Dispersal in *Campylobacter* spp. of *aphA-3*, a Kanamycin Resistance Determinant from Gram-Positive Coci

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DNA annealing studies indicated that kanamycin resistance in *Campylobacter* strains from various geographical areas is encoded by a gene structurally related to *aphA-3* of gram-positive cocci. This finding confirms the transfer of genetic material between gram-positive and gram-negative bacteria under natural conditions.

*Campylobacter coli* and *C. jejuni* are gram-negative bacteria often responsible for bacterial acute gastroenteritis in humans (5). Resistance to ampicillin, chloramphenicol, erythromycin, kanamycin, streptomycin, and tetracycline has been detected in the genus *Campylobacter* (11, 18, 20). The genes conferring resistance to chloramphenicol, kanamycin, and tetracycline are usually borne by plasmids self-transferable to other *Campylobacter* species but not to *Escherichia coli* (11, 18, 20).

### TABLE 1. Bacterial strains used

| Strain   | Species         | Phenotypic characteristics | Origin         | Transfer of Km and Tc determinants | Plasmid size (kilobases) | Reference |
|----------|-----------------|---------------------------|----------------|-----------------------------------|--------------------------|-----------|
| BM2633   | *C. jejuni*     | Km Sm Tc                  | Thailand, human| +                                 | 45                       | This work |
| BM2634   | *C. jejuni*     | Km Sm Tc                  | Thailand, human| +                                 | 61                       | This work |
| BM2635   | *C. coli*       | Ap Km Tc                  | Thailand, animal| +                                 | 48                       | This work |
| BM2636   | *C. coli*       | Km Sm Tc                  | Vietnam, animal| +                                 | 49                       | This work |
| BM2637   | *C. coli*       | Vietnam, animal           | Vietnam, animal| +                                 | 49                       | This work |
| BM2638   | *C. coli*       | Ap Km Sm Tc               | France, human  | –                                 | 31                       | This work |
| BM2509   | *C. coli*       | Ap Cm Em Km Sm Sp Tc      | France, human  | +                                 | 47.2                     | 11        |
| 981      | *C. coli*       | Em Km Sm Tc               | Spain, animal  | +                                 | 64                       | 17        |
| BM2560   | *C. fetus* subsp.| Nal Rif                   | France, spontaneous | +                   | 47.2                     | 11        |
| *C. fetus* | mutant of 5396 |                          |                               | +                   | 47.2                     | 11        |
| MK175    | *C. jejuni*     | Ap Tc                     | Canada, human   | +†                                | 45                       | 20        |

* Phenotypic characters of plasmids according to Novick et al. (14). Chromosomal resistances: Nal, nalidixic acid; Rif, rifampin.

† Mating was on a filter for 24 h with strain BM2560 as a recipient (11). Selection was with 50 μg of nalidixic acid per ml plus either 15 μg of erythromycin, 20 μg of kanamycin, or 8 μg of tetracycline per ml.

‡ Size estimated by 0.8% agarose gel electrophoresis of purified plasmid DNA cleaved by HincII or BglII with bacteriophage λ DNA digested with *PstI* as the internal standard.

† Transfer of tetracycline resistance only.

Bacterial resistance to kanamycin and structurally related antibiotics is generally the result of the synthesis of 3'-aminoglycoside phosphotransferases [APH(3')] (7). In human pathogens, three types (I, II, and III) of APH(3') can be discriminated according to their in vitro substrate profiles (3, 15, 22), and the corresponding genes *aphA-1*, *aphA-2*, and *aphA-3* do not cross-hybridize (11, 24).

We recently reported that high-level resistance to kanamycin in *C. coli* BM2509 was due to the presence of a plasmid-borne *aphA-3* gene (11, 23) and that a chromosomally located *aphA-1* gene was responsible for kanamycin resistance in *Campylobacter*-like organism strain BM2196 (16). This genetic heterogeneity prompted us to study the distribution of *aphA* genes in clinical *Campylobacter* isolates resistant to high levels of kanamycin.

A total of 160 strains of *Campylobacter* spp. from humans and animals, obtained from diverse geographical areas during 1984 to 1985, were screened for high-level resistance to kanamycin (MIC, >2,000 μg/ml). Six strains that were found to be resistant to kanamycin were also resistant to tetracycline (MIC, >64 μg/ml) (Table 1). All the strains, except BM2635, were resistant to streptomycin (MIC, >1,024 μg/ml), and two strains (Table 1) were resistant to ampicillin (MIC, >128 μg/ml). Resistance to kanamycin and tetracycline was transferable by conjugation in all strains except BM2638. Resistance to ampicillin and streptomycin never transferred, and we did not succeed in transferring erythromycin resistance (MIC, ≥1,024 μg/ml) from strains 981 and BM2509.

The six strains studied were resistant to kanamycin but susceptible to tobramycin (3' deoxykanamycin B), indicating that the 3'-hydroxyl group is the site of modification. The
distribution of nucleotide sequences related to *aphA*-1, *aphA*-2, and *aphA*-3 genes (Table 2) in total DNA of kanamycin-resistant *Campylobacter* strains was studied by dot blot hybridization under high-stringency conditions (19). We detected homology with a probe specific for *aphA*-3 (Fig. 1) but not with DNA fragments internal to *aphA*-1 and *aphA*-2 (data not shown). In similar experiments, we did not find homology between total DNA of strains BM2509 and 981 resistant to erythromycin and probes specific for the genes encoding erythromycin esterases (*ereA* and *ereB*) and ribosomal methylases (*ermA*, *ermB*, *ermC*, *ermD*, and *ermF*) (2).

Plasmid DNA from transconjugants and strain BM2638 was purified (4), digested with *Hin*II (Fig. 2A) or *Bgl*II (Fig. 3A), and analyzed by agarose gel electrophoresis. The kanamycin resistance plasmids had distinct restriction patterns. Strains BM2633 and BM2634, isolated from different patients in Thailand, harbored similar plasmids which appeared different from the plasmid present in strain BM2635, also isolated in Thailand but from an animal source. The plasmid in strain BM2638, which did not transfer kanamycin and tetracycline resistance to other *Campylobacter* strains, had a distinct restriction endonuclease profile and a smaller molecular weight (Fig. 2A, Table 1).

The plasmid DNA digested by *Hin*II was transferred to a nitrocellulose filter (12) and hybridized to the 32P-labeled M13mp8*aphA*-3 probe (Fig. 2B). The kanamycin resistance gene was located on the largest *Hin*II-DNA fragment of all plasmids, except that of strain BM2638, which did not hybridize (Fig. 2B). Since total DNA of BM2638 hybridized with the same probe (Fig. 1), the kanamycin resistance gene was tentatively assigned to a chromosomal location.

We previously suggested that kanamycin resistance in *Campylobacter* spp. results from the acquisition of a gene from a gram-positive bacterium (11, 23). Plasmid DNA digested by *Bgl*II was transferred to a nitrocellulose filter (12) and hybridized to pMAK175 DNA 32P labeled in vitro by nick translation (Fig. 3B). This plasmid is representative of the tetracycline resistance plasmids of *Campylobacter* spp. (20). Although they had different restriction endonuclease patterns (Fig. 3A), plasmids mediating kanamycin resistance shared extensive sequence homology with pMAK175 (Fig. 3B). The plasmid in strain BM2638 which does not encode kanamycin or tetracycline resistance did not hybridize to the pMAK175 probe. This observation and the stability of the plasmids in their original hosts and also in *C. fetus* subspp. *fetus* BM2560 support the notion that kanamycin resistance in *Campylobacter* spp. results from the acquisition of a gene rather than of a replicon in bloc.

We recently established that resistance to kanamycin in *C. coli* BM2509 was due to a plasmid-encoded APH(3') of type III, an enzyme not detected previously in gram-negative bacteria (11, 23). In this report, we extend this notion to *Campylobacter* strains independently isolated in various countries. The fact that the corresponding *aphA*-3 genes are

![FIG. 1. Analysis of DNA by dot blot hybridization. Total DNA of *Campylobacter* strains was transferred to a nitrocellulose filter and hybridized to the M13mp8*aphA*-3 probe labeled with 32P in vitro (2.7 × 106 cpm). Bacteriophage M13 DNA has no homology with *Campylobacter* DNA (result not shown). A1, strain BM2509; A2, BM2633; A3, BM2634; A4, BM2635; A5, BM2636; A6, BM2637; A7, BM2638; B1, B2, and B3 correspond to the intragenic fragments of *aphA*-1, *aphA*-2, and *aphA*-3, respectively; B4, pMAK175; B5 to B8, kanamycin-susceptible *Campylobacter* strains.](http://aac.asm.org/)

![FIG. 2. Analysis of plasmid DNA by agarose gel electrophoresis (A) and by hybridization (B). Plasmid DNA (4) was digested with *Hin*II, and the resulting fragments were separated by 0.8% agarose gel electrophoresis, transferred to a nitrocellulose filter, and hybridized to the in vitro 32P-labeled M13mp8*aphA*-3 probe. Plasmid DNA of strain BM2509 and plasmid pMAK175 served as positive and negative controls, respectively. Bacteriophage λ DNA digested with *Pst*I was used as the internal size standard. The arrow indicates the 530-bp *Hpa*I fragment intragenic to *aphA*-3 of pAT93.](http://aac.asm.org/)
fractionated and genetic nitrocellulose a transposon compatible with bacteria these indicate a bacteriophage λ DNA digested with PstI was used as the internal size standard.

located on different plasmids or in the chromosome with the presence of this resistance determinant on a transposable element. However, direct evidence for a transposon in Campylobacter species is still lacking.

Although emergence of kanamycin resistance in Campylobacter spp. constitutes the first example of transfer of genetic material between gram-positive and gram-negative bacteria under natural conditions (23), the gene flux between these two groups of microorganisms is not limited to this resistance gene or to this bacterial genus. Tetracycline resistance in Campylobacter spp. is due to the presence of the determinant tetO (19). This gene exhibits 76% sequence identity with tetM from Streptococcus species, and the substitutions are scattered throughout, suggesting that the two genes diverged from a common ancestor (19). Analysis of the optimal codon usage and of the transcription and translation signals of tetO and the finding of this gene in Enterococcus and Streptococcus species of various groups indicate that tetO, as well as tetM, originates in gram-positive cocci (19; R. Zilhao et al., submitted for publication). It also appears that streptomycin resistance by adenyllylation of the antibiotic in Campylobacter spp. is due to the presence of a 6-amino glycoside nucletidyltransferase, an enzyme confined so far to gram-positive cocci (H. Pinto-Alphandary et al., manuscript in preparation). In all the cases studied to date, antibiotic resistance in Campylobacter spp. appears to originate in gram-positive cocci. The fact that a fundamental difference in gene expression seems to exist between members of the family Enterobacteriaceae and Campylobacter species is consistent with this observation (9). The bla gene and the replication machinery of pBR322 (9) and genes apHA-2 and aadA (A. Labigne, personal communication) from members of the Enterobacteriaceae are not expressed in Campylobacter spp. In contrast, apHA-1 and aadA encoding kanamycin and streptomycin resistance, respectively, in gram-negative bacteria have been found in a Campylobacter-like organism (16; Pinto-Alphandary et al., in preparation).

Transfer of genetic material from gram-positive bacteria to members of the family Enterobacteriaceae (1, 6) and to Neisseria gonorrhoeae (13) has also been documented. Since this gene flux involves microorganisms that are extremely distant related, we postulated that transfer under natural conditions could have occurred only by transformation or conjugation followed by illegitimate recombination (23). In view of this hypothesis, it is all the more interesting that transfer of plasmid DNA by conjugation from gram-negative to gram-positive bacteria (21) and vice versa (P. Trieu-Cuot et al., submitted for publication) has recently been obtained under laboratory conditions.

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