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MECHANISMS OF RESISTANCE

Emerging *erm*(B)-Mediated Macrolide Resistance Associated with Novel Multidrug Resistance Genomic Islands in *Campylobacter*

Dejun Liu,[a,b] Weiwen Liu,[a,c] Ziquan Lv,[c] Junjie Xia,[c] Xing Li,[a] Yuxin Hao,[a] Ying Zhou,[a] Hong Yao,[a] Zhihai Liu,[a] Yang Wang,[a] Jianzhong Shen,[a] Yuebin Ke,[c] Zhangqi Shen[a]

[a]Beijing Advanced Innovation Center for Food Nutrition and Human Health, College of Veterinary Medicine, China Agricultural University, Beijing, China
[b]State Key Laboratory of Agro-Biotechnology, College of Biological Science, China Agricultural University, Beijing, China
[c]Key Laboratory of Molecular Epidemiology of Shenzhen, Shenzhen Center for Disease Control and Prevention, Shenzhen, China

ABSTRACT The rapid dissemination of the macrolide resistance gene *erm*(B) will likely compromise the efficacy of macrolides as the treatment of choice for campylobacteriosis. More importantly, *erm*(B) is always associated with several multidrug resistance genomic islands (MDRGIs), which confer resistance to multiple other antimicrobials. Continuous monitoring of the emergence of *erm*(B) and analysis of its associated genetic environments are crucial for our understanding of macrolide resistance in *Campylobacter*. In this study, 290 *Campylobacter* isolates (216 *Campylobacter coli* isolates and 74 *Campylobacter jejuni* isolates) were obtained from 1,039 fecal samples collected in 2016 from pigs and chickens from three regions of China (344 samples from Guangdong, 335 samples from Shanghai, and 360 samples from Shandong). Overall, 74 isolates (72 *C. coli* isolates and 2 *C. jejuni* isolates) were PCR positive for *erm*(B). Combined with data from previous years, we observed a trend of increasing prevalence of *erm*(B) in *C. coli*. Pulsed-field gel electrophoresis analyses suggested that both clonal expansion and horizontal transmission were involved in the dissemination of *erm*(B) in *C. coli*, and three novel types of *erm*(B)-associated MDRGIs were identified among the isolates. Furthermore, 2 *erm*(B)-harboring *C. jejuni* isolates also contained an aminoglycoside resistance genomic island and a multidrug-resistance-enhancing efflux pump, encoded by RE-*cmeABC*. Antimicrobial susceptibility testing showed that most of the isolates were resistant to all clinically important antimicrobial agents used for the treatment of campylobacteriosis. These findings suggest that the increasing prevalence of *erm*(B)-associated MDRGIs might further limit treatment options for campylobacteriosis.

KEYWORDS *Campylobacter*, MDRGIs, *erm*(B), macrolide resistance

Campylobacter species, especially *Campylobacter jejuni* and *Campylobacter coli*, are the leading bacterial foodborne pathogens (1). Therefore, the World Health Organization (WHO) has listed *Campylobacter* as one of four key causes of diarrheal diseases worldwide (2). In clinical settings, macrolides are the drugs of choice for the treatment of campylobacteriosis (3); however, the widespread use of antimicrobial agents in medical science and veterinary practice has hastened the emergence of macrolide resistance among *Campylobacter* strains in the past few years (4–6). Tylosin, tilmicosin, tulathromycin, and the new drug tildipirosin are macrolides that have been widely used in the livestock and poultry industries in China, Europe, and the United States (7, 8). Macrolide resistance rates are much higher in China than in most developed countries, in which rates have remained below 10% (6, 9–11). The three major mechanisms of macrolide resistance in *Campylobacter* include mutations in target genes (23S rRNA and

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Address correspondence to Yuebin Ke, keyke@szu.edu.cn, or Zhangqi Shen, szq@cau.edu.cn.

D.L. and W.L. contributed equally to this study.

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ribosomal proteins L4 and L22), antibiotic efflux pumps (CmeABC and resistance-enhancing CmeABC [RE-CmeABC]), and ribosomal methylation carried out by an *erm*(B)-encoded ribosomal methylase (12, 13). The *erm*(B)-based mechanism is of particular concern because the gene is horizontally transferable among *Campylobacter* strains and confers high-level resistance (14). Additionally, most *erm*(B) loci in *Campylobacter* are associated with multidrug resistance genomic islands (MDRGIs), which mediate resistance to multiple classes of antibiotics, including aminoglycosides, tetracyclines, and fosfomycin (15).

Interestingly, worldwide macrolide resistance rates for *C. jejuni* isolates are much lower than those for *C. coli* (16). Similarly, while nine classes of *erm*(B)-harboring MDRGIs have been detected in *Campylobacter* strains from China, Spain, and the United States, only two of those classes (types VII and IX) were detected in *C. jejuni*, with the remaining seven classes all being identified in *C. coli* isolates (15, 17, 18). Of note, novel *erm*(B) genetic environments are continually emerging in *Campylobacter*. Therefore, determining the structure of *erm*(B)-harboring MDRGIs is key to understanding the mechanisms of resistance gene transmission in *erm*(B)-carrying isolates.

In this study, we analyzed the prevalence of *erm*(B)-harboring MDRGIs in *Campylobacter* strains isolated from pigs and chickens in Guangzhou, Shanghai, and Shandong, China, in 2016. We identified three novel *erm*(B)-harboring MDRGI types in *Campylobacter* isolates from chickens, with one of the types, designated type X, being identified in 2 *C. jejuni* isolates. Sequence analysis revealed that those 2 isolates not only contained the *erm*(B)-harboring MDRGI but also harbored aminoglycoside resistance genomic islands (ARGIs) and the enhanced multidrug efflux pump-encoding locus RE-*cmeABC* (19). Thus, the isolates exhibited resistance to all antibiotics commonly used for clinical treatment of *Campylobacter* infections, including macrolides, fluoroquinolones, aminoglycosides, tetracyclines, lincosamides, and amphenicols.

RESULTS AND DISCUSSION

Prevalence of *erm*(B) in *Campylobacter*. A total of 290 *Campylobacter* isolates (216 *C. coli* isolates and 74 *C. jejuni* isolates) were obtained from 1,039 samples collected from the three regions of China (344 samples from Guangdong, 335 samples from Shanghai, and 360 samples from Shandong). Seventy-four *erm*(B)-positive isolates, including 72 *C. coli* isolates and 2 *C. jejuni* isolates, were isolated from chicken samples, while no *erm*(B)-positive isolates were obtained from swine samples (Table 1). The percentages of *erm*(B)-positive *Campylobacter* isolates from the three regions are shown in Table 1. Compared with our previous study examining *Campylobacter* isolates collected between 2013 and 2016 (20), the prevalence of *erm*(B) among *C. coli* isolates from Guangdong Province increased from 4.2% (6/144 isolates) in 2013 to 37.3% (57/153 isolates) in 2016. A chi-square test revealed a significant linear trend in prevalence from 2013 through 2016 ($P < 0.001$), while the prevalence rate among isolates from Shanghai significantly increased from 5.9% (6/102 isolates) in 2015 to 25.9% (15/58 isolates) in 2016 (chi-square test, $P < 0.001$). The prevalence of *erm*(B) among isolates from Shandong Province remained low across the examined time period (chi-square test, $P > 0.05$). These results strongly support the hypothesis that *erm*(B)-positive *Campylobacter* strains are more extensively disseminated in Guangdong Province, particularly as both *erm*(B)-positive *C. jejuni* isolates were obtained from samples from Guangdong.

Genotyping. Pulsed-field gel electrophoresis (PFGE) analysis was performed on the 72 *erm*(B)-positive *C. coli* isolates and 2 *erm*(B)-positive *C. jejuni* isolates collected in 2016. We also analyzed 4 human isolates obtained from clinical gastroenteritis cases in Shanghai in 2011 and 1 reference isolate each from pigs and chickens collected from Shanghai, Guangdong, and Shandong in 2013 and 2014 in our previous study (15, 20). Using a cutoff value of 80% pattern similarity, the *erm*(B)-positive *C. coli* isolates were clustered into 16 PFGE patterns, including 3 unique patterns and 13 clusters (Fig. 1). The isolates from Guangdong showed the greatest number of common patterns (14 patterns, including 3 unique patterns), while isolates from Shanghai showed only 5

TABLE 1 Prevalence of *erm*(B) in *Campylobacter* isolates from livestock and poultry collected over 4 successive years (2013 to 2016) (20)[a]

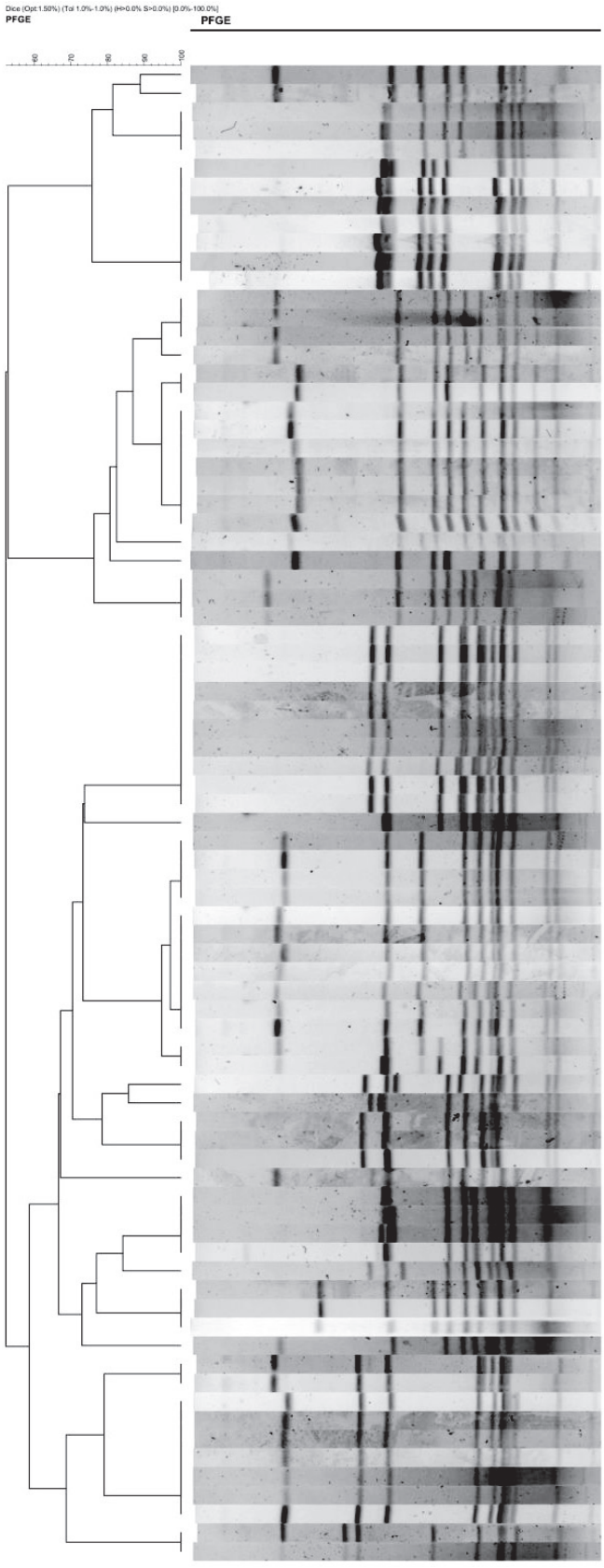
Location and year	No. of samples	No. of isolates (% [95% CI])			
		C. coli	*erm*(B) in *C. coli*	*C. jejuni*	*erm*(B) in *C. jejuni*
Guangdong					
2013	524	144 (27.5 [23.7–31.5])	6 (4.2 [1.5–8.8])	16 (3.1 [1.8–4.9])	0
2014	176	119 (67.6 [60.2–74.5])	28 (23.5 [16.2–32.2])	16 (9.1 [5.3–14.3])	0
2015	250	132 (52.8 [46.4–59.1])	31 (23.5 [16.5–31.6])	4 (1.6 [0.4–4.0])	0
2016	**344**	**153 (44.5 [39.1–49.9])**	**57 (37.3 [29.6–45.4])**	**17 (4.9 [2.9–7.8])**	**2 (11.8 [1.5–36.4])**
Shanghai					
2013	490	105 (21.4 [17.9–25.3])	4 (3.8 [1.0–9.5])	27 (5.5 [3.7–7.9])	0
2014	163	104 (63.8 [55.9–71.2])	3 (2.9 [0.6–8.2])	25 (15.3 [10.2–21.8])	0
2015	467	102 (21.8 [18.2–25.9])	6 (5.9 [2.2–12.4])	32 (6.9 [4.7–9.5])	0
2016	**335**	**58 (17.3 [13.4–21.8])**	**15 (25.9 [15.3–39.0])**	**11 (3.3 [1.7–5.8])**	**0**
Shandong					
2013	460	140 (30.4 [26.3–34.9])	3 (2.1 [0.4–6.1])	142 (30.9 [26.7–35.3])	0
2014	431	163 (37.8 [33.2 42.6])	4 (2.5 [0.7–6.2])	1 (0.2 [0–1.3])	0
2015	417	98 (23.5 [19.5–27.9])	0	0	0
2016	**360**	**5 (1.4 [0.5–3.2])**	**0**	**46 (12.8 [9.5–16.7])**	**0**
Total					
2013	1,474	389 (26.4 [24.2–28.7])	13 (3.3 [1.8–5.6])	185 (12.6 [10.9–14.4])	0
2014	770	386 (50.1 [46.5–53.7])	35 (9.1 [6.4–12.4])	42 (5.5 [4.0–7.3])	0
2015	1,134	332 (29.3 [26.6–32.0])	37 (11.1 [8.0–15.0])	36 (3.2 [2.2–4.4])	0
2016	**1,039**	**216 (20.8 [18.4–23.4])**	**72 (33.3 [27.1–40.0])**	**74 (7.1 [5.6–8.9])**	**2 (2.7 [0.3–9.4])**
All	4,417	1,323 (29.9 [28.6–31.3])	157 (11.9 [10.2–13.7])	337 (7.6 [6.9–8.5])	2 (0.6 [0.07–1.1])

[a]Data from both our previous study and the current study are included. Bold type indicates data from the current study.

patterns. Fewer patterns were identified than in our previous study, with 4 predominant clones (C, E, G, and O) accounting for the majority of isolates (62.5% [45/72 isolates]). Interestingly, strain 16SHKX69-2C, isolated from Shanghai, showed PFGE pattern B and had 100% homology to animal and human isolates collected from Shanghai in 2011 and 2014 and to animal isolates collected from Guangdong in 2014. This finding is consistent with our previous study showing that *erm*(B) was disseminated among *Campylobacter* isolates from humans and food-producing animals (20). Additionally, the 2 *C. jejuni* isolates, 8C and 36C, showed 100% homology, indicating that they belonged to the same clonal type (see Fig. S1 in the supplemental material). These results suggest that both regional expansion of a particular clone and horizontal transmission were involved in the dissemination of *erm*(B) among *Campylobacter* strains, although clonal expansion appears to have accounted for a larger proportion of the dissemination.

Identification of *erm*(B)-harboring MDRGIs. Long-range PCR assays based on integration sites and the genetic environment in 8 different MDRGIs identified in *erm*(B)-positive *Campylobacter* isolates were performed as described previously (21). Sequencing of the PCR products revealed that 40.54% of the *erm*(B)-positive isolates (30/74 isolates) contained regions that resembled a type VI MDRGI (>98% identity), while type III MDRGIs were detected in 23 *C. coli* isolates (31.08% [23/74 isolates]). Only 8 isolates contained a type V MDRGI, which was the predominant type in our previous study (20). It is noteworthy that types VI and III were identified as the predominant MDRGI types in this study, as this is consistent with the profiles of *erm*(B)-positive *Campylobacter* isolates from humans (15, 22). Moreover, PFGE pattern B, which was detected in isolates from animals and humans from different regions and years, was associated with the type VI MDRGI (Fig. 2a). These findings suggest that type VI MDRGIs may be frequently transferred between animal- and human-derived *Campylobacter* strains.

Overall, 11 *C. coli* isolates contained MDRGIs that did not belong to any currently recognized type. Therefore, we used Illumina HiSeq sequencing and primer walking to obtain the sequences of the complete MDRGI segments. Sequence alignment revealed



	Region	PEGE Type	MDRGI Type	Year
16SHKX21C	SH	A1	III	2016
16SHKX25-2C	SH	A2	III	2016
2C	GD	A3	VI	2016
38C	GD	A3	VI	2016
49C	GD	A3	VI	2016
16SHKX69-2C	SH	B	VI	2016
JX3	GD	B	VI	2014
SC49	SH	B	VI	2014
SH-CCD11C287	SH	B	VI	2011
SH-CCD11C416	SH	B	VI	2011
SH-CCD11C490	SH	B	VI	2011
SH-CCD11C518	SH	B	VI	2011
13C	GD	C1	-	2016
35C	GD	C1	III	2016
37C	GD	C1	III	2016
155C	GD	C2	V	2016
16SHKX23-1C	SH	C3	VI	2016
16SHKX65C	SH	C3	-	2016
100C	GD	C4	V	2016
115C	GD	C4	V	2016
16SHKX2-2C	SH	C4	V	2016
16SHKX20-1C	SH	C4	V	2016
16SHKX6-1C	SH	C4	V	2016
16SHKX7-2C	SH	C4	V	2016
GC63	GD	C4	V	2015
1C	GD	C5	VI	2016
16SHKX68-1C	SH	C6	-	2016
12C	GD	D	VI	2016
15C	GD	D	VI	2016
31C	GD	D	VI	2016
101C	GD	E	VI	2016
105C	GD	E	VI	2016
110C	GD	E	VI	2016
126C	GD	E	VI	2016
135C	GD	E	VI	2016
23C	GD	E	VI	2016
27C	GD	E	VI	2016
40C	GD	E	VI	2016
77C	GD	E	VI	2016
94C	GD	E	VI	2016
22C	GD	F	III	2016
28C	GD	G1	III	2016
99C	GD	G1	III	2016
58C	GD	G1	III	2016
74C	GD	G1	III	2016
119C	GD	G2	III	2016
144C	GD	G2	III	2016
170C	GD	G2	III	2016
174C	GD	G2	III	2016
43C	GD	G2	III	2016
85C	GD	G2	III	2016
90C	GD	G2	III	2016
88C	GD	G3	III	2016
93C	GD	G3	III	2016
102C	GD	H1	V	2016
25C	GD	H2	VI	2016
16SHKX43-2C	SH	I	III	2016
16SHKX63-2C	SH	I	III	2016
16SHKX70-1C	SH	I	III	2016
137C	GD	J	VI	2016
17C	GD	K1	VI	2016
18C	GD	K1	VI	2016
19C	GD	K1	XI	2016
67C	GD	K1	VI	2016
3C	GD	K2	VI	2016
16SHKX37-1C	SH	L	III	2016
16SHKX67-2C	SH	L	III	2016
SM26-1	SD	L	II	2013
16C	GD	M	VI	2016
39C	GD	N	VI(B)	2016
96C	GD	N	VI(B)	2016
117C	GD	O	VI(B)	2016
122C	GD	O	VI(B)	2016
123C	GD	O	VI(B)	2016
162C	GD	O	VI	2016
20C	GD	O	VI	2016
26C	GD	O	VI(B)	2016
89C	GD	O	VI(B)	2016
10C	GD	P	VI	2016
34C	GD	P	VI	2016

FIG 1 PFGE typing of *erm*(B)-positive *C. coli* isolates. Seventy-two isolates were collected in 2016, while 8 reference isolates were collected between 2011 and 2015. SmaI was used for PFGE analysis. Regions included Shandong (SD), Shanghai (SH), and Guangdong (GD). A minus sign indicates that the MDRGI type could not be assigned.

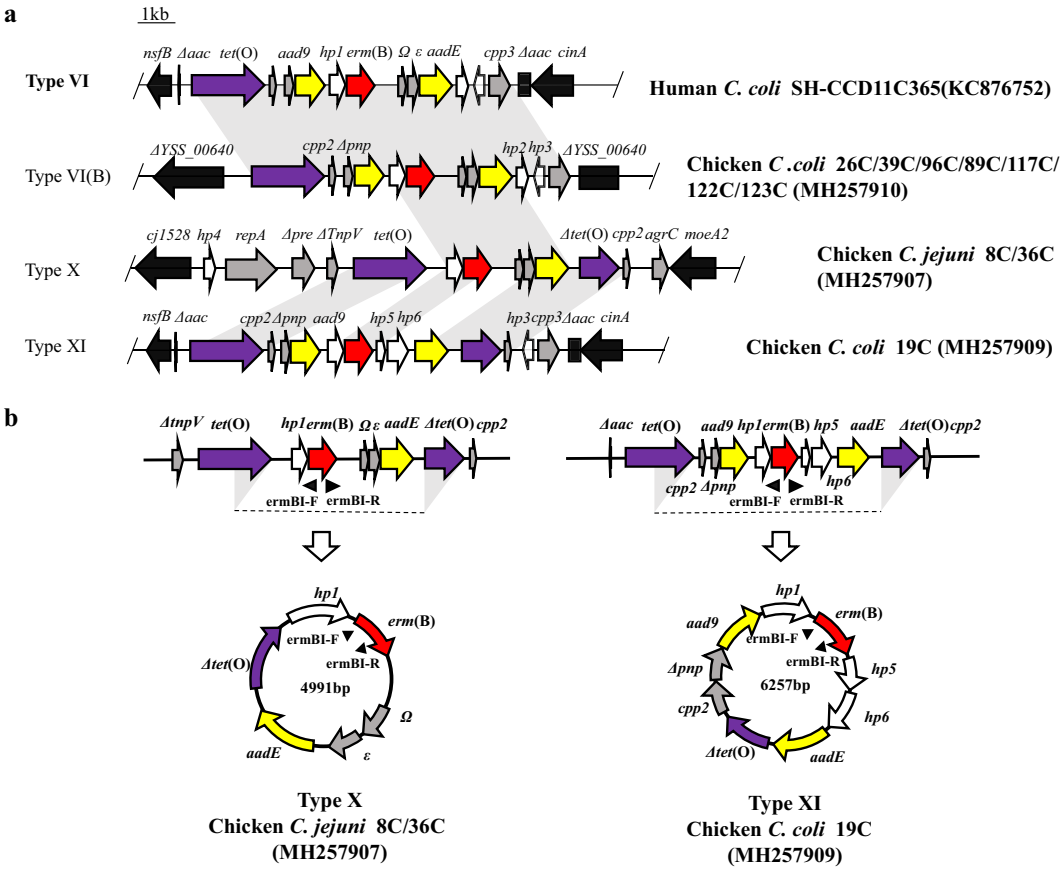


FIG 2 (a) Chromosomal organization and comparison of three novel MDRGI types in *erm*(B)-positive *C. coli* isolates. *erm*(B) is in red, aminoglycoside resistance genes are in yellow, tetracycline resistance gene *tet*(O) is in purple, genes with predicted functions are in gray, and genes coding for hypothetical proteins are in white. Genes flanking the MDRGIs are depicted by black arrows. Gray shading indicates regions with >98% nucleotide sequence identity. Representative strains for each type of MDRGI are indicated at the right. (b) Circular products. Circular products were detected by inverse PCR, while sequence analysis confirmed that the intermediates of both the type X and type XI MDRGIs contained the regions between the two triangular shadows for *tet*(O).

that *erm*(B) was associated with MDRGIs in the 11 *C. coli* isolates. In 8 of the isolates, we identified the genetic boundaries of the MDRGIs in the chromosome; however, we were unable to determine the complete genetic environment of the MDRGIs in the remaining 3 isolates. Two novel classes of MDRGIs were detected in the *C. coli* isolates. Interestingly, an 8,870-bp segment showing 100% nucleotide sequence identity to type VI MDRGIs was inserted into filamentous-hemagglutinin-encoding gene *YSS_00640* in 7 of the *C. coli* isolates. Thus, we designated this novel MDRGI type subtype VI(B). In *C. coli* isolate 19C, the *erm*(B) gene was located between *nfsB* and *cinA*, as is observed in MDRGI types V and VI. Therefore, the entire 10,541-bp segment in this isolate was designated MDRGI type XI. BLAST analysis revealed that the two MDRGIs also shared a common origin of similar nucleotide sequences with Gram-positive bacteria, implying that the two MDRGIs in *Campylobacter* were derived from Gram-positive bacteria, as described previously (15). Furthermore, the type VI(B) and type XI MDRGIs were flanked by identical conserved genes or had the same gene arrangement as type VI MDRGIs, the predominant type in human- and animal-derived *Campylobacter* isolates, hinting at the possibility that these MDRGIs are stably transferrable and highly adaptable.

Characterization of *erm*(B)-carrying *C. jejuni* isolates. To determine the genetic environment of *erm*(B) in *C. jejuni* isolates 8C and 36C, we sequenced and analyzed the genomes of the isolates using a hybrid PacBio (8C only) and Illumina (8C and 36C) sequencing approach. Sequence analysis indicated that the two isolates belonged to the same epidemic clonal lineage. In both isolates, *erm*(B) was located within an

TABLE 2 Types of *erm*(B)-harboring MDRGIs in *Campylobacter* isolates from all reported studies

MDRGI type	No. of isolates belonging to type [% of *erm*(B)-positive isolates]				
	This study, animal	Previous studies[a]		Total	
		Animal	Human	Animal	Human
Total[b]	74	153	28	227	28
I	0	2 (1.3)	0	2 (0.9)	0
II	0	2 (1.3)	0	2 (0.9)	0
III	23 (31.1)	20 (13.1)	2 (7.1)	43 (18.9)	2 (7.1)
IV	0	0	1 (3.6)	0	1 (3.6)
V	8 (10.8)	44 (28.8)	1 (3.6)	52 (22.9)	1 (3.6)
VI	30 (40.5)	30 (19.6)	9 (32.1)	60 (26.4)	9 (32.1)
VI(B)	7 (9.5)	0	0	7 (3.1)	0
VII	0	1 (0.7)	0	1 (0.4)	0
VIII	0	1 (0.7)	0	1 (0.4)	0
IX	0	0	1 (3.6)	0	1 (3.6)
X	2 (2.7)	0	0	2 (0.9)	0
XI	1 (1.4)	0	0	1 (0.4)	0
Unknown	3 (4.1)	44 (28.8)	4 (14.3)	47 (20.7)	4 (14.3)

[a]Data were collected from all reported studies of *erm*(B)-positive *Campylobacter* strains (14, 15, 17, 18, 22–25).
[b]Total number of *erm*(B)-positive isolates from different hosts.

11,766-bp MDRGI (type X) on the chromosome, which was inserted into the intergenic region between *cj1528* and *moeA2* (Fig. 2a). The MDRGIs had a G+C content of 38.9%, similar to those of other MDRGIs but higher than that of the genome of reference *C. jejuni* strain NTCT 11168 (G+C content of 30.5%). The MDRGI contained 10 complete open reading frames (ORFs) and 3 truncated ORFs, 3 of which corresponded to antibiotic resistance genes, including *tet*(O), *erm*(B), and *aadE*. Intriguingly, *repA*, which was located upstream of a complete *tet*(O) locus, shared a high degree of homology (60% coverage and 99% identity) with the corresponding gene in a type VII MDRGI from another *C. jejuni* isolate (23). It is noteworthy that, since the first report of *erm*(B) in *Campylobacter* (14), novel genetic environments have continuously been described. Analysis of all published studies of *erm*(B) in *Campylobacter* (14, 15, 17, 18, 22–25) shows that type VI and type III are the predominant MDRGI types in this genus, with both types consistently being represented in isolates from humans and animals. This finding suggests that these two MDRGI types are widely disseminated among *Campylobacter* isolates (Table 2).

Sequence analysis of *C. jejuni* isolates 8C and 36C showed that, along with an *erm*(B)-harboring MDRGI, both isolates contained an ARGI and the enhanced multidrug efflux pump-encoding locus RE-*cmeABC*. The ARGI showed 99% nucleotide sequence identity to the ARGI in *C. coli* strain HS11B (which was also isolated from Guangdong Province) and was flanked by *cj0073c* and *cj0072c* (GenBank accession number MH257908) in both isolates (Fig. S2) (26). The horizontally transferable enhanced multidrug efflux pump gene RE-*cmeABC*, which was first identified in 2016, mediates resistance to multiple classes of antibiotics, including fluoroquinolones, amphenicols, tetracyclines, and macrolides (19). Thus, *C. jejuni* isolates 8C and 36C contained a multitude of antimicrobial resistance genes, including RE-*cmeABC*, *erm*(B), *tet*(O), *aadE*, *cat*, *aphA3*, *aad9*, *aph2*, *aadA*, *aac*, and *aph(2″)-If*, and exhibited high-level resistance to all major antimicrobials, including fluoroquinolone, macrolide, tetracycline, and aminoglycoside drugs that are currently being used in the clinical treatment of *Campylobacter* infections (Table S1) (27). Therefore, these *Campylobacter* isolates could be considered "superbugs" and represent a significant threat to public health.

Mode of horizontal transfer of *erm*(B) in *Campylobacter*. To assess the transferability of the novel MDRGIs, natural transformation and reverse PCR were performed. Although we were unable to obtain transformants after repeated attempts, two circular intermediates were detected, i.e., a 4,991-bp circular intermediate in *C. jejuni* isolates 8C and 36C and a 6,257-bp intermediate in *C. coli* 19C (Fig. 2b). In all cases, the formation

of the circular intermediates occurred via the two *tet*(O) direct repeats (one complete gene and one truncated gene) located at the ends of the MDRGIs. The presence of the circular intermediate in *C. jejuni* 8C/36C and *C. coli* 19C indicates that *erm*(B) could change its location in the chromosome along with other resistance genes, such as *aadE*, *aad9*, and Δ*tet*(O). Upon integration of the circular intermediate into a plasmid, *erm*(B) could be disseminated via the plasmid and could be coselected by streptomycin and tetracycline. Additionally, because the circular intermediate is formed by recombination between the *tet*(O) genes, it is likely to be able to integrate into other plasmids or chromosomal regions containing homologous sequences. *tet*(O) is one of the most prevalent resistance genes in *Campylobacter*; therefore, *tet*(O) may serve as a key integration site for horizontal transfer of other resistance genes, as well as increasing the risk of *erm*(B) acquisition (28).

In summary, this study investigated the prevalence of *erm*(B) in *Campylobacter* isolates from pigs and chickens in China and then classified the *erm*(B)-carrying MDRGIs. The results confirmed that *erm*(B) is widely prevalent among *Campylobacter* strains in Guangdong Province, with an obvious trend of increasing prevalence over the past few years. PFGE analysis suggested that both clonal expansion and horizontal transmission were involved in the dissemination of *erm*(B) in *Campylobacter*, although clonal expansion accounted for a greater proportion of the dissemination. Examination of the genetic environment of the *erm*(B) genes showed that type VI and type III MDRGIs are the predominant types in isolates from both humans and animals, hinting at the possibility of wide dissemination of these MDRGIs among *Campylobacter* isolates. We also identified three novel *erm*(B)-carrying MDRGIs, two of which were found in *C. coli* isolates, while one was identified in a *C. jejuni* isolate. Significantly, this appears to be the first report of the coexistence of *erm*(B)-harboring MDRGIs, ARGIs, and RE-*cmeABC* in *C. jejuni* isolated from chickens. These isolates were resistant to all antimicrobial agents frequently used in clinical therapy of campylobacteriosis and thus pose a significant threat to public health.

MATERIALS AND METHODS

***Campylobacter* isolates and detection of *erm*(B).** In total, 290 *Campylobacter* isolates (74 *C. jejuni* isolates and 216 *C. coli* isolates) were isolated from 1,039 chicken cecal and swine fecal samples collected from Shanghai and from Guangdong and Shandong provinces in 2016. Detailed information on the isolates is provided in Table S1 in the supplemental material. All *Campylobacter* isolates were cultured on Mueller-Hinton agar (Oxoid, Hampshire, UK) at 42°C under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2). All isolates were subsequently screened for *erm*(B) by PCR, as described previously (15), and the resulting PCR products were sequenced. Data regarding *erm*(B)-positive isolates recorded in previous studies were also obtained, to allow composite analysis. Collected data included prevalence, PFGE profiling, and MDRGI clustering information (14, 15, 17, 18, 22–25).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing of *erm*(B)-carrying *Campylobacter* isolates was carried out using the agar dilution method, as described by the Clinical and Laboratory Standards Institute and the National Antimicrobial Resistance Monitoring System (29, 30). The *Campylobacter* isolates were tested to determine their resistance to six classes of antimicrobials, including macrolides, lincosamides, aminoglycosides, tetracycline, fluoroquinolone, and amphenicol. *C. jejuni* ATCC 33560 was used as the quality control strain.

Molecular typing. All *erm*(B)-positive *Campylobacter* isolates were genotyped by PFGE, which was conducted with a CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, CA, USA) according to the protocol for *Campylobacter* (31). Genomic DNA extracted from the *Campylobacter* isolates was digested with SmaI, while XbaI-digested genomic DNA from *Salmonella enterica* serotype Braenderup strain H9812 was used as the reference marker. Results were analyzed according to the Dice coefficient method, using InfoQuest FP version 4.5 (Bio-Rad Laboratories).

Identification of MDRGIs and whole-genome sequencing. Except for type IX (17), which may have been located within a plasmid, all classes of *erm*(B)-carrying MDRGIs in *Campylobacter* (types I to VIII) have been chromosomally located. PCR primers, the sequences of which are listed in Table S2, were designed to amplify all type I to VIII MDRGIs. Because of difficulties with obtaining complete sequences, *erm*(B) primers were used in conjunction with primers specific for each MDRGI type, to obtain two half segments. The resulting amplicons were visualized by electrophoresis and used to confirm the MDRGI type. However, this method did not allow precise discrimination of types IV, V, and VI. Therefore, specific reverse primers were designed for these three MDRGI types, which were used with the forward *erm*(B) primer (Table S2). The genomes of *erm*(B)-positive type X and type XI isolates that could not be assigned to any class of MDRGIs were then sequenced using the Illumina HiSeq 2500 system (Illumina, San Diego, CA, USA). CLC Genomics Workbench version 9 (CLC bio, Aarhus, Denmark) was used to *de novo* assemble the generated reads into contigs, and the regions flanking the *erm*(B)-carrying contigs were identified

using a primer walking strategy. To assess the genomic background of *erm*(B)-positive *C. jejuni* isolate 8C, a hybrid of both the PacBio and Illumina sequencing methods was used. The assembly statistics for the PacBio genome are shown in Table S3. Whole-fragment analysis was conducted using the RAST annotation server (http://rast.nmpdr.org) and BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Natural transformation was performed to assess the transferability in 10 isolates carrying the 3 novel MDRGIs, as described previously (14). Primer sequences for inverse PCR, which was performed to detect the existence of a circular intermediate, as described previously (32), are listed in Table S2.

Accession number(s). Whole-genome sequencing data that support the findings of this study have been deposited in the NCBI BioProject database under accession number PRJNA492384. The sequences of novel MDRGIs described in this paper have been deposited in the GenBank database under accession numbers MH257910, MH257907, and MH257909.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.00153-19.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.
SUPPLEMENTAL FILE 2, XLSX file, 0.01 MB.
SUPPLEMENTAL FILE 3, XLSX file, 0.01 MB.
SUPPLEMENTAL FILE 4, XLSX file, 0.01 MB.

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