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Chromosomal *tet*(O)-Harboring Regions in *Campylobacter coli* Isolates from Turkeys and Swine

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In turkey-derived *Campylobacter coli* isolates of a unique lineage (cluster II), the tetracycline resistance determinant *tet*(O) was chromosomal and was part of a gene cassette (transposon) interrupting a *Campylobacter jejuni*-associated putative citrate transporter gene. In contrast, the swine-derived *C. coli* strain 6461 harbored a chromosomal *tet*(O) in a different genomic location.

Campylobacter spp. are leading diarrhea-causing bacterial agents in humans. *Campylobacter jejuni* causes the majority (>85%) of campylobacteriosis cases, while the remainder are caused primarily by *C. coli* (6, 21). *Campylobacter* is a zoonotic agent that commonly colonizes poultry and other food animals, including swine, cattle, and sheep (12). *Campylobacter* spp. frequently display resistance to antibiotics, such as tetracycline and quinolones (1, 15). Tetracycline resistance is especially widespread and is encountered even in isolates from animals raised without antibiotics (7, 11, 14, 16, 20, 24).

The gene responsible for tetracycline resistance in *Campylobacter*, *tet*(O), has been most commonly reported on plasmids (8, 9, 15, 22, 23). Plasmids harboring *tet*(O) frequently harbor kanamycin resistance determinants as well (22). However, there is also evidence of *tet*(O) being harbored on the chromosome. For instance, an estimated 33% of tetracycline-resistant *C. jejuni* isolates from Canada lacked plasmids (9). The prevalence of chromosomally borne *tet*(O) was even higher (76%) among isolates from Australia (18). Such chromosomal determinants, however, have not yet been characterized.

Tetracycline resistance is highly prevalent among *C. coli* and *C.*

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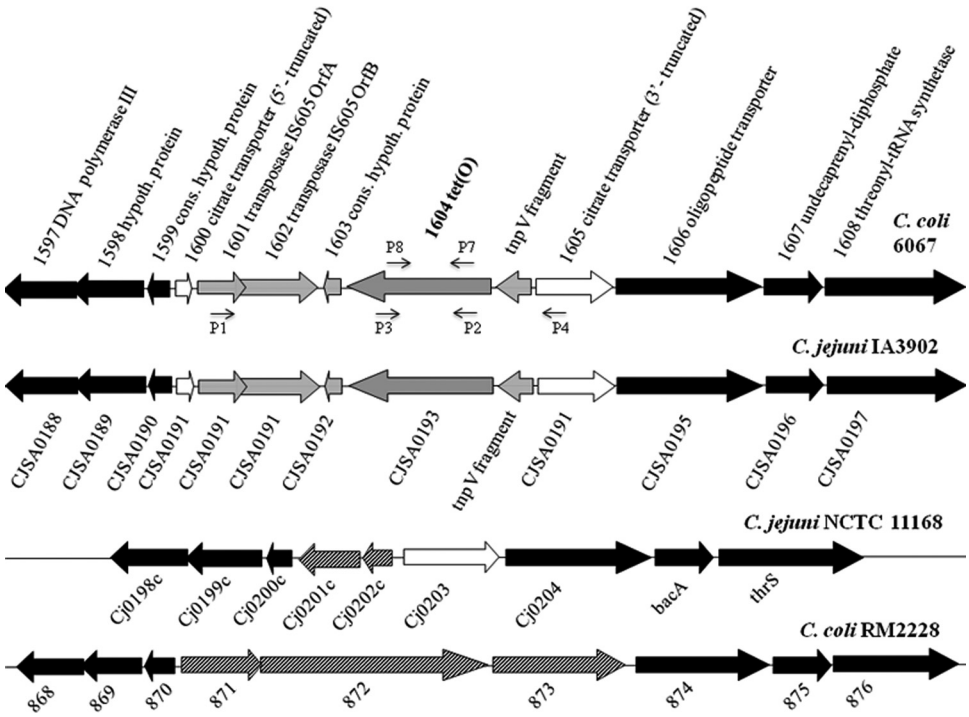

FIG 1 Organization of the *tet*(O)-harboring region in *C. coli* 6067 and *C. jejuni* IA3902 and genomically equivalent regions in other *Campylobacter* genomes. Arrow orientations indicate the putative directions of transcription. ORFs flanking the *tet*(O) cassette and conserved (cons.) among the genomes are in black. ORFs composing the cassette are in gray, while the putative citrate transporter ORF harboring the *tet*(O) cassette is in white. ORFs unique to specific genomes are striped. P1, P2, P4, P5, P7, and P8 are primers, which are also listed in Table 2. hypoth., hypothetical.

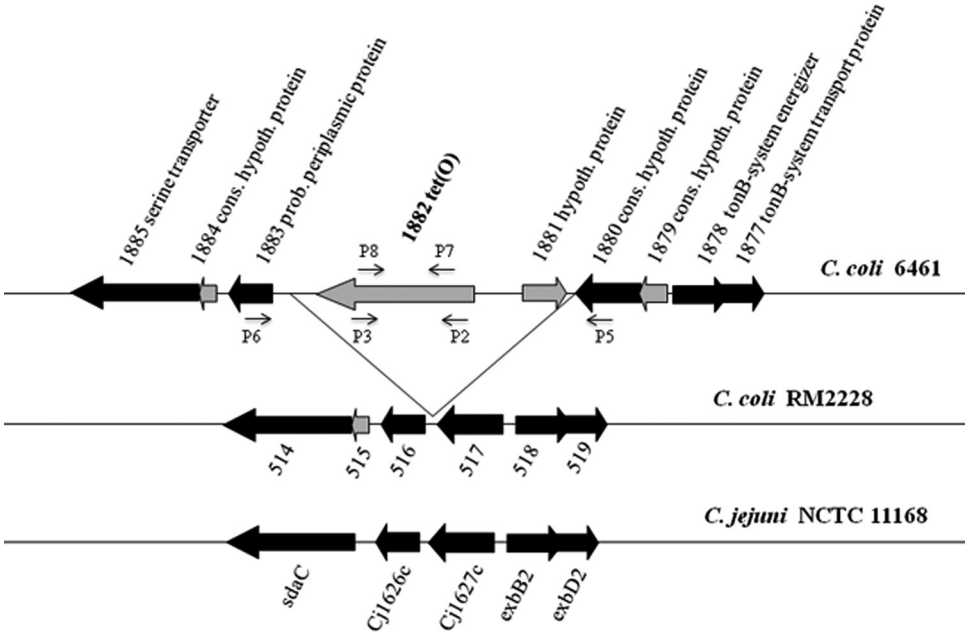

FIG 2 Genomic organization of the *tet*(O)-harboring-region in *C. coli* 6461 and homologous regions in other *Campylobacter* genomes. Arrow orientations indicate putative directions of transcription. ORFs flanking the *tet*(O) cassette and conserved among the genomes are in black. *C. coli*-specific ORFs in the flanking regions are in gray. P2 to P8 are primers, which are also listed in Table 2.

jejuni isolates from turkeys and swine in North Carolina and other regions (7, 10, 16). In turkey-derived *C. jejuni* isolates, resistance to kanamycin was typically accompanied by tetracycline resistance, suggesting the presence of plasmids harboring tetracycline and kanamycin resistance determinants (10).

Data on possible chromosomal carriage of *tet*(O) among turkey- or swine-derived *Campylobacter* spp. have not been available. Chromosomal carriage of resistance determinants has important implications, including higher stability compared with plasmids and potential to be disseminated via transformation. The latter is especially relevant for *Campylobacter*, which is known to be naturally competent (25).

In this study, we describe chromosomal regions harboring *tet*(O) in two *C. coli* strains, *C. coli* 6067 and *C. coli* 6461. *C. coli* 6067 (ST-1150) was obtained from drinking water available to turkeys inside a turkey house, while strain 6461 (ST-854) was isolated from a swine fecal sample. Animals were grown conventionally in eastern North Carolina; the farms and bacterial isolations were previously described (27). *C. coli* 6067 is a member of cluster II, a lineage primarily associated with turkeys and highly distinct from other animal-derived *C. coli* strains (17). *C. coli* cluster II strains constitute more than 40% of turkey-derived *C. coli* isolates in eastern North Carolina (26; R. M. Siletzky and S. Kathariou, unpublished findings) and exhibit several unique attributes: they harbor a *C. jejuni*-like *aspA* allele, lack intervening sequences in 23S rRNA genes, and are invariably susceptible to erythromycin (4, 5, 17).

In *C. coli* 6067 (cluster II), *tet*(O) is part of a putative IS*605* element inserted in a *C. jejuni*-associated citrate transporter gene. The complete genome sequence of *C. coli* 6067 was determined at the Genome Core Facility, Duke University, Durham, NC. Sequence annotation using the updated GAMOLA suite (2) and subsequent comparative analyses using the Functional Genome Distribution algorithm (3) revealed a chromosomal *tet*(O) gene with >99% identity at the nucleotide sequence level to other *Campylobacter tet*(O) sequences in GenBank.

Analysis of the *tet*(O)-harboring region of *C. coli* 6067 revealed that the gene was part of a chromosomal gene cassette (transposon) that carries, in addition to *tet*(O), putative IS*605* transposases ORF A and ORF B and the putative TnpV gene (Fig. 1). The transposon has interrupted a putative citrate transporter gene, the 5′ portion of which (167 nucleotides [nt]) is designated ORF 1600, with the remaining 3′ portion of the coding sequence designated ORF 1605 (Fig. 1). Sequence analysis suggests that the transposon has become inserted in the putative citrate transporter gene recently: there is no evidence of sequence degeneration in the insertionally disrupted gene, with ORFs 1600 and 1605 exhibiting 96 and 98% identity, respectively, at the nucleotide sequence level to the corresponding fragments of ORF Cj0203 of *C. jejuni* NCTC 11168. The interrupted putative citrate transporter was flanked on both sides by genes conserved among other sequenced *Campylobacter* genomes (e.g., *C. jejuni* IA3902, *C. jejuni* NCTC 11168, *C. jejuni* RM1221, and *C. coli* RM2228) (Fig. 1).

Comparative genomic analysis of this region and nucleotide BLAST searches revealed that the putative citrate transporter gene was highly associated with *C. jejuni*; it was absent from sequenced *C. coli* genomes, with the sole exception of *C. coli* 317/04, a human clinical isolate (Fig. 1) (data not shown). In fact, this gene has been identified as one of the *C. jejuni* core genes that are dispensable in *C. coli* (13).

Surprisingly, the genomic organization of the region containing *tet*(O) in *C. coli* 6067 was identical to that of *C. jejuni* IA3902, a tetracycline-resistant strain representing a clonal group implicated in sheep abortions in the United States (19) (Fig. 1). The cassette harboring *tet*(O) was highly conserved (99% identity at the nucleotide sequence level) between *C. coli* 6067 and *C. jejuni* IA3902. However, in other *C. jejuni* and *C. coli* strains with completely sequenced genomes (e.g., *C. jejuni* NCTC 11168, *C. jejuni*

TABLE 1 *C. coli* strains employed in this study

Strain	Source[a]	Date (mo/yr)	Antibiotic susceptibility profile[b]	ST[c]
6067	Water, turkey house	11/2003	TSQ	1150
6077	Turkey	12/2003	TSQ	1161
6100	Turkey	12/2003	T	1161
6252	Turkey	03/2004	TKQ	1487
6690	Turkey	05/2004	T	1833
6890	Turkey	06/2004	TQ	1161
6979	Turkey	06/2004	TKS	1150
7725	Turkey	08/2004	T	1150
8023	Turkey	08/2004	TQ	1192
8901	Turkey	10/2004	TQ	1192
WP145	Swine	12/2002	T	ND
5973	Swine	10/2003	TK	1142
5974	Swine	10/2003	TSE	1151
5979	Swine	10/2003	TSK	1153
5997	Swine	11/2003	TSEK	1246
6008	Swine	11/2003	TSEK	1246
6022	Swine	11/2003	TEK	1142
6029	Swine	11/2003	TSE	1151
6084	Swine	12/2003	TE	829
6087	Swine	12/2003	TEK	828
6093	Swine	12/2003	TEK	1157
6094	Swine	12/2003	TE	1157
6123	Swine	11/2003	TEK	1164
6461	Swine	04/2004	TSE	854

[a] With the exception of *C. coli* 6067 (isolated from water in the turkey house), bacteria were obtained as described previously (27) from fecal samples from the indicated animal source.
[b] Antimicrobial susceptibility profiles were determined as described previously (10) for a panel of antibiotics, including tetracycline (T), streptomycin (S), erythromycin (E), kanamycin (K), and (fluoro)quinolones (nalidixic acid and ciprofloxacin [Q]).
[c] Sequence types (STs) were determined by multilocus sequence typing as described previously (17). ND, not determined.

RM1221, and *C. coli* RM2228), this region lacked *tet*(O) and instead harbored unrelated ORFs (Fig. 1). In *C. jejuni* NCTC 11168 and *C. jejuni* RM1221, these included the gene encoding the putative citrate transporter (also harbored by *C. coli* 6067 and *C. jejuni* IA3902, as discussed above), a putative integral membrane protein, and a hypothetical protein. In *C. coli* RM2228, ORFs 871 to 873 encoded two putative alpha-2 macroglobulin family proteins and a putative penicillin binding protein 1C (Fig. 1).

Nucleotide BLAST analysis revealed that in *C. jejuni* and *C. coli*, the sequences in the regions genomically equivalent to the *tet*(O) region of *C. coli* 6067 were associated with the respective species; for instance, ORFs 871 to 873 of *C. coli* RM2228 were harbored by diverse *C. coli* strains but not by any of the *C. jejuni* genomes in GenBank (data not shown). Similarly, ORFs Cj0201c and Cj0202c in *C. jejuni* NCTC 11168 were harbored by other *C. jejuni* strains but were absent from *C. coli* (with the exception of *C. coli* 317/04, as also described above for the putative citrate transporter gene Cj0203) (Fig. 1) (data not shown).

Swine-derived *C. coli* 6461 harbors a *tet*(O) cassette in a different chromosomal location. In the swine-derived *C. coli* strain 6461, a chromosomal *tet*(O) was identified in a different location from that in *C. coli* 6067 and was accompanied by an additional ORF (ORF 1881) encoding a hypothetical protein. The sequences flanking *tet*(O) and ORF 1881 in *C. coli* 6461 were generally highly conserved among other genomes of *C. coli* and *C. jejuni* (Fig. 2). Nucleotide BLAST analysis of ORF 1881 revealed a homologous ORF (100% identity) in the tetracycline resistance plasmid pTet of a *C. jejuni* strain implicated in a Guillain-Barré syndrome outbreak (28). In this plasmid, the ORF 1881 homolog was also adjacent to *tet*(O), suggesting the possibility that the *tet*(O) cassette in *C. coli* 6461 may have resulted from the chromosomal integration of a plasmid.

TABLE 2 Primers employed in the study[a]

Primer (alternative name)	Sequence (5′→3′)
6067_1601F (P1)	GCTAGACTTTATGGCTCACG
6461_1882F (P2)	ATGGAGGGGGTTCTTTATGG
6461_1882R (P3)	ATGCCATCCTTTGCAGAAAC
6067_1605R (P4)	GTATCCGTTACGCTTTTGAC
6461_1880F (P5)	GCTTGAGGTTTGTGATGCAA
6461_1883R (P6)	CACCCAATAAAGCCGCTAAA
Tet(O) F (P7)	CAAAGGGGAATCACTATCC
Tet(O) R (P8)	AACCTGCCCGCATAGTTC

[a] Primer locations are indicated in Fig. 1 and 2.

Conservation of chromosomal *tet*(O) cassette in cluster II *C. coli*. As mentioned above, *C. coli* 6067 belongs to the distinct clonal group cluster II, which is primarily associated with turkeys (17). To determine whether the chromosomal *tet*(O) genomic organization observed in this strain was conserved in other cluster II strains, we analyzed a panel of strains (Table 1) using PCR with primers derived from *tet*(O) and the sequences flanking the *tet*(O) cassette in *C. coli* 6067 (Table 2). The results showed that chromosomal *tet*(O) found in *C. coli* 6067 was indeed harbored in the same region by all other tested cluster II *C. coli* strains (Fig. 3). Two strains (6100 and 6252) produced weak amplicons with both sets of flanking primers, while their *tet*(O) amplicon was of normal intensity (Fig. 3). This was reproducibly observed and may reflect diversity in the primer sequences of these strains.

PCR using primers P6 and P2, as well as P3 and P5 (Table 2 and Fig. 2), was also employed to determine whether the chromosomal *tet*(O) found in the swine-derived *C. coli* strain 6461 was harbored in the same region in other tetracycline-resistant *C. coli*

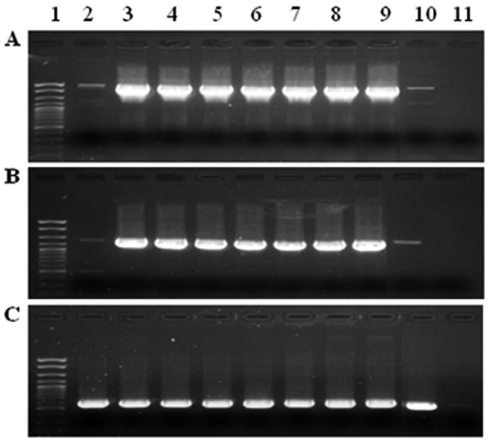

FIG 3 Conservation of the genomic location of the *tet*(O) cassette among cluster II *C. coli* strains. The PCR employed primers derived from the *tet*(O) cassette of *C. coli* 6067. (A) PCR using primers for region downstream of *tet*(O) (P1 and P2); (B) PCR using primers for the region upstream of *tet*(O) (primers P3 and P4); (C) PCR using *tet*(O) primers (internal primers P7 and P8). Lane 1, molecular weight markers (exACTGene cloning DNA ladder; Fisher Scientific International, Inc.); lanes 2 to 10, cluster II *C. coli* strains 6100, 6077, 6690, 6890, 6979, 7725, 8023, 8901, and 6252, respectively; lane 11, negative control (no DNA).

strains from swine (Table 1). None of the 13 strains tested yielded evidence of a *tet*(O) cassette in the same region as *C. coli* 6461 (data not shown).

In conclusion, our findings suggest that a chromosomal transposable unit (IS*605*) harboring *tet*(O) has become inserted in a *C. jejuni*-associated citrate transporter gene and has been acquired by cluster II *C. coli*, a unique lineage commonly associated with turkeys, and possibly by additional *C. coli* strains. Remarkable conservation was noted in the sequence content, genomic location, and organization of this *tet*(O) cassette in cluster II *C. coli* strains and in a *C. jejuni* strain (IA3902) responsible for outbreaks of sheep abortions (19). One may speculate that the chromosomal cassette became transferred, likely via transformation, between strains such as *C. jejuni* IA3902 and the cluster II *C. coli* strains. Further studies are needed to characterize the distribution and potential impact on fitness (including the animal colonization potential) of this cassette, as well as the chromosomal *tet*(O) cassette identified in *C. coli* 6461.

Nucleotide sequence accession numbers. The nucleotide sequences of the *tet*(O) regions in *C. coli* 6067 and 6461 have been submitted to GenBank under accession no. JQ613155 and JQ613156, respectively.

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