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DNA Probes for Identification of Tetracycline Resistance Genes in *Campylobacter* Species Isolated from Swine and Cattle

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Tetracycline-resistant strains of *Campylobacter jejuni* and *Campylobacter coli* from swine and cattle colons were isolated and characterized by hybridization with DNA probes. A probe consisting of the 1.8-kilobase (kb) *Hin*cII fragment from pUA466 was highly specific for the detection of tetracycline resistance (Tc^r) in *C. jejuni* and *C. coli*. The 5-kb *tetM* DNA probe from *Streptococcus agalactiae* plasmid pJI3 which has homology with the 1.8-kb *Hin*cII fragment from pUA466 could also be used to detect Tc^r *Campylobacter* strains. However, the *tetM* probe had a much lower sensitivity and required a lower stringency of hybridization. Therefore, the 1.8-kb *Hin*cII fragment appeared to be more appropriate for the classification of Tc^r in *Campylobacter* spp. No homology was detected between the Tc^r determinant from *Campylobacter* spp. and the *tetL* and *tetN* probes from *Streptococcus* spp. DNA homology was demonstrated between pUA649, a derivative of plasmid pUA466 which had lost most of the Tc^r region, and Tc^r plasmids from *C. jejuni* and *C. coli* isolated from animal and human sources. There was also homology between pUA649 and the chromosomes of *C. jejuni* and *C. coli* strains. In this study, all but one of the tetracycline-resistant *C. coli* and *C. jejuni* strains contained plasmids of approximately 50 kb which hybridized with the 1.8-kb *Hin*cII probe. In one *C. coli* strain (UA703), Tc^r appeared to be chromosomally mediated.

Epidemiological studies of *Campylobacter jejuni* and *Campylobacter coli* have used plasmid profiles as a parameter for strain differentiation (1, 3, 4, 31–33). The function of most of these plasmids is unknown. In contrast, plasmids which encode tetracycline resistance (Tc^r) have been extensively studied. Taylor et al. (29) reported that tetracycline resistance was encoded on a 38-megadalton plasmid in both *C. coli* and *C. jejuni*. More recently, these plasmids were shown to be about 45 kilobases (kb) in size (25). Lambert et al. (8) reported a 47.2-kb plasmid, pIP1433, in *C. coli* which encodes both Tc^r and kanamycin resistance.

Conjugative plasmids encoding Tc^r in six *Campylobacter* isolates from Canada, Belgium, and the United States had a high degree of homology (29). However, these same plasmids did not share DNA homology with four classes of Tc^r determinants (A, B, C, and D) found in members of the family *Enterobacteriaceae* (15, 29, 33). In *C. jejuni* plasmid pUA466, the region responsible for Tc^r includes a 4.2-kb *Acc*I fragment which overlaps with a 1.8-kb *Hin*cII fragment (Fig. 1), and these fragments are conserved in all of the *Campylobacter* plasmids studied so far (27). Plasmid pUA649, in which the 4.2-kb *Acc*I fragment was deleted from pUA466, lost Tc^r. The Tc^r determinant from pUA466 was cloned and expressed in *Escherichia coli* (25, 30). Moreover, the *Campylobacter* Tc^r determinant was shown to have homology with a 5-kb *Hin*cII fragment containing the *tetM* determinant in plasmid pJI3 from *Streptococcus agalactiae* B109 (25). This *tetM* determinant has also been found in a number of unrelated organisms, such as *Ureaplasma urealyticum*, *Mycoplasma* spp., and *Gardnerella vaginalis* (20, 21) and *Neisseria gonorrhoeae* (16).

Classification of Tc^r determinants in gram-positive and gram-negative bacteria is based on DNA hybridization studies using Tc^r DNA probes (6, 9, 15). To investigate the epidemiology of Tc^r in *Campylobacter* species from swine and cattle, we used a DNA probe consisting of the 1.8-kb *Hin*cII fragment from pUA466, as well as a DNA probe consisting of the 5-kb *Hin*cII fragment (*tetM* probe) from streptococcal plasmid pJI3 (21). The Tc^r determinant from *C. jejuni* plasmid pUA466 was homologous only with the 5-kb *Hin*cII fragment from pJI3 (*tetM*) (21) and not with pVB.A15 (*tetL*) (6) or pMV120 (*tetN*) (5). Although most tetracycline-resistant strains of *C. jejuni* and *C. coli* carried a Tc^r plasmid, in one case Tc^r was found to be chromosomally located.

MATERIALS AND METHODS

Bacterial strains. Strains of *C. jejuni*, *C. coli*, *Campylobacter fetus* subsp. *fetus*, and *Campylobacter laridis* used in this study in mating and hybridization experiments are shown in Table 1. Other strains were isolated from the colons of 40 cattle and 52 swine (L.-K. Ng, D. E. Taylor, and M. E. Stiles, manuscript in preparation) from Agriculture Canada, Lacombe, Alberta, during the period July 1985 to February 1986. Altogether, 171 strains were identified as either *C. coli* or *C. jejuni*, of which 27 strains were tetracycline resistant (9 strains of *C. jejuni* and 18 strains of *C. coli*). Twelve of the tetracycline-resistant strains were isolated from four animals with no previous exposure to antibiotics.

Plasmid DNA isolation and analysis. *Campylobacter* strains were screened for plasmid DNA by using the method described by Birnboim and Doly (2). Minipreparations of *E. coli* carrying pJI3 (*tetM*) and pVB.A15 (*tetL*) were obtained by using the same method. For *Streptococcus (Enterococcus) faecalis* carrying pMV120 (*tetN*), the cells were incubated with lysozyme solution at 37°C for 30 min before being lysed with an alkaline sodium dodecyl sulfate solution. Plasmids pJI3, pVB.A15, and pMV120 were supplied by V. Burdett (Duke University Medical Center, Durham, N.C.).

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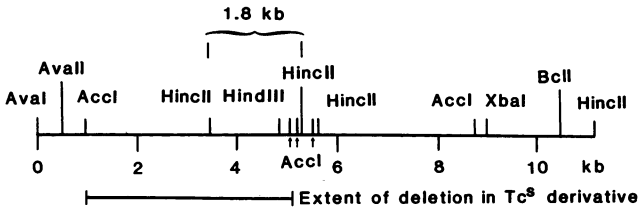

FIG. 1. Restriction map of *C. jejuni* Tc^r region of plasmid pUA466. The Tc^s deletion derivative of pUA466 is named pUA649.

E. coli plasmids of known molecular size (kb) were used as standards, including: pUC8, 2.7; pBR322, 4.0; RSF1030, 8.5; S-a, 35.4; R4776, 50.8; RP4, 58.5; RIP69, 72.4; pSLT2, 92.4; RA-1, 132.4; R40-a, 147.8; R27, 172.5; Rts-1, 194.0; and R478, 255.6. The isolated plasmid DNA was analyzed by electrophoresis at 60 V for 5 h in 0.7% agarose and Tris-borate EDTA (TB) buffer (90 mM Tris, 90 mM boric acid, 2.5 mM disodium EDTA [pH 8.3]) or at 35 V for 14 h in 0.7% agarose and Tris-acetate buffer (40 mM Tris hydrochloride, 20 mM sodium acetate, 2 mM EDTA [pH 8.0]). Gels were stained with ethidium bromide (0.4 μg/ml for 15 min), and DNA bands were visualized on a UV light box at 256 nm.

A modified procedure of Birnboim and Doly (2) was used to prepare purified plasmid DNA for restriction endonuclease digestion. Cells from 15 Mueller-Hinton agar plates of an overnight culture of a *Campylobacter* strain were harvested and washed with 50 ml of TE buffer (50 mM Tris, 5 mM EDTA [pH 8.0]). The washed cells were suspended in 1.7 ml of solution 1 (50 mM glucose, 10 mM EDTA, 0.25 M Tris [pH 8.0], 2 mg of lysozyme per ml) and held on ice for 30 min. The cells were lysed by adding 3.3 ml of solution 2 (0.2 N NaOH containing 1% sodium dodecyl sulfate). After 5 min on ice, 2.5 ml of solution 3 (3 M sodium acetate, pH 4.8) was added. The lysate was held on ice for 1 h and then centrifuged for 15 min at 18,900 × *g* (rotor JA20, J2-21 centrifuge; Beckman Instruments, Inc., Fullerton, Calif.). DNA in the supernatant was precipitated by adding 2 volumes of ice-cold 95% ethanol and held at −20°C for 18 h. The DNA was purified on a cesium chloride gradient (29).

Fragments of plasmids from restriction endonuclease digestions were subjected to electrophoresis for 18 h in horizontal 0.6% agarose gels in TB buffer with bacteriophage λ digested with *Hin*dIII, *Xho*I, and *Kpn*I and plasmid R27 digested with *Xba*I (26) as molecular weight standards. Plasmid pUA466 in *C. jejuni* UA466 and plasmid pMAK175 in *C. jejuni* UA1 were included as positive controls.

Restriction endonucleases. Restriction enzymes *Acc*I, *Xba*I, *Xho*I, *Hin*dIII, *Hin*cII, and *Kpn*I were obtained from Boehringer Mannheim Canada Ltd., Dorval, Quebec, Canada. Enzyme digestions were performed according to manufacturer instructions.

Preparation of ^{32}P-labeled DNA. The 1.8-kb *Hin*cII probe from pUA466 was cloned into pUC8 to give plasmid pUOA1 in *E. coli* JM105 (25). The 1.8-kb *Hin*cII fragment from pUA466 and the *tetM* probe were prepared by electroelution from agarose gel onto DEAE paper (7). The DNA fragments (1.8-kb *Hin*cII probe and *tetM* probe) and plasmid pUA649 were labeled in vitro with [α-^{32}P]dCTP (New England Nuclear Corp. Boston, Mass.) by nick translation (11). The labeled DNA had specific activities of 10^7 to 10^8 cpm/μg.

Dot blot procedure. The sensitivity and specificity of the 1.8-kb *Hin*cII fragment from pUA466 and the *tetM* probe were determined by using two temperatures of hybridization for each probe to represent two stringency conditions. Hybridization at 37°C was used to represent standard stringency (melting temperature, 20°C) of hybridization, and 42°C was used to represent higher stringency (melting temperature, 15°C) of hybridization (12, 14, 22). Overnight cultures in Mueller-Hinton broth were concentrated 10-fold to give about 10^9 to 10^{10} CFU/ml. The actual number of cells in these concentrated cultures was determined by a plate count method, so that a known number of cells was loaded on each dot. A range of sample sizes was loaded onto nitrocellulose filters to give 10^5 to 10^7 CFU per dot. The membrane filters were then processed and hybridized with DNA probes as described by Totten et al. (34).

Southern transfer hybridization. DNA was transferred from agarose gels to nitrocellulose filter membranes (BA85; Schleicher & Schuell, Inc., Keene, N.H.) by the method of Southern (23). Hybridizations were performed as described by Portnoy et al. (19) at 42°C for 16 h. For each blot, 2×10^5 to 5×10^5 cpm of ^{32}P-labeled DNA probe was used.

MIC determination. The MICs of tetracycline for the *Campylobacter* isolates were determined by the agar dilution method, as described previously (18).

Mating experiments. A plate mating method described by Taylor et al. (28) was used for transfer of Tc^r plasmids. Antibiotic plates containing nalidixic acid (48 μg/ml) and tetracycline (8 μg/ml) were used to select for transconjugants. Control plates were monitored for spontaneous mutation of either the donor or the recipient strains.

TABLE 1. *Campylobacter* strains used in mating and hybridization experiments

Expt and organisms	Strain no.	Antibiotic resistance	Source[a]
Mating			
Donor			
C. jejuni	UA466	Tc	Human[b]
C. coli	UA703	Tc	Swine (this study)
Recipient			
C. jejuni	UA67	Nal[c]	Human[d]
C. fetus subsp. *fetus*	ATCC 27374 (UA60)	Nal	ATCC
C. laridis	UA487	Nal	Human[e]
Hybridization			
C. jejuni	UA466	Tc	Human[b]
	UA649	None	Deletion mutant of UA466
	UA650	None	Plasmidless derivative of UA466
	UA1	Tc	Human[d]
	UA124	None	Plasmidless derivative of UA1
	UA705	Tc	Human[f]
	NCTC 11392	None	NCTC
	NCTC 11168	None	NCTC
C. coli	NCTC 11353	None	NCTC
	UA706	Tc	Human[f]
	UA704	Tc	Swine (this study)

[a] ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, Central Public Health Laboratory, London, England.
[b] E. Lee, Naval Research Institute, Bethesda, Md.
[c] Nal, Nalidixic acid resistance.
[d] M. A. Karmali, The Hospital for Sick Children, Toronto, Ontario, Canada.
[e] H. Lior, Laboratory Centre for Disease Control, Ottawa, Ontario, Canada.
[f] L. Mueller, Provincial Laboratory of Public Health, Edmonton, Alberta, Canada.

RESULTS

MICs for tetracycline-resistant *Campylobacter* species. The MICs of tetracycline for the nine *C. jejuni* strains were 32 and 64 μg/ml. Those for the 18 *C. coli* strains ranged from 16 to >64 μg/ml. For *C. coli* UA703, the tetracycline MIC was 64 μg/ml.

Sensitivity and specificity of dot blot hybridization. The sensitivities of the 1.8-kb *Hin*cII fragment and the 5-kb *tetM* probes for the detection of Tc^r were compared using four *Campylobacter* strains (UA466, UA469, and UA650 [Table 1] and a tetracycline-resistant *C. coli* strain isolated from a swine colon). Using the 1.8-kb *Hin*cII probe, the minimum number of cells required for the hybridization at 37 or 42°C to be detected by autoradiography was 10^5 per dot. When the *tetM* probe was used, hybridization was detected at 37°C but not at 42°C, and a minimum of 10^6 CFU per dot was required. Plasmid pUA649, a tetracycline-susceptible (Tc^s) deletion derivative of pUA466, also hybridized to the 5-kb *tetM* fragment. Therefore, the sensitivity and specificity of the 1.8-kb *Hin*cII fragment is higher than that of the *tetM* probe for detecting the Tc^r determinant in *Campylobacter* spp. For subsequent dot blot experiments using the 1.8-kb *Hin*cII probe, hybridization was conducted at 42°C with about 10^5 to 10^6 CFU per dot.

Homology between Tc^r DNA probes. The 1.8-kb *Hin*cII probe was hybridized independently with *tetL*, *tetN*, and *tetM*, the 5-kb *Hin*cII fragment determinants from *Streptococcus* spp. (5, 6). In the cases of *tetL* and *tetN*, the whole plasmids (pVB.A15 and pMV120) were used in hybridization experiments with the 1.8-kb *Hin*cII probe. No homology was detected between the 1.8-kb *Hin*cII probe and either the *tetL* or the *tetN* determinant. The hybridizations were done at 37°C, which represents standard stringency. When the 5-kb *tetM* probe was hybridized to the *Hin*cII digest of pUA466,

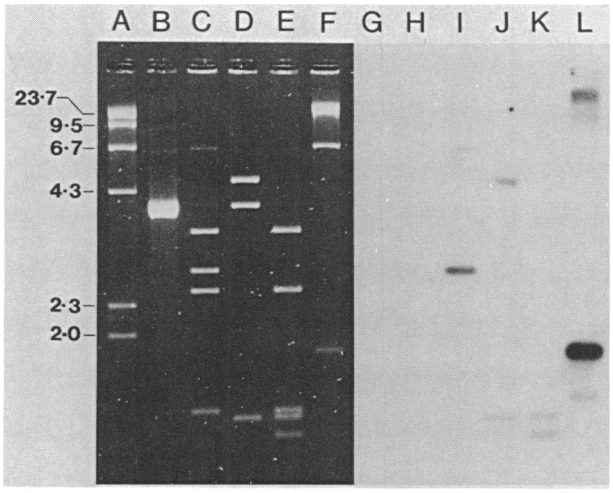

FIG. 2. Southern blot of pJI3 (pACYC177 and the 5-kb *Hin*cII *tetM* fragment) with the 1.8-kb *Hin*cII probe. An agarose gel containing plasmids digested with restriction enzymes and then subjected to electrophoresis is shown in the left panel, and the corresponding autoradiogram hybridized with the 1.8-kb *Hin*cII probe is shown in the right panel. Lanes A and G are the molecular standards of phage λ digested with *Hin*dIII. Their sizes in kilobases are indicated on the left. Lanes: B and H, pACYC177 digested with *Hin*cII; C and I, pJI3 digested with *Hin*cII-*Hin*dIII; D and J, pJI3 digested with *Hin*cII-*Sst*I; E and K, pJI3 digested with *Hin*cII-*Hin*dIII-*Sst*I; and F and L, the positive control (pUA466) digested with *Hin*cII.

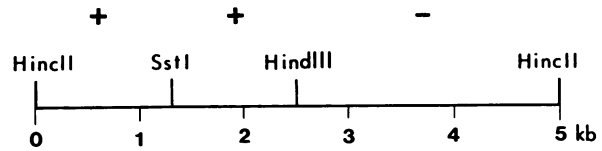

FIG. 3. Linear map of 5-kb *Hin*cII *tetM* fragment from pJI3 (20). +, Hybridization of the fragment with the 1.8-kb *Hin*cII probe; −, no homology between the fragment and the 1.8-kb *Hin*cII probe.

two fragments (1.8 and 6 kb) of pUA466 hybridized with the *tetM* probe, as was shown previously (24). The hybridization between the 1.8-kb *Hin*cII fragment from pUA466 and pJI3 containing the *tetM* determinant digested with *Hin*cII, *Hin*dIII, and *Sst*I is shown in Fig. 2. The 1.8-kb *Hin*cII probe hybridized with the 1.3-kb *Hin*cII-*Sst*I and 1.2-kb *Sst*I-*Hin*dIII fragments (Fig. 3). Therefore, about half of the 5-kb *tetM* probe showed homology with the 1.8-kb *Hin*cII probe. Partially digested DNA fragments of plasmids pJI3 and pUA466 (Fig. 2, lanes C and F) hybridized with the 1.8-kb *Hin*cII probe (Fig. 2, lanes I and L).

Specificity of 1.8-kb *Hin*cII fragment and *tetM* probes. The 27 Tc^r *C. coli* and *C. jejuni* strains were hybridized separately with the 1.8-kb *Hin*cII probe and the 5-kb *tetM* probe. The tetracycline-susceptible *C. jejuni* strains, UA124, UA649, and UA650, as well as six Tc^s *C. coli* strains, three from cattle and three from swine, did not hybridize with the 1.8-kb *Hin*cII fragment at 10^8 CFU per dot. There was no difference between two Tc^r *C. coli* strains, UA705 and UA706, obtained from humans (obtained from L. Mueller, Alberta Provincial Laboratory of Public Health) and the tetracycline-resistant isolates from animals, except *C. coli* UA703, which showed weak hybridization. When the hybridizations were repeated with 8×10^7 to 2×10^8 CFU of *C. coli* UA703 per dot, a strong positive reaction was observed. In contrast, tetracycline-susceptible isolates of *C. coli* from the same colon did not hybridize with the 1.8-kb *Hin*cII fragment (at 2×10^8 CFU) in the dot blot procedure. The 5-kb *tetM* probe hybridized with all of the Tc^r strains; however, because of the lower sensitivity of the probe, a longer exposure time to the X-ray film was required.

Plasmid analysis and Southern transfer hybridization. Most of the Tc^r strains contained single plasmids of approximately the same mobility in agarose gel as the 45-kb plasmids pUA466 from *C. jejuni* UA466 and pMAK175 from *C. jejuni* UA1. To determine the homogeneity and location of the Tc^r determinant in the tetracycline-resistant isolates, plasmids from all of the Tc^r isolates were hybridized separately with the 1.8-kb *Hin*cII fragment and the *tetM* probe. Plasmids from tetracycline-resistant isolates which were of sizes similar to that of pUA466 hybridized with both the 1.8-kb *Hin*cII fragment and the *tetM* probe. In contrast, plasmids in tetracycline-susceptible *C. coli* and *C. jejuni* which were similar in size to pUA466 did not hybridize with the probes. Similarly, the plasmid in Tc^r *C. coli* UA703 (i.e., pUA703) did not hybridize with the probes.

Plasmid restriction endonuclease analysis and Southern transfer hybridization. Further characterization of plasmids pUA703 and pUA704, both of which were present in *C. coli* strains from swine (Table 1), was done by restriction digestions with endonucleases *Acc*I and *Hin*cII. Southern transfer hybridization of the *Acc*I fragments of pUA703 and pUA704 with the 1.8-kb *Hin*cII probe showed that the 1.8-kb *Hin*cII probe hybridized to the 4.2-kb *Acc*I fragment of pUA466 and the 3.6-kb *Acc*I fragment of pUA704. Conversely, the 1.8-kb

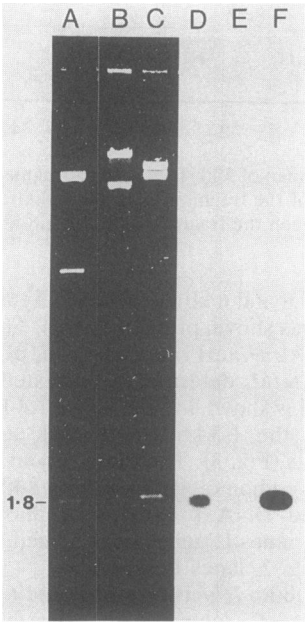

FIG. 4. Southern blot of plasmids pUA703 and pUA704 from *C. coli* hybridized with the 1.8-kb *Hin*cII fragment from pUA466. pUA466 (lane A), pUA703 (lane B), and pUA704 (lane C) were digested with *Hin*cII. The corresponding autoradiogram of lanes A to C after hybridization with the 1.8-kb *Hin*cII probe is shown in lanes D to F.

*Hin*cII fragment did not hybridize to any fragments of pUA703. The 1.8-kb *Hin*cII probe hybridized with the fragment of about the same size in pUA704; however, it did not hybridize with the *Hin*cII fragments of plasmid pUA703 (Fig. 4). This result confirmed that there is no homology between the 1.8-kb *Hin*cII probe and pUA703.

Location of Tc^r on chromosome of *C. coli* UA703. Since the DNA from Tc^r *C. coli* UA703 hybridized with the 1.8-kb *Hin*cII probe in the dot blot experiment but not with the plasmid, the probable location of the Tc^r determinant in this strain is on the chromosome. The chromosomal location of Tc^r in *C. coli* UA703 was confirmed by hybridization of the *Hin*cII digest of the chromosomal DNA of *C. coli* UA703 with the 1.8-kb *Hin*cII probe (Fig. 5). The 1.8-kb *Hin*cII probe was homologous with a *Hin*cII fragment of the same size present in the chromosome of *C. coli* UA703.

Southern blot hybridization of plasmids with pUA649. Since pUA649 (the tetracycline-susceptible deletion derivative of pUA466) lacks most of the Tc^r determinant, it was used to assess the homology of plasmids present in *Campylobacter* strains. Plasmids from seven tetracycline-susceptible and five tetracycline-resistant *Campylobacter* strains were hybridized with pUA649. Four of the former and four of the latter plasmids showed homology with the pUA649 probe at 10^6 CFU per dot (data not shown). All of the plasmids which showed homology with pUA649 had sizes similar to that of pUA466. The plasmid in UA703 did not hybridize with pUA649.

Dot blot hybridization of total genomic DNA with plasmid pUA649. Homology between plasmid pUA649 and total genomic DNA of tetracycline-susceptible *Campylobacter* strains was determined using dot blot hybridization (data not shown). Five *C. jejuni* strains from cattle and *C. jejuni* NCTC 11392 and NCTC 11168 all hybridized with pUA649. Plasmidless derivatives UA124 and UA650 from tetracycline-resistant strains UA1 and UA466, respectively, were also included, and both of these strains hybridized with pUA649. Hybridization of tetracycline-susceptible strains of *C. coli* from cattle showed homology between the DNA of these strains and pUA649. Therefore, either the plasmid or the chromosome in these strains showed homology with pUA649. Since the plasmids present in these strains showed no homology with pUA649, the homology could be attributed to the chromosome. *C. coli* NCTC 11353, *C. fetus* subsp. *fetus* ATCC 27374, *C. laridis* UA487, and one of the tetracycline-resistant *C. coli* strains from swine did not hybridize with pUA649.

Mating experiments. Mating experiments were conducted to determine the ability of the chromosomally located Tc^r determinant in UA703 to transfer from *C. coli* UA703 to other *Campylobacter* strains. *C. coli* UA703 was mated with *C. fetus* subsp. *fetus* UA60, *C. jejuni* UA67, and *C. laridis* UA487. No transconjugants were obtained.

DISCUSSION

Previous studies have demonstrated that there is a high degree of DNA homology among the plasmids in *Campylobacter* spp. (29, 33). In this study, Tc^r plasmid pUA466 was related to plasmids of similar sizes (about 40 to 50 kb). Some of these plasmids do not encode Tc^r, and it is possible that these plasmids lost the Tc^r determinant, as observed with pUA649 (25). Alternately, *C. jejuni* and *C. coli* strains may carry an indigenous plasmid of 40 to 50 kb which subsequently acquired the Tc^r determinant from another source. Although a wide range of sizes (42 to 100 kb) have been

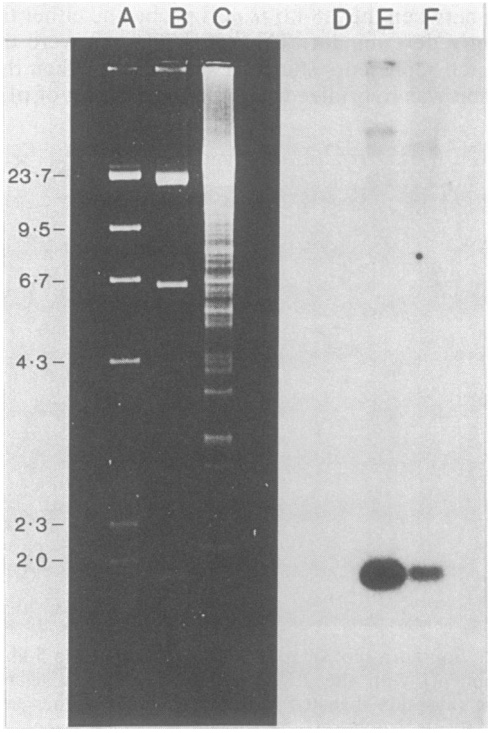

FIG. 5. Southern blot hybridization of total genomic DNA from *C. coli* UA703 with the 1.8-kb *Hin*cII probe. Lanes: B, agarose gel containing pUA466 digested with *Hin*cII; C, the total genomic DNA of UA703 digested with *Hin*cII; D, corresponding autoradiogram prepared by hybridization of the 1.8-kb *Hin*cII probe to phage λ; E, pUA466; and F, the total genomic DNA of UA703. Molecular size standards of phage λ digested with *Hin*dIII are shown in lane A; the sizes in kilobases are indicated on the left.

reported for *Campylobacter* Tc^r plasmids (33), Tc^r plasmids greater than 60 kb were not observed in this study. Comparison of plasmid sizes studied in different laboratories is difficult because of the use of different conditions for plasmid isolation and gel electrophoresis (24, 32).

Based on the hybridization study using the pUA649 probe and total genomic DNA from tetracycline-resistant and -susceptible strains, the Tc^r plasmid (pUA466) showed homology to *C. jejuni* chromosomal DNA. Similar results were reported previously (33). Therefore, unlike other studies on Tc^r (5, 6), it is not appropriate to use the Tc^r plasmid as a DNA probe to detect tetracycline-resistant strains or to study the diversity of the Tc^r determinant in this genus.

The 1.8-kb *Hin*cII fragment from pUA466 is highly specific for Tc^r in *Campylobacter* spp. and can be used as a probe with sensitivity similar to that of *E. coli* DNA probes for heat-labile and heat-stable toxins (17). Only 10^5 to 10^6 cells are required to detect Tc^r. In this study, no false-positives or -negatives were encountered. The 1.8-kb *Hin*cII fragment is located within the Tc^r determinant (25). This cloned fragment is useful for studying Tc^r in *Campylobacter* species. The 5-kb *tetM* DNA probe from pJI3 in *S. agalactiae* has homology with the 1.8-kb *Hin*cII fragment from pUA466 (25). When the 5-kb *tetM* probe is used, tetracycline-resistant strains can also be detected. However, the *tetM* probe has a much lower sensitivity and requires a lower stringency of hybridization (37°C with 50% formamide). This is possibly due to the larger size of the probe and also the lower degree of homology between the *Campylobacter* and streptococcal Tc^r determinants.

The *tetM* probe showed hybridization with tetracycline-susceptible deletion derivative UA649, indicating that false-positives may be encountered when deletion mutants are found in the natural environment. The sequence of the streptococcal *tetM* determinant from *S. faecalis* transposon Tn*1545* has been determined (13), and on the basis of our data on the sequence of the *C. jejuni* Tc^r determinant, we estimate that there is 76% homology between the two determinants (30; D. E. Taylor, K. Hiratsuka, and E. K. Manavathu, 4th Int. Workshop Campylobacter Infections, Göteborg, Sweden, abstr. no. 157, 1987). The *C. coli* Tc^r determinant from pIP1433 (8) has been designated *tetO* (W. Sougakoff, P. Nordmann, and P. Courvalin, 4th Int. Workshop Campylobacter Infections, abstr. no. 154). Moreover, because *C. jejuni* and *C. coli* Tc^r determinants are highly homologous, it is logical to designate both determinants members of class O. Homology among *tetO* determinants can be detected at high stringency, namely 50% formamide at 42°C, whereas *tetM* and *tetO* hybridize only at moderate stringency, namely 50% formamide at 37°C.

In this study, all of the tetracycline-resistant *C. coli* and *C. jejuni* strains hybridized with the 1.8-kb *Hin*cII fragment; therefore, they probably have the same mechanism of resistance. Although the Tc^r determinant is most often carried on a plasmid, in the case of *C. coli* UA703 it was carried on the chromosome. This finding suggests that the Tc^r determinant is located on a transposable element in *Campylobacter* spp. Further work is required to confirm this hypothesis.

In this study, some of the animal colons tested yielded Tc^r *Campylobacter* strains. Four of these colons were from animals that had been raised at a research station and had neither been fed nor treated with antibiotics. These animals must have acquired the antibiotic-resistant strains from the environment. In studies conducted on chicken farms, it was shown that resistant enteric strains could spread from chickens fed with antibiotic-containing feeds to chickens without antibiotics and then to human handlers (10). In fact, since the introduction of the tetracycline antibiotics in 1947, strains of bacteria resistant to tetracycline have been encountered at an increasing rate, and they have traversed many genera (9). This is probably due to the use of tetracycline as a growth promoter in animal feeds and also to its frequent use for treatment of bacterial infections in humans (9).

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