Chloramphenicol Transposons Found in *Salmonella naestved* and *Escherichia coli* of Domestic Animal Origin

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*Salmonella naestved* strain AHI-21, of calf origin, harbors a conjugative R plasmid of group H1, pTE21, which encodes resistance to chloramphenicol (Cm), tetracycline, streptomycin, and sulfadimethoxine. *Escherichia coli* strain AHI-1, of pig origin, also harbors a conjugative R plasmid of group Ia, pTE1, which encodes resistance to chloramphenicol and trimethoprim. When either of these R plasmids coexisted with a nonconjugative plasmid, pMK1, which is a composite plasmid of CoIE1 and a kanamycin transposon (Tn5), transposition of the Cm' gene into pMK1 occurred independently of the host recA gene function, indicating that both R plasmids contained Cm transposons, Tn3351 and Tn3352. Electron microscopic analysis of self-annealed and heteroduplex molecules showed that they were of approximately 1.7 megadaltons in size and were inserted within the CoIE1 loop region of pMK1. However, inverted repeat structures were not seen in these two Cm transposons. Restriction enzyme cleavage analysis showed that both Tn3351 and Tn3352 were indistinguishable in their cleavage patterns, suggesting that they were almost identical in deoxyribonucleic acid sequence despite the difference in their origin. These results suggest that the reciprocal transposition of the Cm' gene might have occurred between *Salmonella* and *E. coli* in nature.

An interesting feature of drug resistance among enteric bacteria isolated from domestic animals is that many *Salmonella* strains have recently become resistant to chloramphenicol (Cm), and most of them carry conjugative R plasmids with chloramphenicol resistance (10, 23, 28). Our previous study also indicated a high incidence of R plasmids conferring chloramphenicol resistance among *Salmonella* strains isolated from animals, especially from calves in Japan (26). An increasing incidence of chloramphenicol-resistant *Salmonella* strains will create serious problems not only for animal hygiene but also for public health, because chloramphenicol is the most effective antibiotic for the treatment of systemic salmonellosis in humans.

There is now clear laboratory evidence that many plasmid genes determining drug resistance reside in discrete deoxyribonucleic acid (DNA) units, termed transposons (4, 6). They can move from one replicon to another independently of the host recA gene function. It seems, therefore, that the sequential transposition of such genetic elements may be the principal mechanisms by which plasmids can accumulate multiple resistance determinants (6). Little information is available, however, on the incidence of such transposable elements among enteric bacteria of animal origin. To obtain more information, the present study was undertaken to demonstrate Cm transposons in *Salmonella* and *Escherichia coli* isolated from domestic animals.

**MATERIALS AND METHODS**

**Bacterial strains and plasmids.** Table 1 lists the bacterial strains and plasmids used.

**Media and drugs.** Heart infusion broth (Difco Laboratories, Detroit, Mich.), Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.), and L broth (16) were used as liquid media. DHL agar (Eiken Chemical Co., Ltd., Tokyo), heart infusion agar (Difco), and L broth agar (16) were used as solid media. The drugs used were chloramphenicol, kanamycin (Km), tetracycline (Tc), streptomycin (Sm), sulfadimethoxine (Su), nalidixic acid, trimethoprim (Tm), and rifampin.

**Conjugation and transformation.** Conjugal transfer of drug resistance was examined by mixed cultivation (25). Selection for the transconjugant was performed on DHL agar containing appropriate drugs. Transformation with plasmid DNA was performed by the method of Cohen et al. (7), with *E. coli* C600 as the recipient. After the 42°C heat pulse, the mixture of recipient cells (0.2 ml) and DNA (0.5 pg/0.1 ml) was diluted in L broth and incubated overnight at 30°C to allow phenotypic expression. Samples of bacterial cul-
ture were then plated on L broth agar supplemented with chloramphenicol (25 μg/ml) and kanamycin (25 μg/ml) and incubated at 37°C for 24 h.

Isolation of plasmid DNA. Plasmid DNA was isolated by the method of Womble et al. (29). Bacteria were cultured with shaking in 200 ml of Trypticase soy broth at 30°C for 18 to 24 h. The cells were harvested by centrifugation and lysed with Triton X-100. The subsequent cleared lysate was concentrated with polyethylene glycol (molecular weight, 6,000) in the presence of 1 M NaCl and subjected to CsCl-ethidium bromide equilibrium density gradient sedimentation at 37,000 rpm for 40 h.

Restriction endonuclease digestion. The endonucleases EcoRI and PstI were obtained from Takara Shujo Co. Ltd., Kyoto. The endonuclease PvuII was obtained from New England Biolabs, Beverly, Mass. All DNA digestions were conducted at 37°C for 2 h in 50 μl of the reaction mixture. Buffers used were as follows: for EcoRI, 100 mM tris(hydroxymethyl)-aminomethane (Tris)-hydrochloride (pH 7.5)-10 mM MgCl2-50 mM NaCl; for PstI and PvuII, 6 mM Tris-hydrochloride (pH 7.5)-6 mM MgCl2-60 mM NaCl.

Analytical gel electrophoresis. Gel electrophoresis was performed by the method of Meyers et al. (18). An agarose electrophoretic apparatus (Atto Co., Ltd., Tokyo) was used with 1.5% (wt/vol) agarose-1 (Doojin Chemical Co., Ltd., Tokyo) containing Tris-borate buffer (89 mM Tris-25 mM ethylenediaminetetraacetic-8.9 mM boric acid, pH 8.4). The gel plate was 14 by 13.8 by 0.3 cm. Gels were run at 100 V for 6 h and then stained for 15 min with ethidium bromide (0.5 μg/ml). The size of the DNA fragments produced by digestion was usually estimated from the mobility of each fragment relative to HindIII-digested lambda phage fragments.

Electron microscopic analysis. Homologous and heteroduplex molecules were prepared by the method of Davis et al. (8). Samples were spread from a hyperphase containing 0.5 to 1.0 μg of DNA per ml, 0.1 mM Tris-hydrochloride (pH 8.5), 10 mM ethylenediaminetetraacetic acid, and 5% (vol/vol), formamide in 10 mM Tris-hydrochloride (pH 8.5). The cytochrome c film was picked with Parlodion membranes, stained with uranyl acetate and shadowed with platinum-palladium (80:20) at an angle of 7°.

RESULTS

Transposability of Cm' genes found on pTE21 and pTE1. To examine the transposability of the Cm' gene, both R-plasmids, pTE21 from S. naestved and TE1 from E. coli, were transferred by conjugation to recA strain AB2463, carrying pMK1. The transformation was then carried out with DNA preparations extracted from the transconjugants AB2463(pMK1, pTE21) and AB2463(pMK1, pTE1). When selection was carried out with both kanamycin and chloramphenicol, transformants of C600 were found with each DNA preparation at a frequency about 10⁻⁷ per recipient. However, some of these transformants were found to carry two plasmids simultaneously in their cells, suggesting that double transformation might have occurred in such cells. The remaining transformants examined were found to carry a single plasmid DNA of approximately 9.4 megadaltons (Mdal) by agarose gel electrophoresis and electron microscopy. It was also found that E. coli C600 transformed with these 9.4-Mdal DNAs acquired the parental resistance characters (Km Cm) and produced active colicin. When a polA strain, P3478, was used as the recipient, however, no resistant transformants could be obtained. Since the ColE1 plasmid requires an active polA gene for its replication system (14), these results indicated that the Cm' gene was transposed from both pTE21 and pTE1 to the pMK1 plasmid DNA. Thus, the 9.4-Mdal plasmids obtained in this experiment were designated pMK1::Tn3351 and pMK1::Tn3352, respectively.

Electron microscopic analysis of pMK1::Tn3351 and pMK1::Tn3352. As described above, both pMK1::Tn3351 and pMK1::Tn3352 had a molecular mass of about 9.4 Mdal. On the other hand, their parental pMK1 was estimated to be about 7.7 Mdal; this estimate was slightly lower than that reported by Yamamoto and Yokota (30). It was therefore suggested that the transposable Cm' gene might be present in the 1.7-Mdal element. To confirm this hypothesis, pMK1::Tn3351, pMK1::Tn3352, and pMK1 DNAs were denatured and then self-annealed and examined by electron microscopy. All of the DNA samples showed the presence of inverted repeat structures which are characteristic of Tn5 (30) (Fig. 1-3). The sizes of Tn5 structures observed in all preparations were identical and were calculated to be about 3.5 Mdal. However, the sizes of single-stranded ColE1 loops observed
a pMK1::Tn3351/ColEl heteroduplex molecule. As is clear from this figure, a single-stranded insertion loop of about 1.7 Mdal was observed within the double-stranded ColEl loop region. However, no inverted repeat structure was seen except for that of Tn5. A similar result was obtained in a pMK1::Tn3352/ColEl heteroduplex. From these results, we concluded that the insertion loop observed under electron microscopy consisted of the Cm transposon.

Restriction enzyme cleavage of pMK1::Tn3351 and pMK1::Tn3352. To examine the similarity between Tn3351 and Tn3352, both plasmid DNAs were digested with three restriction endonucleases, and the resulting DNA fragments were subjected to agarose gel electrophoresis. Both plasmid DNAs were cleaved by EcoRI into two, by PvuII into seven, and by PstI into eight sections (Fig. 5). Moreover, the cleavage patterns were found to be identical, suggesting that they have homologous DNA sequences. On the other hand, the numbers of cutting sites on the pMK1 DNA supplied as controls were found to be one for EcoRI, five for PvuII, and six for PstI. These results indicate that both Tn3351 and Tn3352 have a single cleavage site for EcoRI and two each for PvuII and PstI.

DISCUSSION

The transposability of the gene encoding chloramphenicol resistance from one replicon to another was first reported by Kondo and Mitsuhashi (15). They found that E. coli phage P1 could receive the Cm' gene from an R plasmid, pMS14. Further studies revealed that such transposition of the Cm' gene can occur independently of the host recA gene function (9), and this transposable Cm' gene is now called

![Fig. 1-3. Electron micrographs of formamide-spread single-stranded, self-annealed molecules. ColEl was used as an internal reference. Bars, 0.5 μm.](image1)

**Fig. 1.** Structure of pMK1::Tn3351.
**Fig. 2.** Structure of pMK1::Tn3352.
**Fig. 3.** Structure of pMK1.

in pMK1::Tn3351 and pMK1::Tn3352 were found to be about 1.7 Mdal larger than that of pMK1: 5.9 Mdal for pMK1::Tn3351 and pMK1::Tn3352 and 4.2 Mdal for pMK1. Figure 4 shows...
plasmids among *Salmonella* strains. Frequent use of drugs, especially chloramphenicol in treating animals will undoubtedly give a selective advantage to such events in nature.

Tn3351 and Tn3352 are quite similar in both size (about 1.7 Mdal) and a structural feature (no inverted repeats) to Tn9 and other Tn9-like Cm transposons originally recognized on pMS14 and RNR1 (1, 5, 17, 24, 31). In addition, restriction enzyme cleavage analysis showed that these two Cm transposons are very similar to Tn9. For instance, the number of cutting sites with EcoRI (one site) and PstI (two sites) on both Tn3351 and Tn3352 was found to be consistent with that of Tn9 (1), although a small difference was observed with PvuII digestion, e.g., two sites on both, whereas one on Tn9 (1). These results indicate that both have DNA sequences in common with Tn9. Considering that Tn9 and other Tn9-like Cm transposons originated from naturally occurring *Shigella* strains of human origin, it is suggested that such Tn9-like Cm transposons might be widely distributed among various enteric bacteria of human and animal origin.

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**LITERATURE CITED**


