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MINIREVIEW

The Current State of Macrolide Resistance in *Campylobacter* spp.: Trends and Impacts of Resistance Mechanisms

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ABSTRACT *Campylobacter* spp., especially *Campylobacter jejuni* and *C. coli*, are leading bacterial foodborne pathogens worldwide. In the United States, an estimated 0.8 million cases of campylobacteriosis occur annually, mostly involving *C. jejuni*. Campylobacteriosis is generally self-limiting, but in severe cases, treatment with antibiotics may be mandated. The increasing incidence of fluoroquinolone resistance in *Campylobacter* has rendered macrolides such as erythromycin and azithromycin the drugs of choice for human campylobacteriosis. The prevalence of macrolide resistance in *C. jejuni* remains low, but macrolide resistance can be common in *C. coli*. Substitutions in the 23S rRNA gene, specifically A2075G, and less frequently A2074C/G, remain the most common mechanism for high-level resistance to macrolides. In *C. jejuni*, resistance mediated by such substitutions is accompanied by a reduced ability to colonize chickens and other fitness costs, potentially contributing to the low incidence of macrolide resistance. Interestingly, similar fitness impacts have not been noted in *C. coli*. Also noteworthy is a novel mechanism first reported in 2014 for a *C. coli* isolate from China and mediated by *erm*(B) harbored on multidrug resistance genomic islands. The incidence of *erm*(B) appears to reflect clonal expansion of certain strains, and whole-genome sequencing has been critical to the elucidation of *erm*(B)-associated macrolide resistance in *Campylobacter* spp. With the exception of one report from Spain, *erm*(B)-mediated macrolide resistance has been restricted to *Campylobacter* spp., mostly *C. coli*, of animal and human origin from China. If *erm*(B)-mediated macrolide resistance does not confer fitness costs in *C. jejuni*, the range of this gene may expand in *C. jejuni*, threatening to compromise treatment effectiveness for severe campylobacteriosis cases.

KEYWORDS *Campylobacter*, *Campylobacter coli*, *Campylobacter jejuni*, *erm*(B), erythromycin, macrolide, multidrug resistance, resistance

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In 2013, the Centers for Disease Control and Prevention listed fluoroquinolone- and macrolide-resistant *Campylobacter* as one of the serious antibiotic resistance threats to public health (1). *Campylobacter* spp. are major etiologic agents for human foodborne illness (campylobacteriosis), resulting in an estimated 0.8 million annual cases of disease in the United States alone (2–4). *Campylobacter jejuni* is responsible for about 90% of campylobacteriosis cases, with *Campylobacter coli* accounting for the majority of the remainder (2, 3). Poultry, contaminated water, and raw milk are the most frequently identified vehicles for illness, with most outbreaks attributed to the latter two (5–8). In addition to acute gastroenteritis, campylobacteriosis may result in severe autoimmune sequelae, including reactive arthritis and in about one of every 1,000 cases, Guillain-Barré syndrome (GBS). Campylobacteriosis is considered to be the most frequent antecedent for GBS (9, 10).

Antimicrobial treatment is not routinely recommended, as most human cases resolve on their own within 3 to 5 days, but it may be recommended for patients with

unusually severe and prolonged symptoms, especially those with AIDS, the elderly, or other vulnerable categories (4, 11). Treatment with fluoroquinolones such as ciprofloxacin has been challenged by a high incidence of fluoroquinolone resistance among human isolates, which led to the 2005 ban of the fluoroquinolone enrofloxacin for use in poultry (12). Resistance to the fluoroquinolone ciprofloxacin among human clinical isolates continues to be frequently encountered, e.g., 21.6% of *C. jejuni* and 24.5% *C. coli* at the time of the 2005 ban versus 26.7% and 35.6% of *C. jejuni* and *C. coli*, respectively, in 2014 (13). In view of the continuing relatively high incidence of fluoroquinolone resistance in *Campylobacter* spp. from human cases, macrolides such as erythromycin and azithromycin are considered the drugs of choice for treatment of human campylobacteriosis (1, 4, 14).

Historically, the incidence of resistance to erythromycin and other macrolides has been low, especially in *C. jejuni*, even though there are several mechanisms by which *Campylobacter* can acquire resistance to these antimicrobial agents. Data from the most recent National Antimicrobial Resistance Monitoring System (NARMS) report indicate that the prevalence of erythromycin resistance (MIC $\geq$ 8 μg/ml) in *C. jejuni* has remained below 4% in human and chicken isolates since NARMS testing began in 1997 and 2001, respectively (13). Macrolide resistance was noted in 22% of *C. jejuni* isolates from hogs, but this was based on only nine isolates, and *C. jejuni* is generally uncommon in swine (16). In contrast to the stable, low prevalence of macrolide resistance in *C. jejuni*, an overall trend for increased prevalence of macrolide resistance (MIC $\geq$ 16 μg/ml) was noted in *C. coli*; erythromycin resistance in *C. coli* derived from humans more than tripled in 2014 compared to 2011 (10.3% versus 2.7%) and more than doubled in retail chicken isolates in the same time frame (11.4% versus 5.2%) (13). Analysis of *C. coli* from samples collected at slaughter revealed macrolide resistance in 21% of isolates from sows, 40% from market swine, 11% from chickens, and 6.7% from turkeys (13). Analysis of 678 *C. jejuni* isolates and 119 *C. coli* isolates from human cases in Spain indicated that the prevalence of erythromycin resistance (MIC $\geq$ 32 μg/ml) was at relatively low levels (3.8%) similar to those in the U.S. NARMS report and that most (28/30) of the resistant isolates were *C. coli* (17).

Macrolide resistance in *C. jejuni* and *C. coli* has been the focus of intense study due to its potential to compromise therapeutic effectiveness of macrolides, and several excellent reviews previously addressed resistance mechanisms and implications (14, 18–20). Nonetheless, more-recent investigations have expanded our understanding of the biological implications of macrolide resistance, especially in regard to associated fitness costs in *C. jejuni* (21–24). In addition, employment of whole-genome sequencing (WGS) has not only advanced understanding of the distribution of previously known resistance-associated mutations but, starting in 2014, also revealed the emergence of *erm*(B), a macrolide resistance determinant not previously detected in *Campylobacter* spp. (25). For these collective reasons, we considered the timing appropriate for an updated minireview of mechanisms and implications of macrolide resistance in this pathogen.

MECHANISMS OF RESISTANCE

Target mutations in 23S rRNA genes. *Campylobacter* spp. may evade macrolide binding by alteration of the antimicrobial's 23S rRNA target at position 2074 or 2075. The substitutions A2075G, A2074G, A2074C, and the more rarely encountered A2074T have been shown to confer high-level resistance (>512 μg/ml) to erythromycin when present in all three copies of the 23S rRNA gene in *C. jejuni* and *C. coli* (14, 17, 20, 26, 27). High-level resistance also requires an intact CmeABC efflux system as will be discussed in detail below (14, 26, 27). The ribosomal substitutions may not always occur in all three copies of the gene, resulting in a lower level of resistance (14, 19, 28). Additionally, isolates may have different substitutions on different copies of the 23S rRNA gene. For example, one *C. jejuni* strain with an erythromycin MIC of >128 μg/ml harbored A2074C and A2075G substitutions in different copies of the 23S rRNA gene (28).

WGS analysis of 114 *Campylobacter* isolates from the United States identified 52 isolates (46%), including 17 *C. jejuni* isolates and 35 *C. coli* isolates, that were resistant to erythromycin; all but one harbored the A2075G substitution in the 23S rRNA gene with the remaining isolate, a *C. jejuni* isolate, harboring A2074T, suggesting that A2075G remains the most prevalent genetic event conferring high-level resistance to erythromycin (29). All erythromycin-resistant isolates were coresistant to azithromycin and vice versa, with the exception of one strain found to be azithromycin resistant but erythromycin susceptible and harboring an amino acid substitution (A86E) in ribosomal protein L22 (29).

Target mutations in ribosomal proteins. In the absence of mutations in 23S rRNA genes, mutations in *rplD* and *rplV* (ribosomal proteins L4 and L22, respectively) are associated with a lower level of resistance to macrolides (erythromycin MIC, 32 μg/ml) compared to isolates with ribosomal substitutions that exhibit erythromycin MICs of $>$512 μg/ml (19, 24, 30). Strains with L4 and L22 mutations developed high-level resistance ($>$256 μg/ml) upon acquisition of mutations in the 23S rRNA gene (30). Numerous macrolide resistance-conferring substitutions and insertions in these ribosomal proteins have been recorded. For instance, various substitutions (e.g., G74D, G67V, R72I, and A71D), and a glycine insertion at position 60 were noted in L4 (14, 26, 30, 31). For L22, A88E and G86E have been noted as well as a nine-base duplication at position 292 of *rplV* and insertions at position 86 or 98 (14, 26, 30).

Ribosomal methylation encoded by *erm*(B). For many years, base substitutions in the 23S rRNA sequence were the only mechanism known to specifically mediate high-level macrolide resistance in *Campylobacter* spp. (14). However, in 2014, a ribosomal methylase encoded by *erm*(B) was reported for the first time in *Campylobacter* spp., in *C. coli* strain ZC113 of swine origin in China (25). Erm(B) dimethylates a single adenine in the 23S rRNA gene, leading to decreased binding of macrolides (32). *C. coli* ZC113 and the majority of other *erm*(B)-harboring strains are constitutively resistant to macrolides and also express *erm*(B) constitutively (33). However, a small number of *erm*(B)-harboring strains were found to be susceptible to erythromycin; such strains expressed *erm*(B) and became resistant to macrolides with MICs as high as strains constitutively expressing *erm*(B) upon preincubation with erythromycin or clindamycin (33). Interestingly, the majority of *erm*(B)-harboring strains appear to harbor deletions in the *erm*(B) regulatory region, which may account for their constitutive resistance to macrolides (33). It is tempting to speculate that such deletion derivatives have been selected upon exposure to macrolides, e.g., in animal production.

In *C. coli* ZC113, *erm*(B) was harbored by a chromosomal multidrug resistance genomic island (MDRGI) composed of 17 open reading frames (ORFs), 8 of which encoded antimicrobial resistance determinants (25). Besides *erm*(B), the MDRGI included a truncated *tet*(O) and the aminoglycoside resistance cassette *aadE-sat4-aphA3*, previously found in conjunction with *erm*(B) in *Enterococcus* (25, 34). The MDRGI-associated *erm*(B) showed 100% identity with *erm*(B) of Gram-positive bacteria such as *Streptococcus suis*, *Enterococcus faecium*, and *Lactobacillus plantarum*, and most other MDRGI ORFs exhibited high homology with counterparts in Gram-positive bacteria, suggesting a Gram-positive origin for the *erm*(B)-harboring island in *Campylobacter* (25, 35, 36).

Subsequent reports have identified *erm*(B) in several additional isolates (25, 37–39). Multilocus sequence typing (MLST) analysis of 58 *erm*(B)-harboring *C. coli* isolates from China identified 30 different sequence types, many of which clustered into one clonal complex (CC 828) (36). *erm*(B)-positive isolates from another study also mostly clustered within CC 828 (40). *erm*(B)-harboring isolates exhibited high levels of erythromycin resistance (MIC, 512 μg/ml) with the exception of two of the five *erm*(B)-positive *C. jejuni* isolates identified thus far (MIC, 16 μg/ml) (25, 37, 41). Interestingly, three *erm*(B)-positive *C. coli* isolates also harbored the A2075G substitution in the 23S rRNA gene (39).

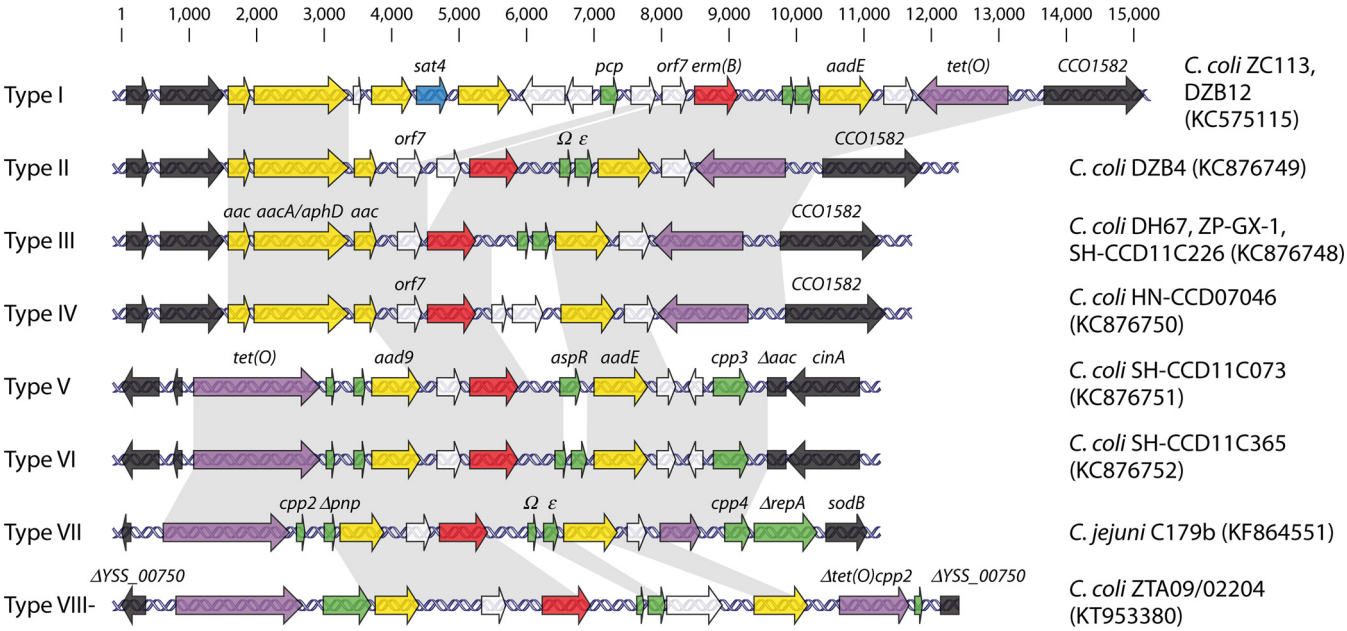

FIG 1 The genetic environment of *erm*(B)-harboring MDRGIs in *Campylobacter* spp. Shaded regions indicate areas with greater than 98% identity. The *erm*(B) gene is shown in red, aminoglycoside resistance genes are shown in yellow, the tetracycline resistance gene *tet*(O) is shown in purple, genes with predicted functions are shown in green, and genes encoding hypothetical proteins are shown in white with bordering regions shown in black in accordance with previously published MDRGIs (25, 36, 37). The comparisons were performed using Geneious (version 9.1.4) (58). Deltas indicate deletions in the corresponding genes.

In all investigated cases, *erm*(B) was harbored by an MDRGI, frequently (57%) on the chromosome or on plasmids of different sizes (35–39). Sequence analysis has revealed at least eight different *erm*(B) genomic organizations, with the arrangement in the first reported *erm*(B)-harboring strain, *C. coli* ZC113, being designated as type I (Fig. 1) (36, 37). Various insertion sites were identified for different MDRGIs, but *erm*(B) was highly conserved (>98% identity at the nucleotide sequence level) among all eight MDRGIs (Fig. 1) (37, 38). The most divergent Erm(B) (type III MDRGI) had only four amino acid substitutions compared to Erm(B) in the type I MDRGI. The lone *erm*(B)-positive strain reported outside China (MDRGI type VIII) had only one amino acid substitution in Erm(B), while *erm*(B) in the type VII MDRGI of *C. jejuni* C179b (accession no. KF864551) had one nucleotide substitution compared to its type I homolog, which did not result in an amino acid change (37, 38).

Detection of *erm*(B) remains uncommon in *Campylobacter*, and as of this writing, *erm*(B) seems to be largely confined to China, with only one *erm*(B)-positive strain reported elsewhere (Spain) (37). WGS analysis of 114 *C. jejuni* and *C. coli* isolates from the NARMS collection failed to identify *erm*(B) among any of the 52 macrolide-resistant isolates (29). It is not clear why reports for *erm*(B)-harboring strains have been largely absent from nations other than China. China's antimicrobial use may have conferred unique selective pressures on *Campylobacter* spp. there. While antimicrobial use is difficult to measure, China has been estimated to be responsible for 23% of the global antimicrobial use, with the United States estimated at 13% (40). It is also conceivable that *erm*(B)-harboring *Campylobacter* strains arose in China via horizontal gene transfer from Gram-positive microbes cooccurring with *Campylobacter*, e.g., in swine, in response to animal husbandry attributes that might be more common in animal production in China than elsewhere.

Even in China, however, incidence of *erm*(B)-harboring strains appears to be low. For instance, only 58 of 1,554 (3.7%) *C. jejuni* and *C. coli* isolates from animal and human cases were positive for *erm*(B) by PCR (36). It is noteworthy that most (53/58) of these *erm*(B)-harboring isolates were *C. coli* obtained in 2011 and 2012, with only 5 isolates from previous years (2007 to 2009), suggesting that *erm*(B) may be expanding (36). The earliest *erm*(B)-positive *Campylobacter* isolate was *C. jejuni* cj94473, isolated from a case

of human diarrheal disease in China in 1994 and exhibiting intermediate (MIC, 16 μg/ml) resistance to erythromycin (41). Another PCR analysis of 858 *C. jejuni* and *C. coli* isolates from human diarrheal cases, chicken, and swine from China identified only 30 (3.5%) that were *erm*(B) positive; all were *C. coli*, with a substantial fraction (13/30 [43%]) originating from human diarrheal cases (39). To the best of the authors' knowledge, fitness impacts of these MDRGIs in *Campylobacter* have not yet been described.

The sole *erm*(B)-positive strain reported outside China may warrant special attention. This strain, *C. coli* ZTA09/02204, harbored *erm*(B) on an MDRGI (type VIII) that was genetically different enough from that of *C. coli* ZC113 (Fig. 1) to prompt the speculation that it may have originated independently (37). The type VIII MDRGI harbors 12 ORFs, including 5 antimicrobial resistance (AMR) genes with an intact *tet*(O), in contrast to the type I MDRGI from *C. coli* ZC113 which contained 17 ORFs with 8 AMR genes and a truncated *tet*(O) (Fig. 1) (25, 37). Especially interesting is the high (99%) similarity of the type VIII MDRGI to a region on the previously characterized plasmid pN29710-1, identified in *C. coli* from retail chicken in the United States and harboring the recently identified aminoglycoside resistance gene *aph*(*2″*)-*Ig* (42). In the type VIII MDRGI, a cluster of genes, including *erm*(B), an omega transcriptional repressor, a toxin-antitoxin system, and *aadE* (Fig. 1), may have been inserted between *aad9* and the truncated *tet*(O) of pN29710-1 (37).

Multidrug efflux pumps. Macrolide resistance mediated by specific mutations or horizontally acquired determinants such as discussed above operates over and above baseline resistance levels conferred by innate efflux systems such as the CmeABC efflux pump. CmeABC is a member of the resistance-nodulation-division (RND) efflux transporter family; it harbors the typical structural motif of 12 transmembrane α-helices and mediates efflux of various compounds (14, 43–46). CmeABC inactivation resulted in up to a 64-fold decrease in the erythromycin MIC of *C. jejuni* harboring the A2075G substitution in the 23S rRNA gene (43, 46, 47). The extent to which CmeABC inactivation may decrease erythromycin MICs of *erm*(B)-harboring strains remains to be determined.

Mutations in the regulatory region of *cmeABC* may affect the MIC of macrolide-resistant isolates, and recently, a *C. jejuni* variant with enhanced macrolide resistance and harboring a single A-to-G substitution in the CmeR-binding site of *cmeABC* (resistance-enhancing CmeABC [RE-CmeABC]) was identified (48). CmeR represses *cmeABC* transcription by binding to an inverted repeat in the *cmeR-cmeA* intergenic region (49, 50). Mutations in the CmeR-binding site have been shown to result in overexpression of CmeABC (51, 52), and indeed one RE-CmeABC variant, which was identified using WGS, exhibited a fivefold increase in *cmeABC* transcript levels (48). Such enhanced-resistance variants may be expanding within *Campylobacter*. The prevalence of RE-CmeABC among chicken- and swine-derived *C. jejuni* and *C. coli* increased from 7% to 20% between 2012 and 2014 (48). It is noteworthy that RE-CmeABC was noticeably more common in *C. jejuni* than *C. coli*, possibly suggesting a fitness advantage in *C. jejuni* (48).

Fitness costs. The finding that macrolide resistance is much more common in *C. coli* than *C. jejuni* prompts the hypothesis that fitness costs associated with substitutions in the 23S rRNA macrolide target keep the prevalence low in *C. jejuni*. *In vitro* competitive fitness assays have in fact indicated markedly impaired fitness associated with erythromycin resistance for certain strains of *C. jejuni* during growth in laboratory media (21, 23, 24, 53). Erythromycin-susceptible strains of *C. jejuni* and their isogenic erythromycin-resistant mutants (harboring either the A2074G or A2075G 23S rRNA substitution) had equal ability to colonize chickens when inoculated as monocultures, but in mixtures and in the absence of erythromycin, the erythromycin-resistant mutants were readily outcompeted by their parental strain counterparts (21, 24). Interestingly, such fitness costs in bird colonization were not noted with macrolide-resistant mutants of *C. coli* (21).

The impaired ability of erythromycin-resistant *C. jejuni* to colonize chickens may account for the overall low prevalence of erythromycin resistance in *C. jejuni* from human disease, for which chicken is a major vehicle. It also raises questions not only

about the mechanisms underlying the observed differences in fitness impacts between *C. jejuni* and *C. coli* but also about mechanisms that allow stable, high-level erythromycin resistance in certain *C. jejuni* strains (19, 22). Compensatory mutations in such strains may counteract the fitness impacts. One study employed inoculation of chickens with mixtures of a *C. jejuni* strain and an isogenic erythromycin-resistant mutant harboring A2074G in the 23S rRNA gene. A spontaneous colonization-proficient derivative of the erythromycin-resistant mutant was recovered from the inoculated birds and harbored a potentially compensatory C2551G substitution in the 23S rRNA gene in addition to the original A2074G substitution, retaining resistance to erythromycin (21). In another study, erythromycin-resistant mutants selected *in vitro* upon exposure to increasing levels of macrolides were found to harbor numerous mutations in addition to 23S rRNA substitutions (30). WGS analysis is expected to further elucidate possible compensatory mechanisms in *C. jejuni* that harbor specific substitutions in 23S rRNA and also exhibit stable, high-level resistance to macrolides.

C. jejuni strains with amino acid substitutions in L4 and L22 also exhibited downregulation of motility and energy metabolism genes; in addition, they had slower growth kinetics and became outcompeted in poultry hosts (24, 30). It is possible that the physiological impacts of L4 and L22 mutations that may precede those in 23S rRNA may contribute to the observed fitness impacts of macrolide resistance in *C. jejuni* (30). However, the roles of ribosomal proteins in macrolide resistance and fitness costs remain to be further elucidated. While some studies identified substitutions in the ribosomal proteins in conjunction with those in the 23S rRNA gene, others did not find such associations (19, 28–30, 41, 54).

The potential impacts of *erm*(B) on *Campylobacter*'s ability to colonize animals or other adaptations remain to be determined. Additionally, while the increasing prevalence of RE-CmeABC in *C. jejuni* may indicate a fitness advantage, experimental fitness assessments of these strains have not been reported.

It should be kept in mind that mutations conferring antimicrobial resistance do not always affect *Campylobacter* fitness in a negative way. Consider fluoroquinolone resistance of *C. jejuni*, mediated by a C257T substitution in *gyrA* (55). Although growth kinetics, motility, and ability to colonize chickens were similar between the parental strain and isogenic resistant mutants in monoculture, when inoculated as mixed cultures, the resistant mutants frequently outcompeted their susceptible parental counterparts, even in the absence of ciprofloxacin (14, 55). This contrasts with macrolide resistance where, as discussed above, resistant *C. jejuni* mutants were outcompeted by their susceptible counterparts.

Dissemination of macrolide resistance. Mutations in the 23S rRNA gene as well as those in *rplV* and *rplD* can be transferred from erythromycin-resistant to erythromycin-susceptible *Campylobacter* strains by natural transformation (19, 31, 56). Interestingly, turkey-derived erythromycin-susceptible *C. coli* strains were more efficiently transformed to erythromycin resistance than *C. coli* from swine, and the transformation frequency was significantly higher at 42°C, the body temperature of poultry, than at 25°C (56, 57). Certain strains of *Campylobacter* have fragmented 23S rRNA stemming from posttranscriptional excision of intervening sequences (IVS) (58). Analysis of erythromycin-resistant *C. coli* from turkeys revealed that, in addition to harboring the A2075G substitution in the 23S rRNA gene, these strains also tended to harbor IVS in all three 23S rRNA genes (59). Both the 23S rRNA A2075G mutation and IVS were transferrable to erythromycin-susceptible, IVS-free *C. coli* by natural transformation, rendering the latter resistant to erythromycin (59). It remains to be determined whether the higher prevalence of A2075G-mediated erythromycin resistance in *C. coli*, in comparison to *C. jejuni*, may reflect differences in the frequency of IVS and 23S rRNA fragmentation in these two species.

erm(B) is also transferable via natural transformation. Even though most of the reported *erm*(B)-harboring isolates have been *C. coli*, with only five *erm*(B)-positive *C. jejuni* strains reported thus far (38, 41), *erm*(B)-mediated resistance was transferable

from *C. coli* to *C. jejuni* by natural transformation with total genomic DNA (25, 36). The type VII *erm*(B)-carrying MDRGI in the chromosome of *C. jejuni* C179b (Fig. 1) was also transferable via natural transformation into erythromycin-susceptible *C. jejuni* (38). Along with macrolide resistance, *C. jejuni* transformants acquired additional AMR genes harbored in the MDRGI, gaining resistance to the corresponding antimicrobials, including lincosamides, tetracycline, ciprofloxacin, and gentamicin (25, 36). Finally, the RE-CmeABC mutation could also be disseminated via natural transformation, with transformants exhibiting up to a 32-fold increase in erythromycin MIC (from 0.5 μg/ml to 16 μg/ml) (48).

Whole-genome sequencing. The massive increase in WGS data for *Campylobacter* spp. (25, 29, 42, 60–63) has yielded a rich resource that can be mined to identify determinants associated with resistance to macrolides and other antimicrobials. The earlier-discussed WGS analysis of 114 *C. jejuni* and *C. coli* isolates from humans, retail meats, and food animals in the United States revealed that detection of specific AMR genes could accurately predict the corresponding resistance phenotype. For example, resistance to tetracycline and fluoroquinolones perfectly correlated with the presence of *tet*(O) or mutations in *gyrA*, respectively (29). WGS was also critical in the identification of *erm*(B) in *Campylobacter* and in analysis of the contents and insertional sites of the *erm*(B)-harboring MDRGIs in *Campylobacter* (25, 37–39). However, differences in the levels of macrolide resistance among isolates meeting the resistance threshold, e.g., $\geq$8 μg/ml and $\geq$16 μg/ml for *C. jejuni* and *C. coli*, respectively (64), may not be readily predictable based on the presence or absence of specific resistance mutations or determinants, as has also been recognized for other bacterial pathogens (65). Further integration of WGS data and phenotypic macrolide resistance assessments may yield WGS signatures with the capacity to predict differences in levels of macrolide resistance in *Campylobacter* spp. beyond the resistance threshold.

CONCLUSIONS

Conserved (primarily A2075G) substitutions in domain V of the 23S rRNA gene still represent the most commonly encountered mechanism for macrolide resistance in *C. jejuni* and *C. coli*. The accompanying fitness costs in *C. jejuni*, especially in colonization of chickens, may be responsible for the relatively low prevalence of macrolide resistance in this species. For reasons that remain to be elucidated, similar fitness costs in colonization have not been observed with *C. coli*, which also exhibits markedly higher prevalence of macrolide resistance, especially among isolates from food and animal sources. The issue of erythromycin resistance in *C. jejuni* is intriguing; in spite of the documented fitness costs, stable erythromycin-resistant strains and poultry flocks colonized by such strains have been reported (19, 66–70). Fitness costs in *C. jejuni* may be dependent on the strain or alleviated by compensatory mutations. Increasing availability and use of WGS data are expected to make major contributions in the further elucidation of these issues.

Even though thus far *erm*(B) remains primarily confined to *C. coli* from China, it has been shown to be transferrable via natural transformation to *C. jejuni*, the species responsible for the majority of cases of campylobacteriosis in humans, and it was recently also identified outside China (Spain). Further clonal expansion of *erm*(B)-harboring strains and infiltration of *erm*(B) into *C. jejuni* or *C. coli* populations in other regions can compromise the clinical effectiveness of macrolides for severe campylobacteriosis. Dissemination of the newly described resistance-enhancing CmeABC mutations (RE-CmeABC) may also have public health implications, especially if they emerge in *C. jejuni* strains already harboring 23S rRNA point mutations or *erm*(B).

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