

Mosaic Structure of a Multiple-Drug-Resistant, Conjugative Plasmid from *Campylobacter jejuni*

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Partial sequence analysis of a *tet(O)* plasmid from a multiple-drug-resistant clinical isolate of *Campylobacter jejuni* revealed 10 genes or pseudogenes encoding different aminoglycoside inactivating enzymes, transposase-like genes, and multiple unknown genes from a variety of pathogenic and commensal bacteria. The plasmid could be mobilized by a P incompatibility group plasmid into *Escherichia coli*, where it apparently integrated into the chromosome and expressed high-level resistance to multiple aminoglycoside antibiotics. This work provides new information about both the nature of drug resistance in *C. jejuni* and the ability of *C. jejuni* to exchange genes with other bacterial species.

Campylobacter jejuni is among the most frequent causes of diarrheal disease worldwide (2, 23), and the incidence is particularly high in Thailand, where both antimicrobial resistance and multidrug resistance are increasing problems (6). Although the levels of resistance to the macrolides erythromycin and azithromycin have remained low, the incidence of resistance to the quinolones has risen to 96% in a 1998 study of U.S. military personnel (22). Plasmid-mediated resistance to tetracycline, chloramphenicol, and kanamycin has been well documented in *C. jejuni* (25, 31, 29), but no other plasmid-encoded resistance genes have been reported, other than a very recent report of streptomycin and streptothricin resistance (12). Recently, comparison of the sequences of transferable *tet(O)* plasmids from a *C. jejuni* strain and a *C. coli* strain isolated on different continents more than 20 years apart revealed high conservation (4, 5). Surveys of clinical isolates of *C. jejuni* in Thailand in our laboratories have revealed a high incidence of both multiple drug resistance and plasmids (unpublished data). Given these observations, we hypothesized that at least some of these antibiotic resistances in recent Thai isolates were plasmid encoded. Here we describe the partial genetic structure of a multiple-drug-resistant plasmid from a Thai clinical isolate of *C. jejuni*. The plasmid is related to other *tet(O)* plasmids but is novel in that it also contains multiple aminoglycoside-inactivating enzymes and transposon-like sequences from a variety of bacteria that have not previously been reported in *C. jejuni*.

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MATERIALS AND METHODS

Bacterial strains and plasmids. *C. jejuni* strain CG8245 was isolated from a diarrhea case of a U.S. soldier deployed to Thailand during a military exercise in 1999. *Escherichia coli* DH5 α was used as the host for cloning experiments, and pBluescript (Stratagene, La Jolla, CA) was used as the cloning vector. For

conjugation experiments with another *C. jejuni* strain, a mutant of *C. jejuni* NCTC 11168 that had been insertionaly inactivated with a chloramphenicol resistance (*cat*) cassette (32) in the Cj1316c gene was used as the recipient (P. Guerry, unpublished data). For conjugation experiments with *E. coli*, *E. coli* C600(RK212.2) (Tet^r Amp^r) (9) and *E. coli* DH5 α (Nal^r) were used.

Bacterial growth conditions. Bacterial strains were maintained at -80°C in brucella broth (Difco) supplemented with 40% glycerol. *C. jejuni* was grown routinely on Mueller-Hinton (MH) agar under microaerobic conditions at 37°C , while *E. coli* was grown on LB agar or MacConkey agar under aerobic conditions at 37°C . Antibiotics were added when appropriate to the following concentrations: 50 μg of kanamycin per ml, 50 μg of spectinomycin per ml, 100 μg of hygromycin per ml, 20 μg of tetracycline per ml, 50 μg of gentamicin per ml, 25 μg of streptomycin per ml, 50 μg of nalidixic acid per ml, 20 μg of chloramphenicol per ml, 62.5 μg of ampicillin per ml.

DNA manipulations. Plasmid DNA from *C. jejuni* strain CG8245 was purified as previously described (3). Plasmid DNA from CG8245 was digested separately with BglII, BclI, and SacII (New England Biolabs, Beverly, Mass.) and cloned into pBluescript that had been digested with either BamHI or SacII. Transformants were selected by blue/white screening on LB supplemented with ampicillin and 1.2 mg/plate of 5-bromo-4-chloro-3-indolyl- β -D-galactopyranoside (X-Gal), kanamycin, or spectinomycin.

DNA sequence analysis. DNA sequencing was facilitated by transposition mutagenesis of the cloned fragments with a previously described (13) EZ::TN MOD transposon containing the *cat* resistance cassette from pRY109 (32). Additional sequencing was done by primer walking, and some contigs were closed using PCR with *Pfu* Ultra High-Fidelity DNA polymerase (Stratagene), followed by cloning into pPCR-Script (Stratagene).

DNA sequencing was done using Big Dye sequencing kits (Applied Biosystems, Foster City, Calif.) and analyzed on an Applied Biosystems 3100 sequencer. Open reading frames (ORFs) predicted to encode peptides of greater than 30 residues were evaluated based on the presence of a suitable initiation codon with appropriate spacing to the ribosome-binding site, as well as physical location with respect to other ORFs. Similarity searches to the predicted proteins were performed using a BLAST algorithm against all available public databases via the National Center for Biotechnology Information, Bethesda, MD.

Mutagenesis of drug resistance genes. Site-specific mutagenesis of each drug resistance gene was performed using clones generated from in vitro transposition as described above. The clones selected had the *cat* gene inserted in the same orientation as the target gene to minimize possible polarity. Selected plasmids were used to electroporate *C. jejuni* CG8245 (14) with selection on MH agar supplemented with chloramphenicol. The successful mutation of each drug resistance gene was confirmed to have occurred by a double-crossover event by PCR using primers flanking the insertion point.

MIC and antibiotic susceptibility testing. MICs were determined using the agar dilution technique as recommended by the National Committee for Clinical Laboratory Standards (20a). Semiquantitative in vitro susceptibility testing by the agar disk diffusion method was performed according to the manufacturer's instructions (BBL, Becton, Dickinson and Company, Sparks, MD).

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TABLE 1. MICs for CG8245, DH5 α and mutants

Substrate	Gene/plasmid ^a	MIC (μ g/ml) ^b for:				
		CG8245	CJ mutant	DH5 α	EC clone	EC mutant
Tetracycline	<i>tet(O)</i> /ORF5/pJW101	150	0.064	1	24	0.75
Streptomycin	<i>aadE</i> /ORF2/pJW101	96	0.094	0.38	12	1
	<i>aadE</i> /ORF23/pJW102	96	96	0.38	0.38	0.38
	<i>aadA</i> /ORF11/pJW102	96	96	0.38	0.38	0.38
Spectinomycin	<i>aad9</i> /ORF8/pJW101	$\geq 1,024$	2	2	$\geq 1,024$	2
	<i>aadA</i> /ORF11/pJW102	$\geq 1,024$	$\geq 1,024$	2	2	2
Kanamycin	<i>aac</i> /ORF12/pJW106	3,500	2,500	0.5	1,500	0.75
	<i>aphA-3</i> /ORF25/pJW107	3,500	700	0.5	1,500	0.5
	<i>aacA</i> / <i>aphD</i> /ORF13/pJW108	3,500	2,500	0.5	1,500	6
Gentamicin	<i>aph2</i> /ORF10/pJW102	400	400	0.047	0.125	0.125
	<i>aacA</i> / <i>aphD</i> /ORF13/pJW102	400	0.38	0.047	0.125	0.125
Streptothricin	<i>sat4</i> /ORF24/pJW102	25	≤ 1.56	≤ 1.56	150	≤ 1.56
Hygromycin	<i>hph</i> /ORF21/pJW102	$\geq 2,800$	1,400	50	$\geq 2,800$	50

^a The plasmid used for mutational analysis for each gene is indicated and shown schematically in Fig. 2, and the transposon insertion point for each mutant allele is shown in Table 3.

^b MICs were determined for wild-type *C. jejuni* CG8245 or a Cm^r insertional mutant of each gene (CJ mutant); MICs were determined for DH5 α , DH5 α carrying the indicated plasmid (EC clone), and the Cm^r insertional mutant of each plasmid gene (EC mutant) that was used to generate the *C. jejuni* mutant.

Conjugation experiments. Conjugation experiments between strains of *C. jejuni* were done using a modification (5) of the method of Taylor (24). The transconjugants were selected by incubation overnight at 37°C under microaerobic conditions on medium that contained chloramphenicol and tetracycline or chloramphenicol and kanamycin.

For conjugation experiments between *C. jejuni* and *E. coli* strains, *C. jejuni* CG8245 was grown overnight on MH plates, while two strains of *E. coli*, C600 and DH5 α , were grown overnight on LB plates. The donor and two recipient strains were resuspended in MH broth to an optical density at 600 nm of 1.0 and mixed in a ratio of 1:1:1 before spotting onto MH agar. The MH agar was incubated for 6 h at 37°C under microaerobic conditions. Bacterial growth was scraped from the plates and resuspended in 1 ml of MH broth. Aliquots of 100 μ l were plated onto MacConkey agar containing kanamycin, spectinomycin, hygromycin, gentamicin, or streptomycin. The cultures were incubated aerobically at 37°C overnight.

Nucleotide sequence accession number. The sequences of the genes described in this paper have been submitted to GenBank under accession number AY701528.

RESULTS

Antibiogram of CG8245. *C. jejuni* strain CG8245, which was serotyped as Lior 19, was resistant to three fluoroquinolones: nalidixic acid (30 μ g), ciprofloxacin (5 μ g), and ofloxacin (16 μ g). It was also resistant to ampicillin (10 μ g) and bactrim (25 μ g). Table 1 shows that CG8245 was also resistant to tetracycline and six aminoglycosides. The strain was sensitive to chloramphenicol (30 μ g), colistin (10 μ g), and azithromycin (15 μ g).

Cloning and sequence analysis of drug resistance genes from pCG8245. *C. jejuni* strain CG8245 contained a single plasmid that hybridized to both *tet(O)* and *aphA-3* probes in Western blot assays (data not shown). Based on these blots, a BglII fragment encoding resistance to kanamycin was cloned into pBluescript and called pJW102. The inserts in this clone and overlapping clones, shown in Fig. 1 and comprising 25.7 kb of contiguous DNA, were fully sequenced. The overall G+C content of this region of the plasmid was 33.4%, which was higher than the two sequenced strains of *C. jejuni* (30.6% and

30.3%, respectively) (11, 21). The G+C content of this region of pCG8245 was also higher than that of the pTet plasmid of strain 81-176 (29.1%) (4, 5) but similar to that of several cryptic *Campylobacter* plasmids (19).

This region of pCG8245 encoded 29 ORFs, as summarized in Table 2 and Fig. 2. Among the ORFs surrounding the *tet(O)* gene were 10 genes predicted to encode aminoglycoside modifying enzymes. These 10 genes are discussed in more detail below.

Mutational analyses of antibiotic markers. Transposon insertions into each of the putative drug resistance genes were generated during sequence analysis of the plasmids shown in Fig. 1, and the insertions used to generate mutants of *C. jejuni* by electroporation are summarized in Table 3. The MICs for the *C. jejuni* CG8245 wild-type strain and the site-specific mutation in each drug gene against its predicted substrate(s) are shown in Table 1. Table 1 also shows the MICs for DH5 α , DH5 α carrying different clones shown in Fig. 2, and the cor-

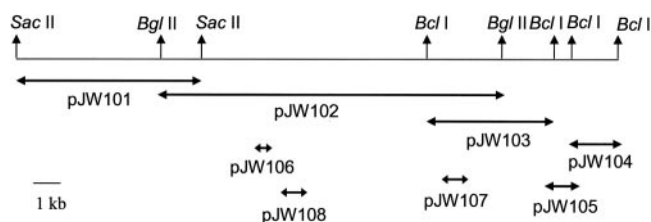


FIG. 1. Schematic of the 25.7-kb region of pCG8245 that was sequenced. DNA sequencing was done on the overlapping regions of DNA either cloned into pBluescript (pJW101, pJW102, pJW103, and pJW104) or PCR amplified and cloned into pPCR-Script (pJW105, pJW106, pJW107, and pJW108). Plasmids pJW106, pJW107, and pJW108 are PCR clones of three different kanamycin resistance genes that were generated for mutational analyses as described in the text.

TABLE 2. Annotation of genes found on the 25 kb sequenced region of pCG8245

ORF	Gene	Codon region (bp)	%G + C content	Length (aa) ^a	Length (aa) ^b	% Identity/% similarity (no. of aa)	Annotation/accession no.
1	Unknown	2–316	37.8	181	92	85/95 (92)	Unknown/ <i>Arcanobacterium pyogenes</i> /AAQ94626
2	<i>aadE</i>	492–1358	35.5	302	281	60/75 (281)	Streptomycin aminoglycoside 6-adenyltransferase/ <i>E. faecium</i> /NP_863159
3	<i>cpp50</i>	2599–2811	26.3	473	70	100/100 (70)	Unknown of pTet plasmid/ <i>C. jejuni</i> strain 81-176/YP_063493
4	<i>cpp51</i>	2830–3009	23.9	59	59	83/83 (59)	Unknown of pTet plasmid/ <i>C. jejuni</i> strain 81-176/YP_063494
5	<i>tet(O)</i>	3368–5287	40.3	639	639	99/99 (639)	Tet(O)/ <i>C. jejuni</i> /YP_063446
6	Unknown Tn916	5339–5512	36.7	62	57	66/82 (57)	ORF6 of Tn916/ <i>E. faecalis</i> /AAB60023
7	Unknown	5841–5990	37.3				Unknown (no significant match in database)
8	<i>aad9</i>	6015–6791	35.8	242	235	55/71 (235)	Spectinomycin adenylyltransferase/ <i>E. faecalis</i> /AAL05551
9	Unknown	6991–7536	37.7	181	180	80/88 (180)	Unknown/ <i>Arcanobacterium pyogenes</i> /AAQ94626
10	<i>aph2</i>	7652–8449	23.7	301	227	22/41 (227)	Aminoglycoside 3'-phosphotransferase, type III/ <i>E. casseliflavus</i> /AAC14693
11	<i>aadA</i>	8450–9244	25.6	261	249	33/57 (250)	Streptomycin 3'-adenylyltransferase/ <i>E. faecalis</i> V583/NP_814808
12	<i>aac</i>	9268–9696	25.1	130	127	50/75 (127)	Acetyltransferase GNAT family/ <i>E. faecalis</i> V583/NP-816983
13	<i>aacA/aphD</i>	9696–10589	23.8	479	281	79/88 (281)	Bifunctional aminoglycoside modifying enzyme/ <i>S. wameri</i> /CAD60199
14	Transposase	10829–11332c	25.2	431	125	40/56 (125)	Transposase IS232/ <i>B. thuringiensis</i> /Q99335
15	Transcriptional regulator	11399–11710c	44.9	117	91	48/72 (91)	Transcriptional regulator/ <i>C. acetobutylicum</i> ATCC 824/NP_349177
16	Unknown	11710–12111c	44.7	150	116	33/54 (118)	Conserved hypothetical/ <i>C. thermocellum</i> /ZP00311549
17	Transposon related	12233–12991c	30.2	250	248	58/76 (248)	IS232 putative ATP-binding protein/ <i>B. thuringiensis</i> /Q99338
18	Transposase	12954–13622c	28.8	431	179	40/53 (184)	Transposase IS232/ <i>B. thuringiensis</i> /Q99335
19	Unknown	13896–14933	39.8	204	143	42/63 (147)	Unknown/ <i>C. acetobutylicum</i> /NP-348122
20	Unknown	14960–15505	39.6	181	181	76/81 (181)	Unknown/ <i>Arcanobacterium pyogenes</i> /AAQ94626
21	<i>hph</i>	15565–16482	47.6	341	296	37/57 (303)	Hygromycin B phosphotransferase (<i>aph7</i>)/ <i>E. coli</i> /P00557
22	<i>pcp</i>	16487–16906	49.3	213	136	53/78 (136)	Pyrrrolidone carboxylate peptidase/ <i>E. faecalis</i> V583/NP_814190
23	<i>aadE</i>	17168–17683	39.5	302	163	100/100 (163)	Streptomycin aminoglycoside 6-adenyltransferase/ <i>E. faecium</i> /NP_863159
24	<i>sat4</i>	17692–18222	37.7	180	176	94/94 (176)	Streptothricin acetyltransferase/ <i>E. faecium</i> /AAM77897
25	<i>aphA-3</i>	18315–19109	44.9	264	264	100/100 (264)	Aminoglycoside 3'-phosphotransferase, type III/ <i>E. faecium</i> /CAD36021
26	<i>tmp</i>	19400–19873	29.3	132	127	65/79 (129)	Transposase/ <i>C. tetani</i> /NP_781296
27	<i>tmpB</i>	19860–21098	27.3	442	442	52/67 (442)	IS606 transposase/ <i>H. pylori</i> /NP_223544
28	pTet13a or <i>cpp14</i>	22645–24315	31.0	1,932	550	100/100 (550)	pTet13/ <i>C. jejuni</i> strain 81–176; homolog of <i>H. pylori</i> JHP0928/YP_063459
29	pTet13b or <i>cpp14</i>	24458–25711	28.0	1,932	417	99/99 (417)	pTet13/ <i>C. jejuni</i> strain 81–176; homolog of <i>H. pylori</i> JHP0928/YP_063459

^a Size of the ortholog protein. aa, amino acids.
^b Length of the pCG8245 peptide.

responding mutated allele of each gene in the respective clones.

There were three genes predicted to encode alleles of streptomycin-inactivating enzymes from *Enterococcus* species. Two

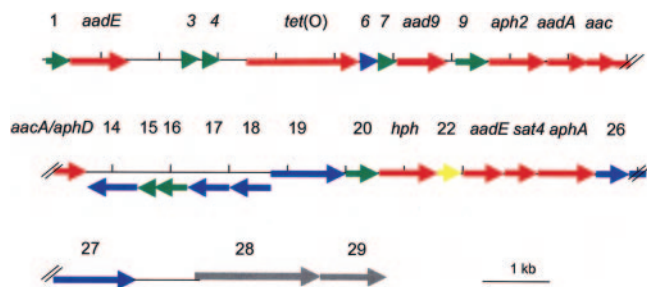


FIG. 2. Schematic of the coding regions within the sequenced 25.7-kb region of pCG8245. The ORFs are color coded based on the putative function of the predicted encoded proteins. Red, antibiotic resistance genes; blue, transposases and transposon associated; green, unknown proteins; gray, similar to genes from the plasticity zones of *H. pylori*; yellow, known but not associated with antibiotic resistance. Vertical hash marks, below the linear plasmid, are located every 1 kb.

genes, ORF2 and ORF23, encoded predicted proteins with similarity to AadE, a streptomycin 6-adenyltransferase, although the homology of the protein encoded by ORF23 appeared limited to the carboxy-terminal 163 amino acids (Table 2). This change apparently resulted in loss of function since ORF23 did not express resistance in either *E. coli* or *C. jejuni*.

TABLE 3. Insertion points of *cat* transposon cassette within antibiotic resistance genes

ORF	Gene	Coding region (bp)	Insertion point (bp)
2	<i>aadE</i>	492–1358	861
5	<i>tet(O)</i>	3368–5287	4101
8	<i>aad9</i>	6015–6791	6703
10	<i>aph2</i>	7652–8449	8258
11	<i>aadA</i>	8450–9244	8785
12	<i>aac</i>	9268–9696	9293
13	<i>aacA/aphD</i>	9696–10589	9977
21	<i>hph</i>	15565–16482	15760
23	<i>aadE</i>	17168–17683	17564
24	<i>sat4</i>	17692–18222	17743
25	<i>aphA-3</i>	18315–19109	18822

However, ORF2 functioned in both hosts. ORF11 encoded a predicted streptomycin/spectinomycin 3'-adenylyltransferase, whose closest homolog was AadA [or Ant(3'')-Ia] from *Enterococcus faecalis*, but the gene appeared not to function in either *E. coli* or *C. jejuni* (Table 1). ORF8, however, encoding an ortholog of Aad-9 or Ant(9)-I from *E. faecalis*, an enzyme that catalyzes ATP-dependent adenylation of the position 9 hydroxyl group of spectinomycin, functioned in both *E. coli* and *C. jejuni*.

There were three genes predicted to encode resistance to kanamycin, all of which functioned in both *E. coli* and *C. jejuni*. ORF12 encoded a protein related to an acetyltransferase enzyme GNAT (GCN5-related N-acetyltransferase) family member from *E. faecalis*. ORF25 encoded a type III aminoglycoside 3'-phosphotransferase or AphA-3, which has been reported on campylobacter plasmids previously (18). ORF13, encoded a homolog of a staphylococcal bifunctional enzyme with acetyltransferase activity at the amino-terminal domain and phosphotransferase activity at the carboxy-terminal domain. However, the homology of the ORF13 product was restricted to the carboxy-terminal region of this bifunctional enzyme, suggesting only phosphotransferase activity. ORF13 also conferred gentamicin resistance on *C. jejuni*, but not on *E. coli* (Table 1). Another gene, ORF10 or *aph2*, encoded an aminoglycoside 3'-phosphotransferase predicted to be active against gentamicin, but the gene did not function in either *E. coli* or *C. jejuni*.

ORF24 encoded a protein with 94% identity to a streptothricin acetyltransferase from *Enterococcus*. ORF21 encoded a protein with 37% identity and 57% similarity to a hygromycin B phosphotransferase (Aph^{7''}) from *E. coli*. This gene was recently found on another *tet*(O) plasmid during genomic sequencing of a *C. coli* strain (11). ORF24 and ORF21 conferred resistance to streptothricin and hygromycin, respectively, in both *E. coli* and *C. jejuni*.

Genes encoding transposases and insertion sequences. Several transposon-related sequences from a variety of bacteria were found on pCG8245. ORF26 encoded a predicted protein with 65% identity to a transposase from *Clostridium tetani* strain E88 (7). The protein also showed 41% identity to OrfA from the *Helicobacter pylori* transposon ISHp608 (16). ORF27 encoded a predicted protein whose best match was OrfB from IS606 from *H. pylori* strain J99 (52% identity) (15). ORF6 encoded a predicted protein with 66% identity to a protein of unknown function from the conjugative transposon Tn916 of *E. faecalis* (10). There were three ORFs related to an insertion sequence, IS232, from *Bacillus thuringiensis* (20). ORF14 and ORF18 have homology to the amino and carboxy regions of the IS232 transposase, respectively. ORF14 encoded a predicted protein with 40% identity to this transposase, but the homology is restricted to the amino end (amino acids 34 to 158 out of 431). ORF18 encoded a protein with 40% identity over 42% of the total length of the IS232 transposase (amino acids 220 to 398 out of 431). Thus, the N and C termini of the IS232 transposase are split and appear reversed, as if there had been a duplication of the IS232 gene, followed by a complex rearrangement. ORF17, which maps between ORF14 and ORF18, encoded a protein that showed 58% identity and 76% similarity to a putative ATP-binding protein from IS232 over 99% of the total length of the protein (amino acids 3 to 250 out of 250). Also mapping between the apparently truncated trans-

posase were two genes encoding proteins with homologs from other species of *Clostridium* (see below).

Other sequenced genes in this region of pCG8245. There were three copies of a gene encoding an unknown protein of 181 amino acids from *Arcanobacterium pyogenes* strain OX-7 (ORF1, -9, and -20). The three alleles encoded proteins that were 75 to 86% identical to each other. PCR was done using primers within these genes and adjacent genes to confirm the presence of these three copies at the indicated positions (data not shown). A homolog of the predicted proteins encoded by ORF1, -9, and -20 has recently been described in *C. coli* RM2228 (11).

ORF3 and ORF4 encoded predicted proteins that showed high levels of similarity to Cpp50 and Cpp51, respectively, from the pTet plasmid of *C. jejuni* 81-176 (5). ORF7 encoded a predicted protein with no significant match in the databases.

ORF15 encoded a predicted protein with a helix-turn-helix motif annotated as a transcriptional regulator in *C. acetobutylicum*. ORF16 encoded a conserved hypothetical protein found in numerous bacteria, with the best match being to a protein from another species of *Clostridium*, *C. thermocellum*.

At the end of the sequenced region are two genes encoding predicted proteins with high homology to the protein encoded by *cpp14* of the pTet plasmid of *C. jejuni* 81-176 (5). The protein encoded by *cpp14* is a large protein (1,932 amino acids) with 99% identity to a protein encoded by JHP0928, a putative methylase encoded in the plasticity zone of *Helicobacter pylori* strain J99 (1). ORF28 of pCG8245 encodes a protein with 100% identity to amino acids 1 to 550 of Cpp14; ORF29 of pCG8245 encodes a protein with 99% identity to amino acids 606 to 1022 of Cpp14. Thus, these two genes appear to represent a split of the larger *cpp14* ORF into two smaller ORFs, the latter of which encodes the putative methylase domain.

Conjugal transfer of pCG8245 to another strain of *C. jejuni*. Similar to previous reports on conjugation of campylobacter *tet*(O) plasmids, pCG8245 could be transferred conjugatively to *C. jejuni* NCTC 11168 Cm^r at a frequency of 4.2×10^{-5} /donor cell. The frequency was slightly lower (6.0×10^{-6} /donor cell) when the mating was done in the presence of DNase to eliminate natural transformation. All transconjugants tested, regardless of whether DNase was added to the mating plates or the selective medium used (chloramphenicol and kanamycin or chloramphenicol and tetracycline) also expressed resistance to hygromycin, spectinomycin, kanamycin, tetracycline, and chloramphenicol. The restriction pattern of plasmid DNA from selected transconjugants was identical to that of pCG8245 (data not shown).

Transfer of drug resistance markers from pCG8245 to *E. coli*. Attempts to directly transfer pCG8245 to *E. coli* DH5 α with selection on kanamycin were uniformly unsuccessful. However, a triple cross among *C. jejuni* CG8245, *E. coli* DH5 α (Lac⁻), and *E. coli* C600 (Lac⁺) containing P incompatibility plasmid RK212.2, encoding resistance to ampicillin and tetracycline (9), was done with selection on MacConkey agar supplemented with either streptomycin, kanamycin, spectinomycin, or hygromycin incubated aerobically. Both Lac⁺ and Lac⁻ *E. coli* transconjugants were obtained at frequencies of approximately 10^{-6} to 10^{-8} /donor cell. Regardless of the selection used, all transconjugants examined were phenotypically resistant to kanamycin, spectinomycin, hygromycin, spectinomycin,

and streptomycin. Attempts to isolate plasmid DNA from both Lac⁻ (DH5 α) and Lac⁺ (C600/RK212.2) transconjugants yielded plasmids that appeared identical to RK212.2 by restriction analysis (data not shown). However, PCR analysis with primers specific for the pCG8245-encoded genes *aadE*, *tet(O)*, *aad9*, *aph2*, *aadA*, *aac*, *aacA/aphD*, *hph*, *sat4*, and *aphA-3* indicated that all of these genes were present in both classes of transconjugants.

DISCUSSION

The plasmid from *C. jejuni* strain CG8245 appears related to pTet from *C. jejuni* strain 81-176 in that, in addition to *tet(O)*, it contains several homologs of other pTet genes in the region sequenced. However, it differs from any previously described *tet(O)* plasmid in that it contains 10 ORFs that encode copies of aminoglycoside-inactivating enzymes from both gram-negative and gram-positive sources. Three of these genes are not functional in either *C. jejuni* or *E. coli* (*aph2/ORF10*, *aadA/ORF11*, and *aadE/ORF23*).

There have been two other recent reports on *tet(O)* plasmids with additional antibiotic resistance markers, although neither was as extensive a repertoire as that found on pCG8245. Gibreel et al. (12) reported on *C. jejuni* plasmids containing the *aadE-sat4-aphA-3* gene cluster that was originally described in gram positives, a cluster similar to that present on pCG8245 as ORF23, -24, and -25. However, in the case of pCG8245, the *aadE* gene copy located adjacent to *sat4* is non-functional. A second, full-length, functional copy of *aadE*, ORF2, is located >15 kb upstream. The *aadE-sat4-aphA-3* gene cluster was originally described as part of a transposon structure, Tn5405 from staphylococci (8). The version of this gene cluster with an intact *aadE* gene reported for *C. jejuni* plasmids from Canada, Egypt, and Sweden was associated with IS607 from *H. pylori*. In the case of pCG8245, the *aadE-sat4-aphA-3* gene cluster mapped next to an apparent hybrid of two *H. pylori* transposons, ISHp608 and IS606 (16, 15). Genomic sequencing of a chicken isolate of *C. coli* has recently revealed a 110-kb plasmid encoding resistance to tetracycline, kanamycin, and hygromycin (11).

Conjugative plasmids of *C. jejuni* are generally considered unable to transfer to other genera (26–30). However, recently Gibreel et al. (12) were able to conjugatively transfer a plasmid encoding *tet(O)* and *aphA-3* from *C. jejuni* to *E. coli*, where the plasmid integrated into the chromosome. The plasmid from CG8245 could be transferred conjugatively to another strain of *C. jejuni*, but we were unable to detect direct transfer from *C. jejuni* to *E. coli*. We could, however, detect mobilization in a triple cross that included P incompatibility plasmid RK212.2 (9). It has been recognized for some time that P incompatibility plasmids can conjugatively transfer from *E. coli* into *C. jejuni* but cannot replicate vegetatively in *C. jejuni*, an observation that has been used in genetic studies to mobilize campylobacter shuttle vectors from *E. coli* into *C. jejuni* (17). Although the molecular details remain to be elucidated, it appears that RK212.2 was able to mobilize pCG8245 from *C. jejuni* into an *E. coli* recipient. It appears that most, if not all, of pCG8245 integrated into the *E. coli* chromosome, similar to the situation described by Gibreel et al. (12), rendering the *E. coli* recipient resistant to multiple aminoglycosides.

The partial DNA sequence of pCG8245 reported here is an eclectic collection of genes from a wide variety of bacterial genera. There are drug resistance genes that appear to have originated from *Enterococcus*, *Staphylococcus*, *Streptococcus*, and *E. coli*. There are homologs of transposons or insertion sequences from two species of *Clostridium*, *Bacillus thuringiensis*, and *H. pylori* and additional genes from *Enterococcus*, *Clostridium*, *Arcanobacterium pyogenes*, and the plasticity zone of *H. pylori*. This is particularly surprising given the fact that *C. jejuni* has not been reported to exchange genes outside of its own genus. Since aminoglycosides are not used clinically to treat *C. jejuni* infections, the high level of resistance in *C. jejuni* strain CG8245 and other Thai isolates is likely due to environmental exposure, perhaps the result of veterinary use. The data suggest that *C. jejuni* can serve as a reservoir of drug resistance that can be disseminated to *E. coli* and perhaps other bacteria.

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