Antimicrobial Resistance of *Campylobacter jejuni* and *Campylobacter coli* with Special Reference to Plasmid Profiles of Japanese Clinical Isolates

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A total of 111 clinical isolates of *Campylobacter jejuni* and 10 clinical isolates of *Campylobacter coli* were characterized by their susceptibility to nine antimicrobial agents and by their plasmid profiles on agarose gel electrophoresis. All of the *C. jejuni* isolates were susceptible to chloramphenicol, ciprofloxacin, erythromycin, kanamycin, and nalidixic acid, but 55% were tetracycline resistant. In the 10 *C. coli* isolates, a high prevalence of multiple-antibiotic resistance was noted. Plasmids were found in 82% of the tetracycline-resistant and 15% of the tetracycline-susceptible *C. jejuni* isolates. Tetracycline resistance in six randomly selected *C. jejuni* isolates, which contained 50- or 135-kilobase (kb) plasmids, was transferred by conjugation to a *Campylobacter fetus* subsp. *fetus* recipient with recovery of a 50- or a 45-kb plasmid from transconjugants. From one multiple-antibiotic-resistant *C. coli* isolate, resistance to tetracycline, kanamycin, and chloramphenicol was transferred concomitantly with a 58-kb plasmid, pNR9589. Nonconjugative 98-kb plasmids, pNR9131 and pNR9581, from *C. coli* isolates with resistance to tetracycline, kanamycin, and erythromycin were shown by cloning experiments to code for at least kanamycin resistance. Restriction digests revealed that 50-kb plasmids from tetracycline-resistant *C. jejuni* isolates were identical, although plasmids from multiple-antibiotic-resistant *C. coli* isolates shared partial DNA homology to each other. Cloning of the kanamycin and chloramphenicol resistance genes of pNR9589 into *Escherichia coli* showed that the two genes are closely linked or clustered. Double-digestion analysis of the fragments encoding the kanamycin resistance of pNR9131, pNR9581, and pNR9589 showed that these three plasmids contain a common fragment related to kanamycin resistance.

*Campylobacter jejuni* is recognized as one of the common causes of acute bacterial enteritis throughout the world (2, 17). More than 200 strains of *C. jejuni* and a small number of *C. coli* strains, 60% of which are from children, are isolated every year in Tokyo Metropolitan Toshima General Hospital.

Taylor et al. (19, 20) and Tenover et al. (21) demonstrated that tetracycline resistance in *C. jejuni* is mediated by conjugative R plasmids with a size of 60 kilobases (kb), and more recently, Tenover et al. (22) showed that all tetracycline-resistant *C. jejuni* harbored R plasmids. Most recently, Taylor (18) mapped the tetracycline resistance plasmid. Studies on plasmid detection in *Campylobacter* spp. have not yet been carried out in Japan, although clinical and epidemiological studies on *Campylobacter* spp. have been performed extensively. In the present study, we examined the MICs of nine antimicrobial agents against *Campylobacter* isolates, the plasmid carriage in these isolates, and the relationship between antimicrobial resistance and plasmid carriage in *C. jejuni* and *C. coli*. Moreover, we analyzed three plasmids isolated from multiple-antibiotic-resistant *C. coli* strains by restriction endonuclease digestion and characterization of the cloned resistance genes.

**MATERIALS AND METHODS**

**Bacterial strains.** We used 111 *C. jejuni* and 10 *C. coli* strains isolated from stools of diarrheal patients at Tokyo Metropolitan Toshima General Hospital during the period from November 1981 to July 1982. All isolates were from sporadic cases. They were suspended in 10% skim milk and stored at −70℃. *C. fetus* subsp. *fetus* C-607 was isolated from bloodstream of a baby with bacteremia.

**Identification of isolates.** All isolates were tested for the following characteristics: Gram stain, production of oxidase, catalase, and H2S (TSI medium [Eiken Chemicals, Tokyo, Japan]) and lead acetate paper strip, growth in the presence of 1% glycine and 3.5% NaCl, growth at 25 and 42℃, and hippurate hydrolysis by the method of Harvey (9).

**Antimicrobial agents and chemicals.** The following antimicrobial agents were tested: chloramphenicol (Sankyo Co. Ltd., Tokyo, Japan), tetracycline (Lederle Japan Ltd., Tokyo, Japan), kanamycin (Meiji Seika Kaisha Ltd., Tokyo, Japan), ampicillin (Meiji Seika Kaisha Ltd.), nalidixic acid (Daichi Seiyaku Co., Ltd., Tokyo, Japan), erythromycin (Dainippon Pharmaceutical Co., Ltd., Osaka, Japan), cephalothin (Shionogi Co., Ltd., Osaka, Japan), metronidazole (Shionogi Co.), and ciprofloxacin (kindly provided by Bayer Yakuhin, Ltd., Tokyo, Japan). Every stock solution of these substances was prepared as specified by the manufacturers. Restriction endonucleases (PstI, BglII, HindIII, HaeIII, and HpaII) and T4 DNA ligase were purchased from Boehringer Mannheim Biochemicals, Tokyo, Japan.

**Antimicrobial susceptibility testing.** Antimicrobial susceptibility testing was done by the standard agar dilution method established by the Japanese Society of Chemotherapy (8). All isolates were subcultured on Trypticase soy agar plates (BBL Microbiology Systems, Cockeysville, Md.) containing 5% horse blood at 37℃ for 48 h in a GasPak jar containing a

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Campy Pak (BBL). These cultures were inoculated into 0.5 ml of heart infusion broth (Difco Laboratories, Detroit, Mich.), incubated for 48 h as described above, and then diluted 100-fold and inoculated onto the test plates with a multipoint inoculator to give a final inoculum size of about 10^5 CFU. The medium used was Mueller-Hinton agar (Eiken Chemicals, Tokyo, Japan) supplemented with 5% horse blood, which was freshly prepared and dried for 1 h. *Staphylococcus aureus* 209P and *Escherichia coli* CHS-2, with known antimicrobial susceptibility, were used as controls on each plate.

**Plasmid detection procedure.** The rapid method of Goto et al. for screening large plasmid DNA (7) was modified. Bacterial strains were cultured on 5% horse blood agar plates at 37°C for 48 h under microaerophilic conditions as described above. One loopful of bacterial cells was suspended and washed with 600 μl of 10 mM Tris hydrochloride (pH 8.0) containing 100 mM NaCl, centrifuged at 5,500 × g for 5 min, and suspended into 100 μl of cold 25% sucrose in 0.05 mM Tris buffer (pH 8.0). After treatment with 2 μl of RNase (5 mg/ml) and then 20 μl of lysozyme (5 mg/ml in the same buffer), the samples were placed in ice for 5 min, 25 μl of 0.25 mM EDTA was added, and the mixture was incubated for 5 min. A 160-μl portion of the detergent mixture (1% Brij 58, 0.4% sodium deoxycholate, 62.5 mM EDTA, 50 mM Tris hydrochloride [pH 8.0]) was added, and the mixture was kept ice-cold for 10 min and then centrifuged at 12,000 × g for 30 min at 4°C to collect cleared lysates. Phenol extraction and centrifugation were performed, and the recovered upper aqueous phase was used for gel electrophoresis. Electrophoresis was carried out on a 0.6% agarose gel dissolved in Tris-borate buffer at 160 V for about 4 h on a horizontal apparatus. The mobilities of plasmid DNA's were compared with those of R6-5 (100 kb) (23), S-a (40 kb) (1), and pNR353 (12 kb), one of our laboratory standards. Values above 100 kb could not be estimated accurately.

**Purification of plasmid DNA.** Plasmid DNAs of *Campylobacter* spp. were purified from cleared lysates by cesium chloride-ethidium bromide centrifugation by the method of Goto et al. (7).

**Conjugation experiments.** For conjugation, six strains of tetracycline-resistant *C. jejuni* and three strains of multiple-antibiotic-resistant *C. coli* were used as donors and *C. fetus subsp. fetus* C-607 was used as a recipient. Broth and plate matings were carried out by the method of Taylor (19), with the following modification. After the bacterial cells had been cultured in flasks containing heart infusion broth (Difco), equal amounts of donor and recipient cells were mixed at 37°C for 48 h. Transconjugants were selected on plates containing 50 μg of nalidixic acid and 25 μg of tetracycline per ml at 25°C for 48 h. For plate matings, each donor and recipient was cultured on horse blood-agar plates at 37°C for 48 h. After being scraped from the plates and suspended in saline, both the donor and the recipient were mixed together, spread over blood-agar plates containing antimicrobial agents as described above, and incubated at 37°C for 48 h. Some transconjugants were tested again for donor ability by plate mating with a tetracycline-susceptible and plasmid-free *C. jejuni* strain as recipient. Tetracycline (25 μg/ml) and cephalothin (50 μg/ml), along with a 42°C growth temperature, were used for counter-selection.

**DNA manipulation.** Reactions for all endonucleases and T4 DNA ligase were carried out under the conditions specified by the supplier. Agarose gel electrophoresis was performed with a 0.6 to 1.0% agarose gel as described previously (4).

**Cloning of resistance genes.** Kanamycin and chloramphenicol resistance genes of pNR9131, pNR9581, and pNR9589 were cloned into *E. coli* X1776 (14) with pUC13 as a vector (15, 27). Transformants were selected under anaerobic conditions on Penassay agar plates (Difco) containing 25 μg of kanamycin or chloramphenicol per ml at 37°C.

## RESULTS

**Identification of isolates.** All the isolates tested had the characteristics of *Campylobacter* spp. When the strains were positive for hippurate hydrolysis, they were considered to be *C. jejuni*; when they were hippurate negative, they were considered to be *C. coli.*

**MICs.** The MIC range and the MICs for 50 and 90% of strains tested (MIC<sub>50</sub> and MIC<sub>90</sub>) for the 111 clinical isolates of *C. jejuni* and 10 isolates of *C. coli* are shown in Table 1. All *C. jejuni* isolates were susceptible to chloramphenicol (MIC, <25 μg/ml), kanamycin (MIC, <25 μg/ml), nalidixic acid (MIC, <32 μg/ml), erythromycin (MIC, <8 μg/ml), and ciprofloxacin (MIC, <12.5 μg/ml). Ciprofloxacin was the most active antimicrobial agent tested (MIC<sub>50</sub>, 0.2 μg/ml; MIC<sub>90</sub>, 0.78 μg/ml). Only one isolate (0.9%) was resistant to ampicillin (MIC, ≥32 μg/ml). For susceptibility to tetracycline, strains were divided into two groups: either very susceptible or highly resistant. Sixty-one isolates (55%) were resistant to tetracycline (MIC, ≥12.5 μg/ml). Metronidazole (MIC<sub>50</sub>, 50 μg/ml; MIC<sub>90</sub>, >100 μg/ml) and cefalothin (MIC, >100 μg/ml) showed poor activity.

**of the *C. coli* isolates, one was resistant to chloramphenicol, six were resistant to tetracycline, five were resistant to kanamycin, one was resistant to nalidixic acid, and four were resistant to erythromycin. The antimicrobial resistance patterns of *C. coli* were considerably different from those of *C. jejuni* in that they showed high-level multiple-antibiotic resistance (Table 2).
TABLE 2. Antimicrobial resistance patterns and plasmids of 10
C. coli isolates

<table>
<thead>
<tr>
<th>Isolate no.</th>
<th>Antimicrobial resistance pattern</th>
<th>Plasmid size (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-127</td>
<td>Tetracycline</td>
<td>50</td>
</tr>
<tr>
<td>C-129</td>
<td>Tetracycline</td>
<td>ND*</td>
</tr>
<tr>
<td>C-131</td>
<td>Tetracycline, kanamycin, erythromycin</td>
<td>98</td>
</tr>
<tr>
<td>C-5816</td>
<td>Tetracycline, kanamycin, erythromycin</td>
<td>98</td>
</tr>
<tr>
<td>C-166</td>
<td>Tetracycline, kanamycin, erythromycin</td>
<td>88</td>
</tr>
<tr>
<td>C-589</td>
<td>Tetracycline, kanamycin, erythromycin, chloramphenicol</td>
<td>58, 5.5</td>
</tr>
<tr>
<td>C-296</td>
<td>Susceptible</td>
<td>46</td>
</tr>
<tr>
<td>C-165</td>
<td>Kanamycin, nalidixic acid</td>
<td>–</td>
</tr>
<tr>
<td>C-195</td>
<td>Susceptible</td>
<td>–</td>
</tr>
<tr>
<td>C-197</td>
<td>Ampicillin</td>
<td>–</td>
</tr>
</tbody>
</table>

* ND, Not done.

Plasmid detection. The results of plasmid detection in 108 isolates of C. jejuni and 10 isolates of C. coli were as follows: 56 C. jejuni (52%) and 7 C. coli (70%) isolates harbored plasmids. Although seven isolates of C. jejuni and one of C. coli had two plasmids, the majority had a single plasmid. The sizes of the plasmids detected ranged from 5.5 to 140 kb. The typical plasmid patterns of the isolates on agarose gel electrophoresis are shown in Fig. 1. The plasmid sizes were 50 kb in C-142, 135 kb in C-147, 50 and 48 kb in C-150 and C-216, 88 kb in C-166, 98 kb in C-131 and C-5816, and 38 and 5.5 kb in C-589.

Correlation between tetracycline resistance and plasmid carriage. Plasmid distributions among C. jejuni isolates with and without tetracycline resistance were examined. A total of 49 (82%) of 60 tetracycline-resistant isolates carried plasmids, the majority of which ranged from 44 to 64 kb in size. About 50% of the plasmids were 50 kb. Further analysis revealed that 49 (89%) of 55 strains for which the tetracycline MIC was 25 μg/ml and higher carried tetracycline resistance plasmids, whereas only one of five strains for which the MIC was 12.5 μg/ml had the plasmid. On the other hand, 41 (85%) of 48 tetracycline-susceptible isolates had no plasmid. The antimicrobial resistance and plasmids of C. coli isolates are summarized in Table 2. For the 10 isolates of C. coli, all of the tetracycline-resistant isolates had plasmids ranging from 5.5 to 98 kb and 3 of the tetracycline-susceptible isolates had no plasmid.

Conjugation experiments. We examined four tetracycline-resistant C. coli isolates and six randomly selected tetracycline-resistant C. jejuni isolates for their ability to transfer tetracycline resistance by conjugation (Table 3). Five C. jejuni isolates containing 50-kb plasmids (pNR9122, pNR9124, pNR9142, pNR9150, and pNR9216) transferred tetracycline resistance to the recipient by conjugation, yielding transconjugants containing the respective plasmids. Strain C-147, containing a 135-kb plasmid, also transferred tetracycline resistance by plate mating, and a 45-kb plasmid was recovered. This unusual finding was obtained twice with 10 colonies examined each time. We could not purify plasmid DNA from C-147 because of its large size. Three plasmids, pNR9166, pNR9131, and pNR9581, from C. coli isolates resistant to tetracycline, kanamycin, and erythromycin were not transferable. From C. coli C-589, which was resistant to chloramphenicol, erythromycin, kanamycin, and tetracycline and contained plasmids pNR9589 (58 kb) and pNR9588 (5.5 kb), resistances to chloramphenicol, kanamycin, and tetracycline but not erythromycin were transferred together with recovery of a 58-kb plasmid. The frequencies of conjugal transfer in broth mating ranged from $1 \times 10^{-5}$ to $2.5 \times 10^{-6}$ transconjugants per donor cell. However, pNR9124 and pNR9147 were not transferable in broth matings and also appeared less transferable than the others in plate matings. Restriction patterns of original and transconjugant plasmids from C. jejuni isolates digested with BglII on agarose gel electrophoresis are shown in Fig. 2. Plasmid pNR9122 and its transconjugant plasmid had identical profiles, whereas plasmid pNR9124 and its transconjugant plasmid shared most fragments but were not completely identical.

Three transconjugants (T-122, T-142, and T-589) were tested again for donor ability by plate mating to a tetracycline-susceptible and plasmid-free C. jejuni strain, and tetracycline resistance was again transferred to the second C. jejuni recipient.

Tetracycline resistance of C. jejuni was not transferred to nalidixic acid-resistant E. coli CHS-2 by conjugation.

Restriction endonuclease digestion analysis of the plasmid DNA. Electrophoretic profiles of plasmids from C. jejuni (pNR9122, pNR9124, and pNR9142), along with those from C. coli (pNR9131, pNR9581, and pNR9589), obtained after PstI, BglII, and HindIII digestion are shown in Fig. 3. Plasmids pNR9122, pNR9124, and pNR9142 showed identical profiles when digested with all three enzymes. Plasmids pNR9131 and pNR9581 showed identical profiles when digested with PstI (Fig. 3A, lanes 4 and 5) but showed diverse profiles with BglII and HindIII digestion, although sharing some fragments of the same size (Fig. 3B and C, lanes 4 and 5). On the other hand, conjugative tetracycline-, kanamycin, and chloramphenicol-resistant plasmid pNR9589 showed different profiles from those of pNR9131 and pNR9581 when digested with the three enzymes. The PstI profile of pNR9589 was identical with that of tetracycline-resistant plasmids from C. jejuni (pNR9122, pNR9124, and pNR9142), except that an extra 2.1-kb fragment was observed in pNR9589 (Fig. 3A, lanes 1 to 3 and 6). However, the electrophoretic profiles of pNR9589, pNR9131, and pNR9581 ob-

FIG. 1. Agarose gel (0.6%) electrophoresis of plasmid DNA from C. jejuni and C. coli isolates. Lanes: 1, C-142; 2, C-147; 3, C-150; 4, C-166; 5, C-131; 6, C-216; 7, C-5816; 8, C-589; 9, standard containing R6-5 (100 kb), S-a (40 kb), and pNR353 (12 kb).
tained after digestion with the other two enzymes were different from those of the conjugative tetracycline-resistant plasmids.

**Cloning of the kanamycin and chloramphenicol resistance genes.** To examine the resistance genes of *C. coli* in more detail, we attempted to clone the genes into *E. coli* Χ1776, since it proved impossible to transfer plasmids from *Campylobacter* strains to *E. coli* by transformation or conjugation. We failed to clone the tetracycline resistance gene of *C. coli* to *E. coli* with pUC13 as a vector but were able to clone the kanamycin resistance genes of pNR9131, pNR9581, and pNR9589 to *E. coli*. The chloramphenicol resistance gene of pNR9589 was also cloned and expressed in *E. coli*. The properties of the recombinant plasmids are summarized in Table 4. When kanamycin and chloramphenicol resistance genes were cloned from pNR9589, the frequency of chloramphenicol-resistant transformants was 100-fold less than that of kanamycin-resistant transformants, and all the chloramphenicol-resistant transformants were also resistant to kanamycin. HindIII digestion profiles of the original and recombinant plasmids are shown in Fig. 4. Recombinant plasmids pNR9010 and pNR9011 contained the kanamycin resistance genes of pNR9131 and pNR9581 in the 7.8- and 5.8-kb HindIII fragments, respectively (Table 4). The kanamycin-resistant recombinant, pNR9012, contained the 1.4-kb HindIII fragment of pNR9589. pNR9013, which mediated kanamycin and chloramphenicol resistances, contained the 1.4- and 3.5-kb HindIII fragments. However, any one of the fragments alone did not express chloramphenicol resistance when cloned separately (data not shown), suggesting that linkage of the two HindIII fragments is necessary for expression of chloramphenicol resistance. To see whether similarities existed among these kanamycin resistance genes, recombinant plasmids were double-digested with HindIII and HaeIII or with HindIII and HpaII (Fig. 5A and B). The fragments indicated by arrows in Fig. 5A and B were found to be common to all recombinant plasmids. At least about 400 to 500 base pairs of the 1,400-base-pair HindIII fragment of pNR9012 was shared by pNR9010 and pNR9011.

**DISCUSSION**

MIC test results for our clinical isolates of *Campylobacter* were in general agreement with those previously reported except for an unusually high incidence of tetracycline resistance in our strains (6, 11, 16, 25, 26, 28). Tetracycline and erythromycin resistances in thermophilic campylobacters have recently been reported (3, 5, 10, 16, 26, 28). Fifty-five percent of our *C. jejuni* isolates were resistant to tetracy-
cline, although none of them were resistant to erythromycin. The reported incidences of tetracycline resistance are 4 to 8% (in Belgium) (25, 26), 12% (in Canada) (11), 30% (in the United States) (28), 38% (in Israel) (16), and 69% (in Japan) (10). In other Japanese reports, tetracycline resistance is also described as being common in human isolates. Vanhoof et al. showed that antimicrobial MIC distributions for C. jejuni isolates of human origin were very comparable to those of C. jejuni isolates of animal origin (24), and many investigators, after determining the MICs for C. jejuni and C. coli isolates from humans, poultry, and swine (5, 24, 28), have suggested that the differences in antimicrobial susceptibility patterns might be related to the ultimate source of the strains tested. Considering this background, the fact that tetracycline resistance in poultry isolates is not as common as that in human isolates in Japan (22%) (10) might suggest the existence of another source of infection besides poultry.

The MIC patterns for the 10 C. coli isolates were considerably different from those for the C. jejuni isolates, and multiple-antibiotic resistance including tetracycline and erythromycin resistance was commonly seen. This was also noticed by Wang et al. (28) and Fliegelman et al. (6), although the antimicrobial agents tested were not necessarily identical. It is of considerable interest that antibiotic resistance occurs far more frequently in C. coli than in C. jejuni, despite the absence of any difference in the antimicrobial agents added to animal feed.

As for the R plasmids encoding tetracycline resistance in C. jejuni, Tenover et al. have shown that they transfer tetracycline resistance and vary in size from 42 to 133 kb (22). Our studies also demonstrate that the majority (82%) of tetracycline-resistant isolates carry plasmids from 44 to 59 kb in size, particularly 89% of strains for which MICs are 25 μg/ml and higher. The reason why 11 tetracycline-resistant isolates had no plasmid cannot be explained at present. Each of six randomly selected plasmid-carrying, tetracycline-resistant C. jejuni isolates transferred tetracycline resistance to the C. fetus subsp. fetus recipient by conjugation. The 50-kb plasmids showed identical patterns for digestion with PstI, BglII, and HindIII, but there were some differences in the frequency of transfer among them. Plasmid pNR9124, with a lower frequency of transfer, did not show an exactly identical digestion profile for the original and transconjugant plasmids. The differences might be related to minor changes of the plasmid during conjugation. Concerning the unusual fact that plasmid pNR9147 was reduced in size from 135 kb to 45 kb after transfer, it is possible that the donor strain contained the 45-kb plasmid but it could not be visualized or that partial deletion of the plasmid might have occurred. The finding that transconjugants were able to transfer tetracycline resistance again to another tetracycline-susceptible C. jejuni, although none of them were resistant to erythromycin. The reported incidences of tetracycline resistance are 4 to 8% (in Belgium) (25, 26), 12% (in Canada) (11), 30% (in the United States) (28), 38% (in Israel) (16), and 69% (in Japan) (10). In other Japanese reports, tetracycline resistance is also described as being common in human isolates. Vanhoof et al. showed that antimicrobial MIC distributions for C. jejuni isolates of human origin were very comparable to those of C. jejuni isolates of animal origin (24), and many investigators, after determining the MICs for C. jejuni and C. coli isolates from humans, poultry, and swine (5, 24, 28), have suggested that the differences in antimicrobial susceptibility patterns might be related to the ultimate source of the strains tested. Considering this background, the fact that tetracycline resistance in poultry isolates is not as common as that in human isolates in Japan (22%) (10) might suggest the existence of another source of infection besides poultry.

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![FIG. 3. Agarose gel (0.7%) electrophoresis of plasmid DNA from C. jejuni and C. coli isolates after digestion with the endonucleases PstI (A), BglII (B), and HindIII (C). Lanes: 1, pNR9122; 2, pNR9124; 3, pNR9142; 4, pNR9131; 5, pNR9581; 6, pNR9589; 7, PstI-digested lambda bacteriophage DNA. Electrophoresis was carried out at 160 V for 4 h for panels A and B and at 40 V overnight for panel C. The numbers at the side indicate the molecular sizes in kilobases.](image1)

![FIG. 4. Agarose gel (1.0%) electrophoresis of recombinant and original plasmids after digestion with HindIII. Lanes: 1, PstI-digested lambda DNA as size standard; 2, pUC13; 3, pNR9131; 4, pNR910; 5, pNR9581; 6, pNR9011; 7, pNR9589; 8, pNR9012; 9, pNR9013. The numbers at the side indicate the molecular sizes in kilobases.](image2)
jejuni strain suggests that tetracycline resistance in C. jejuni is mediated by conjugative plasmids, although other possibilities such as transformation, transduction, or chromosomal mobilization have not been completely excluded. Plasmid-curing or insertion inactivation studies were not done.

Although the high incidence of multiple-antibiotic resistance in C. coli strains is well known (28), there have been few reports concerning the plasmids involved. We isolated three plasmids from multiple-antibiotic-resistant isolates of C. coli. The restriction endonuclease digestion profiles of these plasmids were different from each other, although a few common fragments were observed (Fig. 3), and were also different from those of the tetracycline resistance plasmid in C. jejuni. The PstI digestion profile of pNR9589 was quite similar to that of the tetracycline resistance plasmid of C. jejuni, whereas the digestion profiles with other endonucleases were different. Recently, Taylor et al. (20) and Tenover et al. (22) reported the molecular size distribution of a tetracycline resistance plasmid of Campylobacter spp. that yielded different fragments by digestion with several restriction endonucleases, although these hybridized with each other.

From cloning experiments, the kanamycin and chloramphenicol resistance genes of pNR9589 appeared to be closely linked to each other. Although kanamycin resistance was encoded in the 7.8-, 5.8-, and 1.4-kb HindIII fragments of pNR9131, pNR9581, and pNR9589, respectively (Table 4; Fig. 4), these fragments contained common smaller fragments (Fig. 5), suggesting that the kanamycin resistance genes found in our C. coli strains were closely related. Recently, Lambert et al. isolated a strain of C. coli carrying a 47.2-kb conjugative plasmid (pIP1433) encoding both tetracycline and kanamycin resistance (13). Its kanamycin resistance was due to 3'-aminoglycoside phosphotransferase type III, an enzyme previously confined to gram-positive cocci. More recently, Kotarski et al. reported that a 59-kb self-transmissible plasmid encoding resistance to kanamycin and tetracycline contains a transposable kanamycin resistance gene (12). We have not yet explored these possibilities.

FIG. 5. Polyacrylamide gel (5%) electrophoresis of recombinant plasmids after double-digestion with HindIII and HaeIII (A) and HindIII and HpaII (B). Lanes: 1, pNR9010; 2, pNR9011; 3, pNR9012; 4, pUC13. Arrows indicate the common fragments among recombinant plasmids except those of pUC13. The numbers at the side indicate the molecular sizes in base pairs.

ACKNOWLEDGMENTS

We thank N. Goto and S. Horiuchi for technical advice and critical review.

LITERATURE CITED


