C. coli*, isolated from turkeys and swine in the USA, were resistant to this antimicrobial agent [26,29,31]. Likewise, macrolide resistance among *Campylobacter* isolates

from humans and *C. jejuni* isolates from chickens and cattle has been low and stable in most European countries, especially in Scandinavia, but a high prevalence of macrolide resistance, ranging from 15–80%, was observed in *C. coli*, isolated from chickens and swine [12,25,32–36]. Interestingly, high erythromycin resistance levels were observed among human clinical *Campylobacter* isolates from Africa, but low resistance levels to this antibiotic were noticed in *C. jejuni* and *C. coli* isolated from food-producing animals [25,37,38]. In Asia, less than 5% of *C. jejuni* isolated from humans, broilers, swine and cattle were resistant to macrolides, while 14–62% of *C. coli* isolated from humans, broilers and swine were resistant to this class of antimicrobial agents [39–43]. Similar to findings from other continents, macrolide resistance was mainly observed among *C. coli* isolates from swine in Australia [44]. These surveillance data clearly indicate that *C. coli* isolates harbor more macrolide resistance than *C. jejuni* isolates. The exact reasons for this difference are unknown and warrant further investigation.

Resistance mechanisms

In *Campylobacter*, the resistance to FQs is mainly mediated by point mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase A (GyrA) [45,46]. No mutations in DNA gyrase B have been associated with FQ resistance in *Campylobacter* [47–49]. The genes encoding topoisomeraseIV (*parC*/*parE*) are also involved in FQ resistance in Gram-negative bacteria, however, these genes are absent in *Campylobacter*. Thus, it is not surprising that *parC*/*parE* mutations are not implicated in *Campylobacter* resistance to FQ antimicrobials [47–52]. Unlike FQ resistance in other enteric organisms (e.g., *Salmonella* and *Escherichia coli*), in which acquisition of high-level FQ resistance requires stepwise accumulation of point mutations in *gyrA* and *parC*, a single point mutation in the QRDR of *gyrA* is sufficient to substantially reduce the susceptibility of *Campylobacter* to FQ antimicrobials [45,50,53]. The most frequently observed mutation in FQ-resistant isolates of *Campylobacter* is the C257T change in the *gyrA* gene, which leads to the T86I substitution in the gyrase and confers high-level resistance to FQs [45]. Other reported resistance-associated mutations include T86K, A70T and D90N, which are less common and do not confer FQ resistance as high as that observed for the T86I mutation [6,45]. In addition to the mutations in GyrA, the multidrug efflux pump, CmeABC, also contributes to FQ resistance by reducing the accumulation of the agents in *Campylobacter* cells [50,53,54]. Thus, CmeABC functions synergistically with the *gyrA* mutations in mediating FQ resistance. All of the known FQ resistance determinants in *Campylobacter* are chromosomally encoded and plasmid-mediated quinolone-resistance determinants, such as *qnr*, *aac(6′)-Ib-cr* and *qepA*, have not been reported in *Campylobacter*.

Macrolide resistance in *Campylobacter* is mainly associated with target modification and active efflux [55–59]. Modification of the ribosomal target, leading to macrolide resistance in *Campylobacter*, can occur either by enzyme-mediated methylation or by point mutation in the 23S rRNA and/or ribosomal proteins L4 and L22 [25,45]. To date, macrolide resistance mediated by rRNA methylation has been reported only in *Campylobacter rectus* [60]. Point mutations in domain V of the 23S rRNA, on the other hand, have been recognized as the most common mechanism for macrolide resistance in *C. jejuni* and *C. coli* [25,45,61]. These point mutations occur at positions 2074 and 2075 of the 23S rRNA, corresponding to positions 2058 and 2059, respectively, in *E. coli*. Among the reported resistance-associated mutations, the A2074C, A2074G and A2075G mutations are found to confer a high-level resistance to macrolide antibiotics (erythromycin MIC >128 μg/ml) in *C. jejuni* and *C. coli* [55,57,58,61, 62]. In clinical and field isolates, the A2075G mutation is observed most frequently [25,45, 63]. *C. coli* and *C. jejuni* have three copies of the rrn operon [64]. A mutation associated with macrolide resistance in *Campylobacter* is usually present in all three copies of the 23S rRNA gene; however, some mutations, such as A2074T, which confers a low level of erythromycin resistance, may not be present in all copies of the 23S rRNA gene [55,58,65].

In addition to the target modification, active efflux also contributes to macrolide resistance in *Campylobacter* [57–59,62,63,66,67]. In isolates with intermediate- or low-level macrolide resistance, inactivation of the CmeABC efflux pump completely restored the susceptibility of the isolates [57,58,68]. Even in the highly resistant *Campylobacter* strains with the A2074G or A2075G mutation, inactivation of CmeABC also significantly reduced the resistance level to macrolide antibiotics, suggesting that this efflux system functions synergistically with target mutations [58,59,66,68]. Additionally, the synergy between the CmeABC efflux pump and mutations in the ribosomal proteins L4 (G74D) and L22 (insertions at position 86 or 98), was also shown to confer macrolide resistance in *C. jejuni* and *C. coli* [59,62]. The target mutations and active efflux confer resistance in *Campylobacter* not only to macrolides (e.g., erythromycin, clarithromycin, azithromycin and tylosin), but also to ketolides (e.g., telithromycin) [45,68].

Resistance to tetracycline in *Campylobacter* is conferred by *tet(O)*, which is widely present in *Campylobacter* isolates recovered from various animal species [7]. To date, no other *tet* resistance genes have been found in *Campylobacter*. *tet(O)* encodes a ribosomal protection protein [69]. Recent work demonstrates that this protein recognizes an open A site on the bacterial ribosome and binds it in such a manner that it induces a conformational change that results in the release of the bound tetracycline molecule [70]. Furthermore, the conformational change persists for an extended period of time, thus allowing for continued protein elongation in an efficient manner [70,71]. Based on G–C content, sequence homology, codon usage and hybridization studies, it appears that *Campylobacter tet(O)* was probably acquired by horizontal gene transfer (HGT) from either *Streptomyces*, *Streptococcus* or *Enterococcus* spp. [72,73]. In most strains, the *tet(O)* gene is plasmid-encoded, however, some isolates do have a chromosomally encoded copy of the gene [74,75]. Tetracycline resistance in *C. jejuni* is also associated with the CmeABC multidrug efflux pump [54,66].

Compared to FQs, macrolides and tetracyclines, *Campylobacter* resistance to other antibiotics has received less attention. Aminoglycoside resistance in *Campylobacter* is conferred by drug modification proteins. Multiple aminoglycoside modifying enzymes, including 3′-aminoglycoside phosphotransferase types I, III, IV and VII, 3′,9-aminoglycoside adenyltransferase and 6-aminoglycoside adenyltransferase, have been described in *Campylobacter* [46]. In general, β-lactam antibiotics have limited efficacy against *Campylobacter* spp. and resistance to this class of antibiotics appears to be mediated by both intrinsic resistance and β-lactamase production [46,76].

Campylobacter exhibits intrinsic resistance to a variety of antibiotics, including bacitracin, novobiocin, rifampin, streptogramin B, trimethoprim and vancomycin [77,78]. Although the mechanisms of this intrinsic resistance are unclear, it is likely to be mediated, in part, by low permeability of the *Campylobacter* membrane and active efflux conferred by multidrug-efflux transporters [46].

Dynamics of antibiotic resistance emergence

Resistance to FQs and macrolides in *Campylobacter* occurs spontaneously owing to mutations in target genes. Assessed in culture media, the frequencies of emergence of FQ-resistant mutants range from approximately 10^{-6}–10^{-8}/cell/generation [79]. Different point mutations occur in the QRDR region of *gyrA* and confer varied levels of resistance to FQ antibiotics [79]. Thus, the measured frequencies of emergence of FQ resistance, vary with the concentration of antibiotics used in the media for mutant enumeration. In *Campylobacter*, the elevated expression of *cmeABC* increases the frequency of emergence of FQ-resistant mutants. This enhancing effect on mutant emergence is probably attributable to the synergistic action of CmeABC and *gyrA* mutations in conferring FQ resistance, allowing more mutants to grow

on antibiotic-containing plates. In addition, Mfd (Mutant Frequency Decline), a transcription-repair coupling factor involved in strand-specific DNA repair, promotes the emergence of FQ-resistant mutants in *Campylobacter* [80]. Inactivation of the *mfd* gene in *Campylobacter* resulted in a 100-fold reduction in the number of spontaneous mutants resistant to ciprofloxacin, while overexpression of *mfd* increased the mutant numbers. Given the fact that Mfd does not affect the MIC of FQ antibiotics in *Campylobacter*, the altered mutant number is likely to be a result of the direct effect of Mfd on mutation rates.

If the cell population is sufficiently large ($>10^6$), ciprofloxacin-resistant mutants will inevitably emerge when *Campylobacter* is exposed to FQs. This has been demonstrated both *in vitro* and *in vivo* [80]. Multiple independent studies have demonstrated the rapid development of FQ-resistant mutants in chickens originally infected with FQ-susceptible *C. jejuni*, but treated with enrofloxacin [50,81–84]. In the treated birds, FQ-resistant *Campylobacter* mutants could be detected in feces as early as 24 h after the initiation of treatment, and the FQ-resistant *Campylobacter* population eventually colonized the intestinal tract of the birds. Thus, treatment of *Campylobacter*-infected birds does not eradicate the organisms, but converts an originally FQ-susceptible population to FQ-resistant *Campylobacter*, by selecting for spontaneous FQ-resistant mutants from an originally FQ-susceptible population. Since contaminated poultry meat is a main source of human *Campylobacter* infections, the FQ-resistant *Campylobacter* developed in poultry can be transmitted to humans via the food chain. Owing to this concern, the FDA banned the use of FQ antimicrobials in poultry production in the USA in 2005 [201]. The development of FQ-resistant *Campylobacter* from antibiotic treatment was also observed in pigs infected with *C. coli* and human patients infected with *C. jejuni* [85–89]. Together, these observations indicate that *Campylobacter* is highly adaptable to FQ treatment. Selection of pre-existing spontaneous mutants is likely to be the key reason for the development of the FQ-resistant population (Figure 1), but *de novo* formation of FQ-resistant mutants during the treatment can not be totally excluded, as suggested by the results from an *in vitro* study, in which some FQ-resistant mutants emerged from an originally FQ-susceptible population long after the initiation of the treatment [80].

In contrast to FQ resistance, the mutation frequency for macrolide resistance in *Campylobacter* is low ($\sim 10^{-10}$/cell/generation) and is approximately 10,000-fold lower than that of FQ resistance [58,79]. The mutants obtained by single-step selection tend to have low-to-intermediate levels (MIC = 8–64 μg/ml) of resistance to erythromycin [58,62,90]. These mutants, obtained by a single-step selection, either harbor mutations in the L4 and L22 proteins or have no detectible mutations, and are not stable in the absence of macrolide antibiotics [62,90]. Acquisition of the mutations in 23S rRNA appears to require stepwise selection (increase of antibiotic concentration in steps) and/or prolonged exposure to macrolide antibiotics, suggesting that other mutations or changes in *Campylobacter* may be required prior to the occurrence of the 23S rRNA mutations [58,62]. Although some specific changes in L4 and L22 proteins did not seem to be required for the development of 23S rRNA mutations, the contribution of mutations in other genes to the process can not be excluded. Once acquired, most 23S rRNA mutations confer a high level of resistance to erythromycin (MIC $\geq$ 512 μg/ml) and can be stably maintained in the absence of macrolide antibiotics [55,62]. Although macrolide resistance is generally more prevalent in *C. coli* isolates than in *C. jejuni* isolates, the comparison of a limited number of *C. coli* and *C. jejuni* strains did not show an elevated mutation frequency for erythromycin in *C. coli*, suggesting that *C. coli* is not intrinsically more mutable than *C. jejuni* [58].

Another unique feature of macrolide resistance in *Campylobacter* is the slow development of resistant mutants under antibiotic treatment. Using *Campylobacter*-infected chickens, Lin *et al.* showed that therapeutic treatment of *Campylobacter*-infected birds with tylosin (administered in drinking water for three consecutive days) did not select for erythromycin-

resistant *Campylobacter*, even after three treatments. This is in clear contrast to the development of FQ resistance, which occurs rapidly in birds treated with enrofloxacin. But when tylosin was given to *Campylobacter*-infected birds daily as a feed additive, after several weeks of exposure, erythromycin-resistant *Campylobacter* emerged in the birds [58]. Similarly, Ladely *et al.* found that subtherapeutic use of tylosin in chickens, given continuously in feed, had a more significant impact than therapeutic use on the development of macrolide-resistant *Campylobacter* [91]. These *in vivo* findings are consistent with the low rate of spontaneous mutation to erythromycin resistance observed *in vitro* and suggest that a continuous exposure to macrolides for an extended period is required for the development of macrolide resistance in *Campylobacter*. The differences in the development of FQ resistance and macrolide resistance are illustrated in Figure 1. The low rate of emergence, the requirement for prolonged antibiotic exposure to select macrolide-resistance mutations and the significant fitness cost of erythromycin-resistant mutants (see the persistence section) may collectively contribute to the relatively low prevalence of macrolide resistance in *Campylobacter* compared with FQ resistance.

Transmission of antibiotic resistance determinants

In addition to the mutation-based mechanisms, *Campylobacter* can also acquire antibiotic-resistance determinants via HGT. Horizontal transfer of DNA between *Campylobacter* strains has been shown in bacterial cultures [92,93] and chicken intestine [94,95]. In bacteria, HGT is mediated by natural transformation, conjugation and transduction, all of which can occur in *Campylobacter*. Natural transformation and conjugation are especially well-recognized in *Campylobacter* and are often utilized as genetic tools to manipulate *Campylobacter* [96]. Conjugation is likely to play a major role in the transfer of plasmid-mediated resistance, for example, *tet(O)*, while natural transformation may be a major mechanism for the transfer of chromosomally encoded resistance (e.g., FQ and macrolide resistance). It should be noted that all three HGT mechanisms show strain-to-strain variation in *Campylobacter*.

Natural transformation utilizes sophisticated mechanisms to take up free DNA from the environment. Multiple factors involved in *Campylobacter*s natural transformation have been identified [92,97–101]. The transfer of genes encoding antibiotic-modifying enzymes in *Campylobacter* by natural transformation was demonstrated in several studies utilizing bacterial cultures or animal models [92,93,95]. In these studies, co-cultivation or co-colonization of *Campylobacter* strains carrying different antibiotic resistance determinants, such as *aphA3*, *cat*, or *tet(O)*, generated progeny populations resistant to multiple antibiotics. A definitive role of natural transformation in mediating the transfer of antibiotic resistance determinants was shown in a recent study using bacterial co-cultures, in which a transformation-deficient mutation and DNase I treatment of bacterial cultures abolished the formation of double resistant progeny [92]. Transfer of antibiotic resistance determinants in *Campylobacter* co-cultures occurred quickly and was not prevented by prewashing the cultures prior to mixture, suggesting that *Campylobacter* may actively release DNA to the media during growth [92]. In addition to the genes encoding antibiotic resistance, point mutations responsible for FQ and macrolide resistance can also be transferred to *Campylobacter* by natural transformation [90,93,97].

To determine if natural transformation facilitates the emergence of FQ-resistant *Campylobacter,* Jeon *et al.* measured the frequency of emergence of spontaneous FQ-resistant mutants in culture media by DNase I treatment, which depleted the free DNA available for transformation [92]. The treatment did not affect the measured frequencies of emergence of FQ-resistant mutants from an originally FQ-susceptible population, suggesting that natural transformation does not contribute to the original emergence of FQ-resistant mutants. Additionally, *in vitro* and *in vivo* experiments showed that deficiencies in natural

transformation did not affect the development of FQ-resistant *Campylobacter* during FQ treatment [92], suggesting that the *denovo* development of FQ-resistant mutants from a FQ-susceptible population is not influenced by natural transformation and is primarily owing to selection and enrichment of the spontaneous FQ-resistant mutants. However, natural transformation may contribute to the spread of FQ resistance across different *Campylobacter* populations, strains or species.

Multiple plasmids have been reported in *Campylobacter*, some of which can be transmitted by conjugation [75,98,102,103]. Many of the conjugative plasmids carry genes mediating resistance to tetracyclines [75,104] and aminoglycosides [103,105]. Although interspecies conjugative transfer of drug-resistant plasmids was reported with *Campylobacter*, conjugation was most successful at the intraspecies level [75,94,103,105]. In addition, intergenus conjugation from *C. jejuni* to *E. coli* was occasionally successful [103,105]. It was also reported that the transfer of a conjugative plasmid carrying *tet(O)* occurred between *C. jejuni* strains in the intestinal tract of chickens [94]. Considering the high prevalence of conjugative *tet(O)* plasmids in *Campylobacter*, it is possible that conjugation has contributed to the spread of tetracycline resistance in *Campylobacter*. Interestingly, a recent study reported conjugative transfer of a chromosomally encoded streptomycin resistance gene from *Helicobacter pylori* to *C. jejuni* at the intergenus level [106]. This transfer was believed to have happened by a conjugation-like mechanism, since it required physical contact of the two species and was protected from DNase I treatment.

Integrons and mobile genetic elements, such as transposons and insertional sequences, are important players for the transmission and spread of antibiotic resistance genes in bacteria [107]. However, these elements are not common in *Campylobacter* and do not appear to play a major role in the horizontal transfer of antibiotic resistance in *Campylobacter*. Class I integrons, which are the most common integrons associated with antibiotic resistance, were reported in both *C. jejuni* and *C. coli* and were found to carry aminoglycoside resistance genes (*aadA2* and *aacA4*) [108–110].

Campylobacter-infecting bacteriophages were isolated from different *Campylobacter* species and various sources [111–113]. Recent work in chickens has demonstrated that bacteriophages cause genomic instability in *C. jejuni* and mediate interstrain transfer of large DNA fragments [114,115]. These findings suggest that *Campylobacter*–bacteriophage interactions may be more common than previously recognized and might play a role in HGT in *Campylobacter*. However, the exact role of bacteriophages in transmitting antibiotic resistance determinants between *Campylobacter* awaits further investigation.

Persistence & fitness of antibiotic resistant *Campylobacter*

Resistance-conferring mutations or determinants may affect bacterial physiology (e.g., growth rate) and consequently their adaptability in antibiotic-free environments. In the absence of antibiotic selection pressure, antibiotic-resistant *Campylobacter* may or may not show a fitness burden. Whether antibiotic-resistant *Campylobacter* persists is influenced by its ability to transmit between hosts and to compete with antibiotic-susceptible *Campylobacter*. This competition determines if antibiotic-resistant *Campylobacter* continues to prevail or decline in antibiotic-free environments.

FQ resistance mediated by *gyrA* mutations can be stably maintained in *Campylobacter* in the absence of antibiotic selection pressure [116]. FQ-resistant *Campylobacter*, carrying the T86I mutation in GyrA, colonized chickens persistently without losing the resistance phenotype and the resistance-associated mutation. Both *in vitro* culturing and chicken colonization studies suggested that FQ-resistant *Campylobacter* mutants do not carry a fitness burden. In fact, pairwise competition experiments indicate that FQ-resistant mutants outcompete FQ-

susceptible strains in chickens [116], suggesting that, in fact, the FQ-resistant mutants possess an enhanced fitness. This fitness change is linked to the T86I mutation and does not appear to be owing to a compensatory mutation since transformation of FQ-susceptible *C. jejuni* strains with this mutation changed their fitness in chickens. Recently Zhang and colleagues further confirmed the link between the T86I mutation and the fitness change by creating revertants. Reversion of the T86I mutation to the wild-type allele was accompanied by the loss of the fitness advantage in chickens [Zhang Q; Pers. Comm.]. These laboratory findings are compatible with the results from several surveillance studies, in which FQ-resistant *Campylobacter* was found to continue to prevail in poultry from producers who had discontinued using FQ antimicrobials for up to 4 years [117–119]. Based on the results from the laboratory and surveillance studies, it is tempting to predict that once the prevalence of FQ resistance in *Campylobacter* is high, it will be difficult to reduce the prevalence of resistance.

An intriguing question about FQ resistance is how the resistance-associated *gyrA* mutations affect *Campylobacter* physiology and fitness. DNA gyrase controls DNA supercoiling and is important for DNA replication and transcription. It is conceivable that the resistance-conferring mutations in GyrA may alter the activities of the enzyme and affect DNA supercoiling in *Campylobacter*. Indeed, work using recombinant gyrases demonstrates that the mutant enzyme carrying the T86I change, which is the most common mutation observed in FQ-resistant isolates, has a greatly reduced supercoiling activity [Han J, Zhang Q; Pers. Comm.]. Using a reporter plasmid, it was further found that DNA super-coiling levels are reduced in the FQ-resistant mutant compared with the wild-type strain. These results provide compelling evidence that this resistance-conferring mutation alters the native function of DNA gyrase. Whether this alteration is sufficient to affect *Campylobacter* physiology and its fitness is currently under investigation [Zhang Q; Pers. Comm.].

Campylobacter mutants that show low-to-intermediate levels of erythromycin resistance and lack 23S rRNA mutations, are not stable in culture media or animal hosts and easily lose the resistance phenotype in the absence of macrolide antibiotics [62,90]. However, the macrolide-resistant mutants harboring 23S rRNA mutations are highly resistant to erythromycin and stable in terms of the resistance phenotype, and can persist in chickens in the absence of competition [55,62]. In contrast to FQ-resistant *Campylobacter*, erythromycin-resistant mutants show a clear fitness burden when compared with the wild-type strains. Using a chicken model, Zhang's group conducted pairwise competitions and revealed that erythromycin-resistant mutants carrying the A2074G or A2075G mutation in 23S rRNA were rapidly outcompeted by the isogenic wild-type strains [Luangtongkum T, Zhang Q; Pers. Comm.]. This fitness cost was consistently observed in multiple chicken experiments using different pairs of strains and suggests that, in the absence of the antibiotics, the mutations conferring macrolide resistance render *Campylobacter* less fit in its natural host. This finding is supported by surveillance data from Denmark, where reduced use of tylosin as a growth promoter in swine has led to a significant decrease in the number of erythromycin-resistant *C. coli* isolated from pigs [120]. These observations suggest that removal of the selection force will quickly reduce the prevalence of macrolide-resistant *Campylobacter*.

Tetracycline resistance conferred by *tet(O)* has become highly prevalent in *Campylobacter* worldwide. This gene is usually carried on a plasmid, although it can be chromosomally encoded. Interestingly, recent studies conducted with poultry operations demonstrate that tetracycline-resistant *Campylobacter* are prevalent in both organic and conventional production systems [29,121]. Cui *et al.* also reported the high prevalence of tetracycline-resistant *Campylobacter* in organic chickens from retail stores [122]. In addition, tetracycline-resistant *Campylobacter* were also frequently isolated from organic dairy farms and antibiotic-free pigs [31,123]. Since the majority of these cited references originate from the USA, where organic production is regulated under the National Organic Program, these animals were not

permitted to have any exposure to antibiotics following the last trimester of their gestation for mammals and following the second day of life for poultry. Additionally all forages and grains provided to these animals must be grown in an organic environment that has not been exposed to antibiotics for the 3 years prior to harvest. Different from the USA regulations, many organic certification programs in other regions of the world do allow for a limited use of antibiotics in certain circumstances. Although it is not possible to say that absolutely no antibiotic exposure occurs on the USA organic operations, the known use of such products is strictly prohibited and the local environments (including pastures) are required to meet organic standards for 3 years prior to their use for the production of organic products. Therefore, the prevalence of tetracycline-resistant *Campylobacter* in the USA organic production systems is unlikely to be maintained by antibiotic selection and suggests that *tet(O)*-containing plasmids may have coevolved with *Campylobacter*, such that carrying the plasmid is no longer a burden to the host.

Future perspective

Antibiotic resistance in *Campylobacter* continues to be a challenge for food safety and public health. Owing to the high prevalence of FQ resistance, FQ antimicrobials are losing effectiveness in the clinical treatment of human campylobacteriosis. Enhanced research efforts are needed to understand the factors affecting the transmission and persistence of FQ-resistant *Campylobacter* in various environments and hosts. It will also be interesting to examine how FQ resistance influences *Campylobacter* fitness and if withdrawal of FQ antimicrobials from animal production decreases the prevalence of FQ-resistant *Campylobacter*. Additionally, newer FQs that are effective against ciprofloxacin-resistant *Campylobacter* and novel treatment schemes that avoid the selection of FQ-resistant mutants, should be evaluated. Macrolides are still the most effective antibiotics against *Campylobacter* infections, but the rising trend of erythromycin resistance in *C. coli* and *C. jejuni* in some regions requires prudent use of this class of antibiotics. Additional studies are needed to understand how macrolide-resistant *Campylobacter* emerge under selective pressure. Application of advanced approaches, such as genomics and proteomics, is expected to provide new insights into the molecular mechanisms involved in the development of macrolide resistance in *Campylobacter*. It has become clear that the multidrug efflux pump, CmeABC, plays an important role in mediating antibiotic resistance in *Campylobacter*, but the contributions of other efflux transporters to antibiotic resistance remains to be elucidated. In addition, the natural functions of these efflux transporters in *Campylobacter* physiology await further investigation. Novel approaches that target drug efflux transporters or block the emergence and transmission of resistance determinants can be explored to control antibiotic-resistant *Campylobacter*.

Executive summary

Epidemiology

- *Campylobacter* is increasingly resistant to clinically important antibiotics, which has become a major concern for public health.
- *Campylobacter* isolates resistant to fluoroquinolone (FQ) and tetracycline are highly prevalent in many countries. Although macrolide resistance is relatively low and stabilized in *Campylobacter jejuni*, there is a trend for increased prevalence of macrolide-resistant *Campylobacter* in certain regions of the world and the trend is especially clear in *Campylobacter coli* isolates recovered from swine and turkey.

Resistance mechanisms

- FQ and macrolide resistance in *Campylobacter* is mediated by point mutations in *gyrA* and 23S rRNA, respectively. *tet(O)* is the only *tet* gene currently identified in *Campylobacter* and confers resistance to the tetracycline class of antibiotics.
- As an efflux pump, CmeABC reduces the intracellular concentration of antibiotics, functions synergistically with other resistance mechanisms and contributes to the resistance to multiple antimicrobials.

Emergence of antibiotic resistance

- Resistance-associated *gyrA* mutations occur spontaneously at a relatively high frequency in *Campylobacter* and FQ treatment rapidly selects for FQ-resistant mutants. The C257T change in *gyrA* is the most frequently observed mutation in FQ-resistant isolates and confers high-level resistance to FQs.
- In *Campylobacter*, the rate of spontaneous mutation to macrolide resistance is substantially lower than that observed for FQ resistance and the development of stable macrolide-resistant mutants requires stepwise selection and prolonged exposure to the antibiotics.

Transmission of antibiotic resistance determinants

- *Campylobacter* acquires resistance determinants by mutation and horizontal gene transfer. Natural transformation, conjugation and transduction can all occur in *Campylobacter* and are likely to contribute to the spread of antibiotic resistance determinants.

Persistence & fitness of antibiotic resistance

- The *gyrA* mutation conferring FQ resistance does not incur a fitness cost in *Campylobacter*. Once FQ-resistant *Campylobacter* is prevalent, it may be difficult to reverse the resistance trend because FQ resistance can persist even in the absence of antibiotic selection.
- Erythromycin-resistant *Campylobacter* carries a significant fitness burden and removal of the selective pressure will quickly reduce the prevalence of macrolide resistance.

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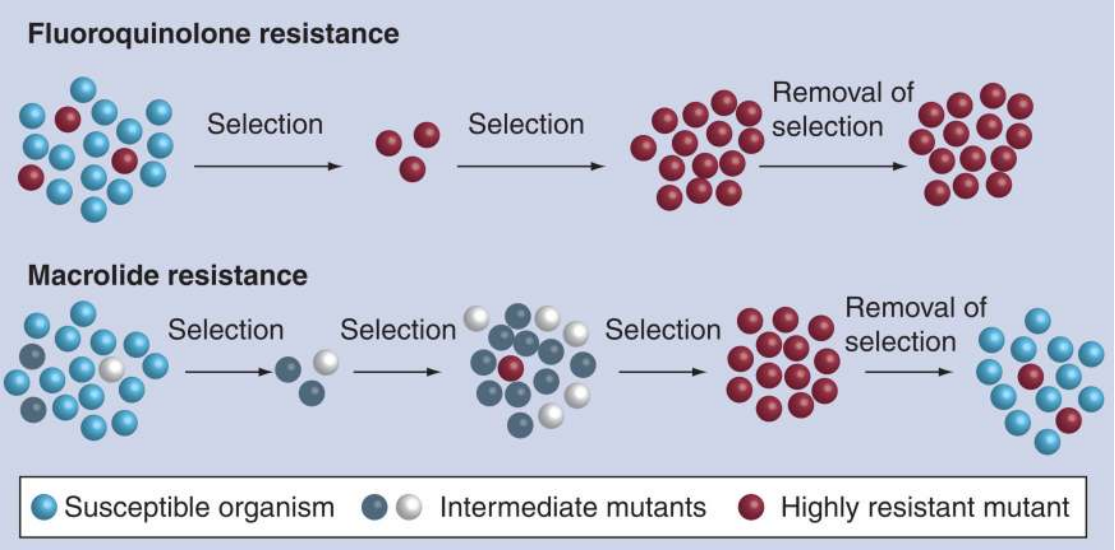

Figure 1. Model for the development and fitness of fluoroquinolone and macrolide resistance in *Campylobacter*

Fluoroquinolone-resistant mutants develop rapidly during antibiotic treatment and the mutant population continues to persist even after removal of the selection pressure. Development of macrolide-resistant mutants involves a multistep process and requires prolonged exposure to the antibiotic. Once the selection pressure is removed, macrolide-resistant mutants cannot compete with macrolide-susceptible *Campylobacter* and will decrease in number.