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Natural Transformation-Mediated Transfer of Erythromycin Resistance in *Campylobacter coli* Strains from Turkeys and Swine

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Erythromycin resistance in *Campylobacter coli* from meat animals is frequently encountered and could represent a substantial barrier to antibiotic treatment of human infections. Erythromycin resistance in this organism has been associated with a point mutation (A2075G) in the 23S rRNA gene. However, the mechanisms responsible for possible dissemination of erythromycin resistance in *C. coli* remain poorly understood. In this study, we investigated transformation-mediated acquisition of erythromycin resistance by genotypically diverse *C. coli* strains from turkeys and swine, with total genomic DNA from erythromycin-resistant *C. coli* of either turkey or swine origin used as a donor. Overall, transformation to erythromycin resistance was significantly more frequent in *C. coli* strains from turkeys than in swine-derived strains ($P < 0.01$). The frequency of transformation to erythromycin resistance was 10^{-5} to 10^{-6} for turkey-derived strains but 10^{-7} or less for *C. coli* from swine. Transformants harbored the point mutation A2075G in the 23S rRNA gene, as did the erythromycin-resistant strains used as DNA donors. Erythromycin resistance was stable in transformants following serial transfers in the absence of the antibiotic, and most transformants had high MICs (>256 μg/ml), as did the *C. coli* donor strains. In contrast to the results obtained with transformation, spontaneous mutants had relatively low erythromycin MICs (32 to 64 μg/ml) and lacked the A2075G mutation in the 23S rRNA gene. These findings suggest that natural transformation has the potential to contribute to the dissemination of high-level resistance to erythromycin among *C. coli* strains colonizing meat animals.

Campylobacter strains are the most common diarrhea-causing bacterial agents in humans in the United States and other industrialized nations (14, 21). Most (80 to 90%) human infections involve *Campylobacter jejuni*, with *C. coli* accounting for the majority of the remainder (16). *Campylobacter* is a zoonotic agent and commonly colonizes poultry and other meat animals, including swine, cattle, and sheep (12). Currently, the acquisition of antibiotic resistance by *C. jejuni* and *C. coli* is of great public health concern. Although most *Campylobacter* infections in humans are self-limited and do not require antibiotic treatment, severe infections and predisposing factors may necessitate antimicrobial treatment (21). The efficiency of fluoroquinolones has been compromised by increasing trends toward fluoroquinolone resistance among human isolates (7, 17). Currently, erythromycin and other macrolides (e.g., azithromycin) are the leading choices for the treatment of severe *Campylobacter* infections (16).

An issue of special concern is *C. coli*'s propensity to acquire resistance to macrolides, including erythromycin. In contrast to *C. jejuni*, which largely remains sensitive to erythromycin, *C. coli* has frequently been found to be resistant to erythromycin and other macrolides (1, 2, 4, 5, 6, 8, 20). *C. coli* appears to have a predilection for swine (1, 20, 24), but recent data indicate that *C. coli* also commonly colonizes commercial turkeys in eastern North Carolina, a region which is a major contributor to turkey production in the United States (15, 22). Erythromycin and other macrolides (e.g., tylosin) are extensively used therapeutically and as growth promoters in animal agriculture, possibly creating selection pressure for resistance to these antibiotics in *C. coli* (7), even though the reasons for the significantly greater prevalence of erythromycin resistance in *C. coli* than in *C. jejuni* remain unclear. A point mutation, A2075G, in the 23S rRNA gene (position 2075 in the 23S rRNA gene of *C. jejuni* NCTC 11168, corresponding to position 2059 in the 23S rRNA gene of *Escherichia coli*) is associated with erythromycin resistance in *Campylobacter* (9, 10, 13, 23).

C. jejuni and *C. coli* are well-known for the ability to acquire exogenous DNA by natural transformation (25, 27), with transformation frequencies for chromosomal DNA markers (nalidixic acid and streptomycin resistance) being as high as 10^{-3} per recipient cell for *C. coli* (25). Chromosomal and plasmid-borne competence genes have been identified in *C. jejuni* (3, 26). However, the role of transformation in the dissemination of resistance to erythromycin and other macrolides in *C. coli* has not been described. Overall, limited data exist on transformation in *Campylobacter* strains isolated from meat animals, and most investigations have involved *C. jejuni*. In this study, we investigated the transformation-mediated transfer of erythromycin resistance in *C. coli* from farm animals (turkeys and swine) in vitro.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The *C. coli* strains used for this study are listed in Table 1. These strains are part of our laboratory's *Campylobacter* strain collection and were obtained from turkeys and swine at different farms in eastern North Carolina, South Carolina, and Virginia between 2001 and 2004. *Campylobacter* strains were isolated from turkey fecal or cecal samples and from swine fecal samples, using direct plating on charcoal cefoperazone deoxycholate agar (Oxoid, Basingstoke, Hampshire, England) at 42°C under microaerobic

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TABLE 1. Transformation indices for transformation-mediated acquisition of erythromycin resistance in *C. coli* strains isolated from turkeys and swine

Source of recipient	Recipient strain[a]	Transformation index for donor strain[c]			Average transformation index[d]
		1705	1800r	2901	
Turkey	961 (TSNKA)	1.0	1.0	1.0	1.0
Turkey	3325 (TSNA)	0.6	1.0	0.4	0.7
Turkey	1536 (TNA)	1.0	1.0	0.9	1.0
Turkey	931g (TNA)	1.0	1.0	1.0	1.0
Turkey	3237 (TKA)	1.0	1.0	1.0	1.0
Turkey	1651 (TKA)	1.0	0.5	1.0	0.9
Turkey	1702rnd (TSA)	0.8	0.9	0.8	0.9
Turkey	1787 (TNKA)	0.9	0.8	1.0	0.9
Turkey	44nec (SN)	1.0	0.8	1.0	0.9
Turkey	6034 (TNA)	1.0	1.0	0.8	0.9
Turkey	37-2nec (A)	0.9	0.5	1.0	0.9
Turkey	7474 (A)	0.7	0.6	0.3	0.5
Turkey	7580 (A)	0.0	0.2	0.3	0.2
Swine	614-3m (TSKA)	1.0	1.0	1.0	1.0
Swine	426 (K)	1.0	0.9	1.0	0.9
Swine	4963 (T)	0.8	1.0	0.8	0.9
Swine	2113 (TA)	0.9	0.8	0.8	0.8
Swine	3175 (TKA)	1.0	0.8	0.8	0.7
Swine	P5 (TKA)	0.8	0.3	0.7	0.6
Swine	WP66 (TSK)	0.8	0.4	0.7	0.7
Swine	5980 (S)	0.0	0.0	0.0	0.0
Swine	WP126 (TA)	0.0	0.0	0.0	0.0
Swine	1684 (TA)	0.0	0.0	0.0	0.0
Swine	WP14[b]	0.0	0.1	0.1	0.1
Swine	WP19[b]	0.0	0.1	0.0	0.1
Swine	WP145 (TS)	0.0	0.0	0.0	0.0

[a] Antibiotics to which the recipients are resistant are listed as follows: T, tetracycline; S, streptomycin; N, nalidixic acid; K, kanamycin; A, ampicillin.
[b] Susceptible to all antibiotics tested.
[c] Strain 1705 (TESNKA) was of turkey origin, and strains 1800r (TESNKA) and 2901 (TEA) were of swine origin. Additional donors included strain 1686 (TEKA), derived from swine, and strains 1420 (TESNKA), 1702 (TESNKA), 2562 (TESNKA), and 2774 (TESNKA), derived from turkeys.
[d] Averages of transformation indices were calculated based on results obtained using donors 1705, 1800r, and 2901 and any additional donor strains.

conditions, as described previously (22). Species determinations, antibiotic susceptibility profiles, and strain fingerprinting by *flaA* typing and pulsed-field gel electrophoresis with the enzymes SmaI and KpnI were done as described previously (15, 22). Bacteria were routinely grown on Mueller-Hinton agar (MHA; Mueller-Hinton broth [MHB] with 1.2% agar) (Becton Dickinson, Sparks, MD) for 20 to 24 h at 42°C under microaerobic conditions generated by the CampyPak microaerophilic system (BBL, Sparks, MD) and then preserved in brain heart infusion broth (Becton Dickinson) supplemented with glycerol (20%) at −70°C, as described previously (22).

Erythromycin MIC determination. Erythromycin MICs were determined following the guidelines of CLSI (formerly NCCLS). Growth was tested at erythromycin concentrations up to 256 μg/ml and monitored following 48 h of microaerobic incubation at 42°C. The threshold for resistance was a MIC of 8 μg/ml, as described previously (9). High-level resistance corresponded to growth of the bacteria in the presence of 128 μg/ml or 256 μg/ml erythromycin.

Transformation assay with Mueller-Hinton agar. Based on their antibiotic resistance profiles, erythromycin-resistant and erythromycin-sensitive strains were selected as donors and recipients, respectively, for transformation assays. All recipients were tested with a panel of three donor strains (1705, 1800r, and 2901) (Table 1). In addition, some recipients were also tested with genomic DNAs from additional donor strains, including swine-derived strain 1686 and turkey-derived strains 1420, 1702, 2562, and 2774. Genomic DNAs of erythromycin-resistant donors were extracted using a QIAGEN DNeasy tissue kit (QIAGEN Inc., Valencia, CA) as described previously (22). Loopfuls of the erythromycin-sensitive recipient (from 20- to 24-h-old cultures on MHA, grown microaerobically at 42°C) were spotted onto MHA plates in triplicate, and then 4 μl genomic DNA of the donor strain was added to each spot and mixed, with the diameter of each spot after mixing being ca. 0.5 cm. The plates were incubated overnight (15 to 17 h) at 42°C under microaerobic conditions, and all of the material from each spot was spread plated on a separate 12-cm-diameter MHA plate containing erythromycin (10 μg/ml) (EMHA). Each transformation included spots of the recipient without donor DNA on the same MHA plate as a negative control. The EMHA plates were incubated for 48 h at 42°C under microaerobic conditions and examined for growth of *Campylobacter*. A quantitative estimate of the extent of growth on these plates, termed the transformation index, was made as follows: scores of 1.0, 0.5, and 0 were given to transformations yielding growth on >50% of the EMHA plate surface (ca. 100 colonies or more), growth on <50% of the surface (<100 colonies), and no detectable growth, respectively. The scores were calculated based on the arithmetic means from two independent experiments, each done in triplicate.

Broth transformation and determination of transformation frequency. Broth transformations followed a previously described protocol (27), with modifications. Briefly, recipient strains were grown on sheep blood agar plates (Remel, Lenexa, KS) at 42°C for 48 h under microaerobic conditions. A single colony was transferred to 5 ml of MHB and incubated at 42°C for 24 h under microaerobic conditions. This culture (0.1 ml) was added to 50 ml MHB preconditioned at 42°C and incubated for 7 h at 42°C under microaerobic conditions to reach exponential phase. For each transformation, 1.0 ml of this culture was transferred to a sterile polypropylene round-bottomed tube (14 ml) (Becton Dickinson, Franklin Lakes, NJ), and 15 μl of total genomic DNA (ca. 3 μg) from the donor was added. Negative controls were processed identically, except that no genomic DNA was added. After 5 h of incubation at 42°C under microaerobic conditions, 100 μl was spread plated on EMHA in triplicate, the plates were incubated microaerobically for 36 to 48 h at 42°C, and colonies were enumerated. Dilutions (10^{-4} and 10^{-6}) at the end of the 5-h transformation period were also plated on MHA and incubated microaerobically for 24 to 36 h at 42°C in order to determine the CFU/ml of the recipient. The transformation frequency was determined as the ratio of the number of transformants/ml to the total CFU/ml of the recipient.

Isolation of spontaneous mutants and determination of mutation frequency. On rare occasions, one or two colonies grew on the negative control plates during the transformation assay. These colonies, presumed to correspond to spontaneous erythromycin-resistant mutants, were subcultured onto EMHA and then stored at −70°C. To further assess the spontaneous mutation frequency for resistance to erythromycin, selected erythromycin-sensitive strains were subcultured on sheep blood agar at 42°C under microaerobic conditions, and single colonies were transferred to MHB (10 ml) and incubated for 40 to 44 h under the same conditions. Aliquots (1 ml) were transferred to three sterile microcentrifuge tubes (ISC BioExpress, Kaysville, Utah), centrifuged at 5,400 × *g* for 10 min, resuspended in 100 μl of MHB, and spread plated on MHA containing 8 μg/ml or 10 μg/ml erythromycin. The plates were incubated microaerobically for 48 h at 42°C, and colonies were enumerated. The cultures were also serially diluted, plated on MHA, and incubated for 24 to 36 h to enumerate total CFU. The mutation frequency (ratio of erythromycin-resistant CFU/ml to total CFU/ml) was determined based on two independent experiments for each strain.

Sequence analysis of 23S rRNA gene. A 470-bp internal fragment of the 23S rRNA gene was amplified using the forward primer F2-campy-23S (5′-AATTG ATGGGGTTAGCATTAGC-3′) and the reverse primer R1-campy-23S (5′-AA CGATTTCCAACCGTTCTG-3′) (23). The PCR cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 34 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 5 min. PCR products were electrophoresed (3% agarose), the band corresponding to the amplified product was excised, and the DNA was purified using a QIAquick gel extraction kit (QIAGEN). The purified products were sequenced (Davis Sequencing, Davis, CA), and the sequences were analyzed by using the online multiple sequence alignment program ClustalW (www.ebi.ac.uk/clustalw/).

Stability of erythromycin resistance in transformants. Transformants 961-1705em and 1651-1705em were subcultured daily in 5 ml MHB (1:10,000 dilution) for 14 days in the absence of erythromycin. The cultures were diluted and plated on both MHA and EMHA on days 7 and 14, and the CFU on MHA and EMHA were compared.

Statistical analysis. The impact of the hosts (turkeys versus swine) of the recipient and donor strains on transformation was analyzed with split-plot analysis of variance in glm, using SAS software (SAS Institute Inc., Cary, NC). Transformation frequency data were arc sine transformed for normal distribution.

RESULTS

Transformation-mediated acquisition of erythromycin resistance in *C. coli* strains of turkey and swine origin. A total of

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26 *C. coli* strains of meat animal origin (13 each from swine and turkeys) were tested for transformation to erythromycin resistance with the agar transformation assay, using total genomic DNAs from at least three different erythromycin-resistant *C. coli* strains (derived from turkeys and swine) as donors (Table 1). The recipient organisms were chosen to include erythromycin-sensitive organisms with different genomic fingerprints, as determined by *flaA* typing and by pulsed-field gel electrophoresis with SmaI and KpnI (data not shown). In addition, the recipients differed in terms of their overall antibiotic susceptibility profiles (Table 1).

The majority (19/26) of the strains frequently yielded erythromycin-resistant transformants on the selective medium (EMHA) (Table 1). Negative controls, which lacked donor DNA, yielded either very few (one or two colonies) or, most commonly, no colonies at all on EMHA (data not shown). Bacteria from turkeys were more likely overall to become transformed to erythromycin resistance than those from swine (92.3% versus 53.8%; $P < 0.01$). Of the seven strains which transformed poorly or not at all (transformation indices of <0.16), six were from swine, and only one (strain 7580) was of turkey origin. The transformation index was not significantly influenced by the origin (swine versus turkey) of the donor DNA ($P > 0.05$), since the efficiency of DNA from the turkey-derived strain 1705 for transforming recipients to erythromycin resistance was similar to those of DNA from the swine-derived strains 1800r and 2901 (Table 1). The use of additional turkey- and swine-derived erythromycin-resistant strains as sources of donor DNA in transformations of a subset of the recipients also failed to reveal a significant impact of the source of the donors (data not shown).

Enumerations of transformed cells and accurate determinations of transformation frequency utilizing the agar assay were hampered by difficulty in obtaining discrete colonies on EMHA plates, possibly due to clumping of the recipient cells during the transformation period on the agar plates and to the highly mucoid colony morphology of the *C. coli* strains. A liquid (MHB)-based transformation assay was therefore employed to accurately determine transformation frequencies. A subset of nine strains (four from turkeys and five from swine) were studied with the MHB assay, using the same panel of donor DNAs employed with the agar assay. Transformation frequencies of turkey-derived organisms were found to be in the range of 10^{-5} to 10^{-6}, whereas they were typically 10^{-7} or lower for strains from swine (Table 2). The transformation frequencies of three of the swine-derived strains (P5, 2113, and 5980) were below the detection limit, with no transformants identified in any of the transformations using these strains as recipients, even though two of these strains (P5 and 2113) had average transformation indices of 0.6 and 0.8, respectively, in the agar assay (Table 1). Similar to the findings with the agar assay, the transformation frequency was not significantly influenced by the source of the donor DNA (Table 2).

Erythromycin-resistant transformants can themselves serve as donors of erythromycin resistance in subsequent transformations. An erythromycin-resistant transformant of strain 3237, designated 3237-1705em (derived from transformation of strain 3237 by DNA from strain 1705), was found to be as effective in transforming the erythromycin-sensitive strain 7474 as DNA from strain 1705 (Table 2). The ability of transformants to serve as donors of erythromycin resistance in transformations of sensitive recipients, including the original parental strain, was also confirmed with the agar transformation assay (data not shown).

TABLE 2. Frequencies of transformation to erythromycin resistance for *C. coli* strains from turkeys and swine

Recipient strain (source)	Donor strain[a]	Transformation frequency
961 (turkey)	1705	7.7×10^{-5}
	1800r	4.2×10^{-5}
	2901	5.0×10^{-5}
3237 (turkey)	1705	1.5×10^{-5}
	1800r	2.7×10^{-6}
	2901	9.2×10^{-6}
3325 (turkey)	1705	8.1×10^{-6}
	1800r	8.5×10^{-6}
	2901	8.4×10^{-6}
7474 (turkey)	1705	2.3×10^{-6}
	3237-1705em[b]	2.8×10^{-6}
	1800r	9.5×10^{-7}
	2901	1.4×10^{-6}
614-3m (swine)	1705	1.4×10^{-7}
	1800r	4.9×10^{-8}
	2901	2.1×10^{-7}
426 (swine)	1705	1.3×10^{-8}
	1800r	$<1.3 \times 10^{-8}$
	2901	1.3×10^{-8}
P5 (swine)	1705	$<3.6 \times 10^{-9}$
	1800r	$<3.6 \times 10^{-9}$
	2901	$<3.6 \times 10^{-9}$
2113 (swine)	1705	$<2.8 \times 10^{-9}$
	1800r	$<2.8 \times 10^{-9}$
	2901	$<2.8 \times 10^{-9}$
5980 (swine)	1705	$<3.2 \times 10^{-8}$
	1800r	$<3.2 \times 10^{-8}$
	2901	$<3.2 \times 10^{-8}$

[a] Donor strains were of turkey (1705) or swine (1800r and 2901) origin, as described in Table 1.
[b] Erythromycin-resistant transformant derived from strain 3237 transformed with chromosomal DNA from strain 1705.

Frequency of spontaneous mutations to erythromycin resistance in *C. coli*. Spontaneous erythromycin-resistant mutants could only rarely be detected in the negative control plates of the transformation experiments. The frequency of spontaneous mutation to erythromycin resistance was determined for seven turkey-derived strains (961, 3325, 1536, 931g, 3237, 1787, and 44nec) and two strains from swine (426 and 614-3m). Spontaneous mutants could be isolated from only three strains (3237, 1536, and 44nec) of the nine that were tested, with frequencies generally lower than 10^{-8}.

High-level resistance to erythromycin was acquired by transformation, in contrast to relatively low levels of resistance in spontaneous mutants. All tested erythromycin-sensitive strains, including those used as recipients in transformation, had uniformly low erythromycin MICs (<4 μg/ml), in contrast to erythromycin-resistant field strains, including those used as sources of donor DNA in transformations, all of which had high erythromycin MICs (>256 μg/ml) (Table 3). Erythromycin MICs were also determined for 34 transformants derived from different recipient/donor combinations. Most (30/34) had similarly high levels of resistance (MICs, >256 μg/ml) (Table 3). Only four transformants (three derived from recip-

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TABLE 3. Distribution of erythromycin MICs for *C. coli* field isolates, erythromycin-resistant transformants, and spontaneous mutants

C. coli isolates (*n*)	No. of isolates with indicated MIC (μg/ml)				
	<4	32	64	128	>256
Erythromycin-sensitive field isolates (15)	15				
Erythromycin-resistant field isolates (16)					16
Erythromycin-resistant transformants (34)		1		3	30
Erythromycin-resistant spontaneous mutants (3)		1	2		

ient 426 and one from WP19) had lower erythromycin MICs (32 to 128 μg/ml). In contrast to the overall high MICs of field strains and transformants, spontaneous erythromycin-resistant mutants had relatively low MICs (32 to 64 μg/ml) (Table 3).

Analysis of 23S rRNA gene sequences reveals A2075G transition in transformants. The nucleotide sequence of a PCR-amplified internal fragment (470 bp) of the 23S rRNA gene that harbors position 2075 was determined for a panel of 30 strains, including 8 erythromycin-sensitive recipients, 8 erythromycin-resistant strains used as sources of DNA in transformations, 11 transformants from different recipient/donor combinations, and 3 spontaneous mutants. A portion of the nucleotide sequences is shown in Table 4. The A2075G transition was detected in all eight erythromycin-resistant strains, whereas all eight erythromycin-sensitive strains harbored adenine at position 2075. No other differences in nucleotide sequence were detected between erythromycin-resistant and -sensitive strains in the 470-bp region (Table 4 and data not shown).

The A2075G transition was detected in 10 of 11 tested transformants. Two transformants, 1536-1705em and 1702rnd-1702em, were found to have both A and G upon visual exam-

TABLE 4. Erythromycin MICs and sequences of an internal fragment of the 23S rRNA gene for *C. coli* field strains, erythromycin-resistant transformants, and spontaneous mutants

Strain[b]	MIC (μg/ml)	Sequence[c] (5′-3′)
11969c[a]	1	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
Recipients		
1536	<4	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
1651	<4	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
3175	<4	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
3237	<4	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
426	<4	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
931g	<4	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
1702rnd	<4	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
WP19	<4	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
Mutants		
1651	32	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
3237	64	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
WP19	64	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
Transformants		
WP19-1420em	32	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
426-2562em	128	GAAAATTCCTCCTACCCGCGGCAAGACGGAAAGACCCCGTGGACCTTTACTACAGCTTGA
426-2774em	128	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
426-2901em	128	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
1536-1705em	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**N**AGACCCCGTGGACCTTTACTACAGCTTGA
1651-1705em	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
3175-1686em	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
3237-1420em	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
931g-2901em	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
931g-1800rem	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
1702md-1702em	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**N**AGACCCCGTGGACCTTTACTACAGCTTGA
Donors		
1420	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
1686	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
1705	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
1702	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
1800r	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
2562	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
2774	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA
2901	>256	GAAAATTCCTCCTACCCGCGGCAAGACGGA**G**AGACCCCGTGGACCTTTACTACAGCTTGA

[a] Erythromycin-sensitive *C. coli* strain from a raw chicken carcass (10) (GenBank accession number AY249915).
[b] "em"indicates erythromycin-resistant transformants obtained from specific recipient-donor combinations. For example, 1536-1705em was strain 1536 transformed by DNA from strain 1705.
[c] Letters in bold correspond to position 2075; N indicates that a mixture of A and G was observed at this position.

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ination of the sequencing chromatograms, suggesting that the transition was absent from at least one of the three copies of the 23S rRNA gene in these transformants. None of the three tested spontaneous mutants harbored the transition (Table 4).

A strong correlation between the erythromycin MIC and the presence of the A2075G transition was noted. All erythromycin-resistant field strains and transformants with high erythromycin MICs (>128 μg/ml) harbored the transition, whereas the putative transformant WP19-1420em, which had a relatively low MIC (32 μg/ml), and spontaneous erythromycin-resistant mutants, which also had MICs of 32 to 64 μg/ml, lacked this transition (Table 4).

Erythromycin resistance is stable in transformants in the absence of antibiotic. The stability of erythromycin resistance in the absence of the antibiotic was investigated in two transformants, 961-1705em and 1651-1705em. In both cases, resistance remained stable after 14 successive daily transfers in the absence of erythromycin, with similar CFU/ml of the cultures on plates with and without erythromycin (data not shown).

DISCUSSION

Even though thermophilic campylobacters have long been known to be naturally competent, limited data exist on the role of transformation in dissemination of high-level erythromycin resistance in *C. jejuni* and *C. coli* strains that colonize meat animals. Considering the prevalence of *C. coli* in animals such as swine and turkeys, the high incidence of resistance to erythromycin and other macrolides in this bacterium, and the rising status of these drugs as the first line of treatment of human infections, it is important to investigate mechanisms that may underlie the dissemination of erythromycin resistance in *C. coli*.

Our findings suggest that transformation can indeed mediate the acquisition of high-level resistance to erythromycin in animal-derived *C. coli* strains, with the majority (12/13) of the strains from turkeys and several (7/13) of the *C. coli* strains from swine yielding transformants under laboratory conditions with the agar assay. Frequencies of transformation, determined using broth assays, were also significantly higher for turkey-derived than swine-derived strains. Transformation frequencies were below the detection level for two of the swine strains that yielded transformants in the agar assay, suggesting that the agar assay may be preferable for determining whether a strain is capable of transformation to erythromycin resistance, even though it does not lend itself to accurate determinations of transformation frequencies. To our knowledge, this is the first documentation that *C. coli* strains from meat animals can be naturally transformed to high-level erythromycin resistance with genomic DNAs from erythromycin-resistant bacteria and that host-related differences in transformation potential may exist.

In contrast to transformants, which had high-level resistance and harbored the A2075G transition in the 23S rRNA gene, spontaneous erythromycin-resistant mutants were resistant to relatively low levels of the antibiotic and lacked the A2075G transition, as also described by others (18). Thus, spontaneous mutations alone are unlikely to be responsible for the frequent occurrence of resistance to erythromycin in *C. coli* strains from meat animals (1, 2, 4, 5, 6, 20), which this study and others (9, 19) have found to be characterized by high erythromycin MICs and to be accompanied by the A2075G transition in the 23S rRNA gene. Our findings suggest that erythromycin resistance acquired by transformation is stable in the absence of the antibiotic. In addition, DNAs from transformants could transform other strains to erythromycin resistance, with transformation frequencies similar to those obtained with DNAs from erythromycin-resistant field strains. Taken together, the available data suggest that transformation may indeed contribute to the high prevalence of high-level resistance to erythromycin in *C. coli* colonizing turkeys and swine. In terms of other modes of transfer of this resistance, conjugation is unlikely since high-level resistance is mediated by a chromosomal marker (the substitution in the 23S rRNA gene), and no evidence currently exists on phage-mediated transfer (transduction) in *Campylobacter*. However, we cannot exclude the possibility that conditions in swine and turkey production systems (especially the extensive use of the macrolide tylosin as a growth promoter) may be such that mutants with high-level resistance (harboring the A2075G transition) are selected for, in contrast to the relatively low-level spontaneous mutants obtained in the laboratory.

The documentation of the A2075G transition in the 23S rRNA gene in erythromycin-resistant *C. coli* strains from swine and turkeys confirms and extends previous detections of this mutation in erythromycin-resistant *C. coli* strains from humans, swine, broilers, and sheep (9, 10, 13, 19). Recently, the role of this transition in high-level erythromycin resistance was confirmed by the transformation of *C. jejuni* 81116, derived from a human clinical case, to high-level resistance, using an amplified fragment of the 23S rRNA gene that harbored the transition as a donor (9). Our data for transformants that had a mixture of the wild-type sequence and the sequence harboring the A2075G transition suggest that high-level resistance to erythromycin in *C. coli* can be conferred even when the transition may be absent from one of the three 23S rRNA genes, as also described recently for *C. jejuni* (9).

In our study, the efficiency of transformation of *C. coli* to erythromycin resistance could be as high as 10^{-5} in certain strains but, overall, was lower than the previously reported transformation frequency (10^{-3}) of this species to nalidixic acid and streptomycin resistance (25). Data from our laboratory, obtained by using two *C. coli* strains (3237 and 614-3m) sensitive to both erythromycin and nalidixic acid, also suggested that transformation to nalidixic acid resistance was markedly more efficient (10^{-3} to 10^{-4}) than transformation of the same strain to erythromycin resistance (10^{-6} to 10^{-8}) (J. S. Kim and S. Kathariou, unpublished). The reasons for the relatively low frequencies of transformation to erythromycin resistance remain unclear, but they may be due to the apparent requirement for the A2075G transition in at least two of the three copies of the 23S rRNA gene in bacteria with high-level resistance (9), thus necessitating multiple transformation events. In contrast, nalidixic acid resistance involves a single chromosomal locus (*gyrA* harboring a specific substitution) (7).

The reasons for the relatively low transformation frequencies for swine-derived *C. coli* strains remain unclear. Molecular subtyping studies have shown that *C. coli* strains from swine are genotypically distinct from those colonizing poultry (11), and this may also suggest physiologic differences and specialized

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environmental requirements for transformation in swine-derived strains. Alternatively, such strains may lack competence due to the absence of proteins involved in the transformation process (26) or to the existence of barriers to gene flow, such as restriction/modification systems. Preliminary data for three swine-derived strains (5980, 1684, and WP145) which could not be transformed to erythromycin resistance showed that they were also unable to acquire resistance to nalidixic acid by transformation; this was even the case when isogenic DNA was used as a donor, as determined with strains 1684 and WP145 (J. S. Kim and S. Kathariou, unpublished). These findings suggest that restriction/modification systems were not responsible for the lack of transformation in these strains. The existence of strains of *Campylobacter* that lack competence has been noted before (25, 27). In *Helicobacter pylori*, an organism genetically closely related to *Campylobacter*, noncompetent strains were less likely to be resistant to metronidazole than competent strains, suggesting that transformation is important for disseminating antibiotic resistance in that organism (28). Further studies are needed to more accurately evaluate the role of transformation in the dissemination of antibiotic resistance in *C. coli*.

In conclusion, our results indicate that under laboratory conditions, transformation can be effective in the acquisition of high-level resistance to erythromycin by *C. coli* strains from turkeys and swine, whereas spontaneous mutations result in a genetically distinct class of mutants which lack the A2075G transition and are resistant to relatively low levels of the antibiotic. Further studies are needed to elucidate the mechanisms underlying the observed differences in the potential for transformation to erythromycin resistance in organisms derived from different hosts and to characterize the potential for transformation under conditions that prevail in the turkey and swine production ecosystems.

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