

Differences in Methylation at GATC Sites in Genomic DNA of *Campylobacter coli* from Turkeys and Swine[∇]

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A significant fraction (46/108, 43%) of swine isolates of *Campylobacter coli* but none of 81 isolates of *C. coli* from turkeys had genomic DNA that was resistant to digestion by MboI, suggesting methylation of adenines at GATC sites. No consistent association was noted between antimicrobial resistance and MboI resistance. Seven swine-associated multilocus sequence typing-based sequence types (STs) were detected among multiple isolates with MboI-resistant DNA. The data suggest host-associated DNA modification system(s) specific for adenine at GATC sites in *C. coli* from swine.

Campylobacter coli commonly colonizes swine but has also been isolated from turkeys (7, 12–15, 23, 26, 29, 31). Applications of several DNA-based typing schemes have revealed that genotypes of *C. coli* isolates from swine are largely different than those from other meat animals (7, 13, 19). Characterization of antimicrobial susceptibility profiles in *C. coli* isolates derived from different animals has shown noticeable differences in the prevalence of resistance to several antibiotics (23). In addition, we have found that *C. coli* isolates from turkeys were more prone to acquire resistance to erythromycin via natural transformation than their counterparts from swine (8). Such findings suggest that *C. coli* isolates from different meat animals harbor genetic attributes that confer on them distinct phenotypes and host-associated adaptations. Other host-associated phenotypes, however, remain unidentified. To date, only one *C. coli* strain (RM2228, a multidrug-resistant strain from a chicken) has had its genome completely sequenced and described in detail (5), and limited information is available on genome-wide comparisons of *C. coli* isolates from different animal hosts (11).

DNA modification at specific sites is a common feature of bacterial genomes and is frequently associated with restriction-modification systems (30). A type of DNA modification that has been extensively identified in various bacterial genomes involves methylation of either adenines or cytosines at GATC sites, rendering the genomic DNA of the organism resistant to enzymes such as MboI and Sau3AI, respectively (18, 22). Cytosine methylation at GATC sites is frequently encountered in bacteria, and in several species it has been found to be characteristic of specific clonal groups (e.g., see references 2, 24, 28, and 32). On the other hand, methyltransferases specific for

adenine at GATC sites are encountered not only as part of type II restriction-modification systems but also as solitary enzymes that are evolutionarily related to the Dam methylase of *Escherichia coli* (16, 20). In *E. coli* and other bacteria, such solitary adenine DNA methyltransferases with specificity for GATC sites have key functions in DNA mismatch repair mechanisms and in a variety of regulatory processes (3, 17, 21).

Surprisingly limited information is available on the presence of either adenine or cytosine modification at GATC sites in *Campylobacter* spp. A survey of 12 *Campylobacter* strains identified three (one each of *C. jejuni*, *C. coli*, and *C. upsaliensis*) that harbored methylated adenines at GATC sites; none of the isolates harbored methylated cytosines at these sites (4). However, only two strains of *C. coli* were included in the survey. In this study, we investigated the prevalence of adenine or cytosine methylation at GATC sites among *C. coli* isolates from turkeys ($n = 81$) and swine ($n = 108$). The isolates were derived from several different conventional turkey and swine farms in eastern North Carolina at various time points between 2003 and 2005 (31).

DNA from all 189 isolates was susceptible to Sau3AI, suggesting the absence of cytosine methylation at GATC sites. Furthermore, DNA from all 81 turkey isolates was readily digestible with MboI (data not shown), suggesting the absence of adenine methylation at the GATC sites of these turkey-derived *C. coli* isolates. In contrast, 46 of the 108 (43%) swine-derived isolates had DNA that was resistant to digestion by MboI (Fig. 1), suggesting adenine methylation at GATC sites. DNA from 18 randomly chosen isolates with MboI-resistant DNA was digested with DpnI, which is specific for GATC sites at which the adenine is methylated (10). The DNA of all 18 isolates was fully susceptible to DpnI digestion (data not shown), confirming that the adenines at GATC sites were methylated.

Multilocus sequence typing (MLST) of swine-derived *C. coli* isolates (47 and 60 with DNA that was MboI resistant and MboI susceptible, respectively) identified 42 sequence types (STs), 20 of which were encountered in one or more of the isolates with MboI-resistant DNA. Most MboI-resistant strains tended to be closely related, being placed in common branches

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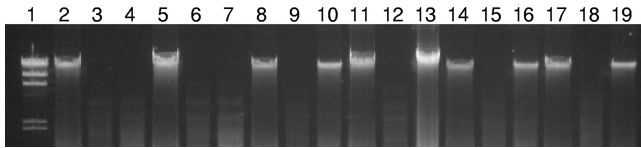


FIG. 1. MboI resistance among swine-derived *C. coli*. Genomic DNA was extracted with a Qiagen DNeasy kit and digested with Sau3AI and MboI. Lanes (after lane 1, the sets of three lanes for the isolates contain DNA that is undigested, digested with Sau3AI, and digested with MboI, respectively): 1, molecular size markers (Roche, Indianapolis, IN); 2 to 4, DNA of the turkey-derived isolate 6017; 5 to 7, DNA of the turkey-derived isolate 6067; 8 to 10, DNA of the swine-derived isolate 6458; 11 to 13, DNA of the swine-derived isolate 6461; 14 to 16, DNA of the swine-derived isolate 6550; 17 to 19, DNA of the swine-derived isolate 7995.

TABLE 1. STs encountered in swine-derived *C. coli* isolates with MboI-resistant DNA

ST	No. of isolates	No. of isolates with MboI-resistant DNA
828	2	1
854	13	2
887	2	2
1096	11	11
1109	11	10
1143	3	3
1246	2	2
1417	4	4
4605	2	1
Others ^a	11	11

^a STs found only in one MboI-resistant isolate were 890, 1055, 1112, 1186, 1829, 1830, 1831, 2869, 4606, 4607, and 4608.

in a minimum spanning tree depicting putative relationships among the STs (Fig. 2). Seven STs (854, 887, 1096, 1109, 1143, 1246, and 1417) were identified among multiple isolates with MboI-resistant DNA (Table 1). All 11 isolates of ST-1096 and most (10/11) isolates of ST-1109 were MboI resistant. In contrast, only two of the 13 isolates of ST-854 were resistant to MboI (Table 1). Several prevalent STs were also detected for which none of the isolates had MboI-resistant DNA (e.g., all six isolates of ST-829, all eight of ST-1151, and all six of ST-1157) (Fig. 2).

No consistent trends in antimicrobial resistance were detected between MboI-resistant and MboI-susceptible swine-derived isolates. With the exception of resistance to erythromycin, no significant differences were noted in resistance to the other antibiotics (tetracycline, streptomycin, kanamycin, and ciprofloxacin) (Fig. 3). The antimicrobial susceptibility profiles observed most frequently among both MboI-resistant and MboI-susceptible isolates were TSE (resistance to tetracycline,

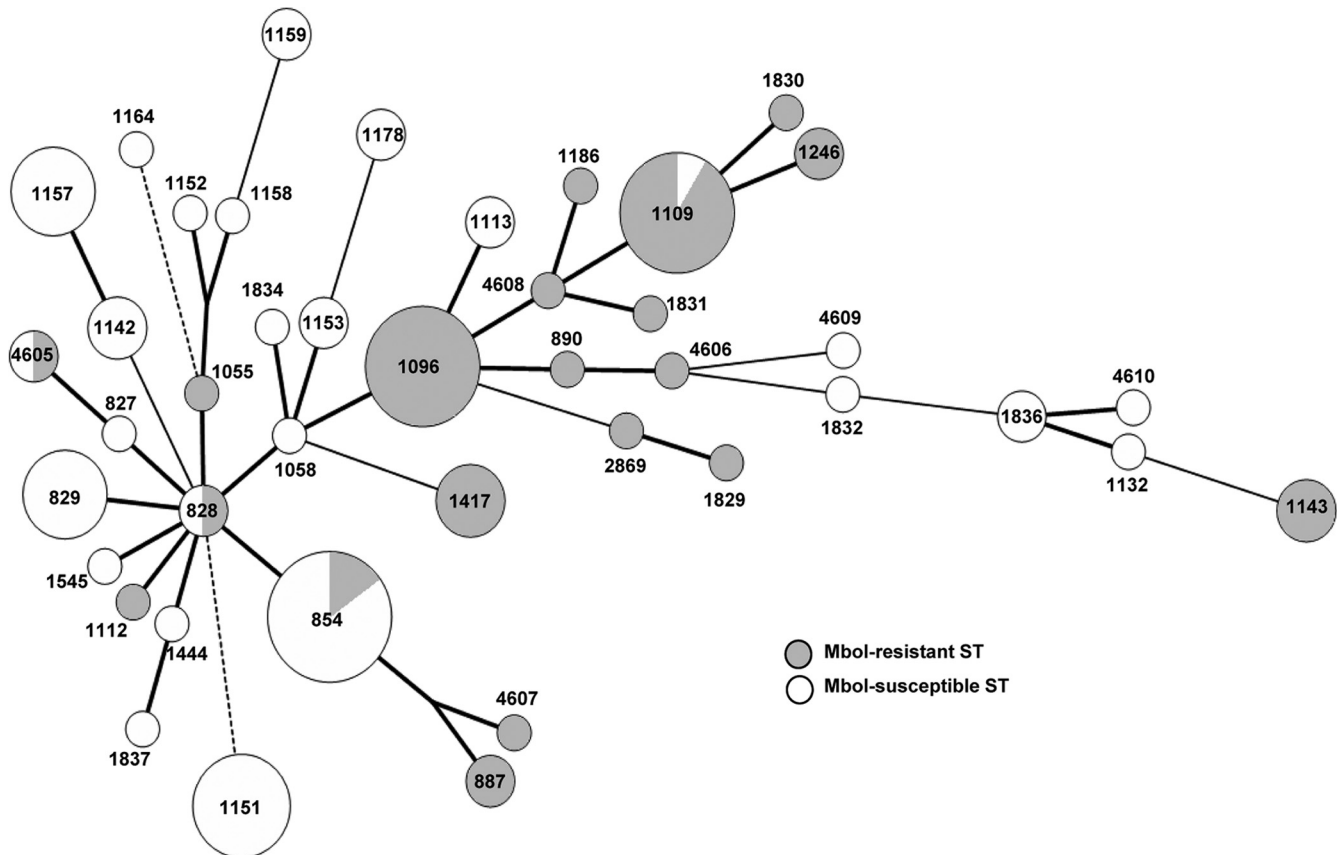


FIG. 2. STs of swine-derived *C. coli*. Thick bold lines indicate one locus difference, thin solid lines indicate 2 locus differences, and dashed lines indicate 3 or more locus differences. The size of the circle indicates the number of isolates with that ST, with the smallest circles indicating unique STs. STs were determined as described previously (19). ST-4605 to ST-4610 were new STs.

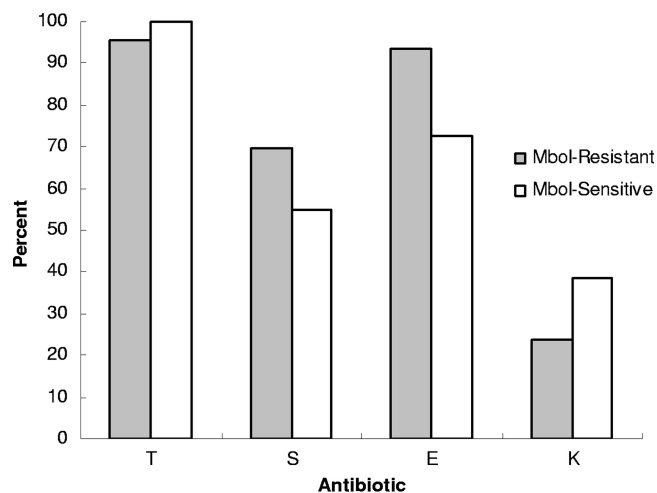


FIG. 3. Antibiotic resistance prevalence among MboI-resistant and MboI-sensitive swine-derived *Campylobacter coli* isolates. Erythromycin resistance prevalence was higher among MboI-resistant than among MboI-susceptible isolates ($P < 0.05$); differences in prevalence in resistance to the other antibiotics were not significant. T, S, E, and K indicate resistance to tetracycline, streptomycin, erythromycin, and kanamycin, respectively. None of the isolates were resistant to ciprofloxacin (data not shown). Resistance thresholds were as described previously (15).

streptomycin, and erythromycin) and TE (resistance to tetracycline and erythromycin) (Table 2), but the TSE profile was more prevalent among MboI-resistant isolates (20/46, 43%) than among MboI-susceptible ones (16/62, 26%) ($P = 0.05931$).

The DNA methylation system detected here is the first to show host animal-associated differences in prevalence in *Campylobacter* species. Several STs of isolates with this methylation (e.g., ST-854 and ST-1096) appear to be prevalent among *C. coli* isolates from swine, having been detected among several isolates in this study and others (9, 13, 19, 25, 27). These findings suggest that the DNA methylation described here may be relatively common among *C. coli* isolates from

TABLE 2. Prevalence of antimicrobial resistance profiles in MboI-resistant versus MboI-susceptible *C. coli* isolates from swine

AMR profile ^a	No. (%) of isolates with:	
	MboI-resistant DNA (n = 46)	MboI-susceptible DNA (n = 62)
Pan sensitive	0 (0)	0 (0)
T	0 (0)	4 (6)
SE	2 (4)	0 (0)
TE	12 (26)	13 (21)
TK	1 (2)	2 (3)
TS	1 (2)	5 (8)
TEK	1 (2)	9 (15)
TSE	20 (43)	16 (26)
TSK	1 (2)	6 (10)
TSEK	8 (17)	7 (11)

^a AMR, antimicrobial resistance; T, tetracycline; S, streptomycin; E, erythromycin; K, kanamycin. "AMR profile" refers to resistance phenotypes. Thus, "T" indicates isolates with resistance only to tetracycline, "TE" indicates isolates with resistance to both tetracycline and erythromycin (but susceptible to the other antibiotics in the panel), etc. "Pan sensitive" indicates isolates that were susceptible to all antibiotics in the panel.

swine but rare or absent among *C. coli* isolates from turkeys, although characterization of DNA methylation in *C. coli* isolates from other animal hosts is needed. The detection of isolates with a common ST but different DNA resistance to MboI suggests that the gene responsible for the methylation may be transferred among closely related isolates via horizontal gene transfer. Alternatively, the gene may have been inactivated or lost in those with MboI-susceptible DNA.

Further studies are needed to identify and characterize the gene mediating the genomic DNA methylation and to determine whether such a gene is accompanied by a cognate restriction endonuclease gene. The responsible gene (or restriction modification system) may have the potential to affect DNA transfer via natural transformation, as has been described in other systems (1, 6). Such involvement, along with possible roles in host adaptation and other adaptations, will need to be investigated, ideally through the use of isogenic mutants.

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