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Emergence of Aminoglycoside Resistance Genes *aadA* and *aadE* in the Genus *Campylobacter*

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Resistance to streptomycin or spectinomycin or both in five *Campylobacter coli* strains, two *Campylobacter jejuni* strains, and a *Campylobacter*-like strain was studied by enzymatic assays and dot blot hybridization. Resistance was due to 6- or 3″,9-aminoglycoside adenylyltransferases and to new types of phospho- and adenylyltransferases.

Campylobacter spp. are among the pathogenic agents most frequently associated with gastroenteric diseases in humans and animals (1). Resistance to ampicillin, chloramphenicol, erythromycin, kanamycin, spectinomycin, streptomycin, and tetracycline in these gram-negative bacteria has been reported (7). However, only chloramphenicol, kanamycin, and tetracycline resistances have been shown to be plasmid mediated (7, 14, 16, 18). Kanamycin resistance may also be chromosomally encoded (12).

Forty human and animal isolates of *Campylobacter* spp. from diverse geographical areas, obtained during 1984 and 1985, were screened for resistance to streptomycin or spectinomycin or both. Seven strains—four *Campylobacter coli*, two *Campylobacter jejuni*, and a CLS—were found to be resistant and were studied further; their relevant characteristics are given in Table 1. *C. coli* BM2509, resistant to streptomycin and spectinomycin (7), and BM2635, susceptible to both antibiotics (14), were also included. *Campylobacter* strains were grown on Mueller-Hinton agar (Diagnostics Pasteur, Marnes-la-Coquette, France) containing 5% defibrinated horse blood at 37°C under microaerobic conditions in a GasPak jar (BBL Microbiology Systems, Cockeysville, Md.) with the catalyst in place containing a CampyPak envelope (BBL). The CLS was grown at 37°C under microaerobic conditions in the presence of a catalyst. Disk susceptibility tests were on Mueller-Hinton agar supplemented with horse blood. The MICs of streptomycin and spectinomycin against the strains studied are indicated in Table 2. Three strains, BM2509 (7), BM2196 (12), and 981 (15), were resistant to high levels of both antibiotics. Three strains, BM2633, BM2634, and BM2639, were highly resistant to streptomycin only. Two strains, BM2638 and BM2640, were resistant to lower levels of streptomycin and remained susceptible to spectinomycin. The majority of the strains were multiresistant to antibiotics, and streptomycin resistance was often associated with resistance to kanamycin and tetracycline or erythromycin (Table 1). In the

TABLE 1. Bacterial strains

Species	Strain	Origin	Phenotype[a]	Reference or source
C. coli	BM2509	France (human)	Ap Cm Em Km Sm Sp Tc	7
C. coli	BM2635	Thailand (animal)	Ap Km Tc	14
C. coli	BM2638	France (human)	Ap Km Sm Tc	14
C. coli	BM2639	France (human)	Em Sm	J. L. Fauchère
C. coli	BM2640	Thailand (human)	Sm Tc	S. Supavez
C. coli	981	Spain (animal)	Em Km Sm Sp Tc	15
C. jejuni	BM2633	Thailand (human)	Km Sm Tc	14
C. jejuni	BM2634	Thailand (human)	Km Sm Tc	14
CLS	BM2196	France (human)	Em Km Sm Sp Nal	12

[a] From the work of Novick et al. (11). Nal, Nalidixic acid.

Campylobacter spp. can acquire antibiotic resistance genes from gram-positive cocci (7, 16), and a *Campylobacter*-like strain (CLS) has been shown to possess a kanamycin resistance gene from gram-negative bacteria (12). We therefore investigated the origins of streptomycin and spectinomycin resistance determinants in *Campylobacter* spp. Resistance to these antibiotics is generally due to inactivation of the drugs by aminoglycoside phosphotransferases (APH) or adenylyltransferases (AAD) (3). The enzymes which modify streptomycin or spectinomycin or both include APH(3″), AAD(6), and AAD(9) in gram-positive bacteria and APH(3″), APH(6), and AAD(3″)(9) in gram-negative bacteria.

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TABLE 2. MICs of streptomycin and spectinomycin for *Campylobacter* spp. and CLS

Antibiotic	MIC (μg/ml) against strain[a]								
	BM2635	BM2509	BM2638	BM2639	BM2640	981	BM2633	BM2634	BM2196
Streptomycin	4	>2,048	256	>2,048	256	>2,048	>2,048	>2,048	>2,048
Spectinomycin	16	>2,048	16	16	16	>2,048	8	16	>2,048

[a] MICs were determined by the method of Steers et al. (17) with 10^5 CFU per spot. Resistance to streptomycin and spectinomycin was defined as MICs of ≥32 and ≥128 μg/ml, respectively.

kanamycin-resistant strains, except BM2638 and BM2196, kanamycin and tetracycline resistances were self-transferable en bloc to *Campylobacter fetus* (14) but transfer of the other resistance markers was never observed. No extrachromosomal DNA was detected in CLS BM2196 (12).

All the strains tested for aminoglycoside-modifying activity possessed an adenylylating activity and, with the exception of strain 981, no streptomycin or spectinomycin phosphotransferase activity (Fig. 1). *Campylobacter* isolates resistant to moderate or high levels of streptomycin alone encoded an adenylyltransferase which was active against streptomycin but not spectinomycin. Strains resistant to both antibiotics synthesized an enzymatic activity which modified the two drugs. There was therefore a good correlation between the resistance phenotype of the hosts and the substrate profile of the adenylyltransferases.

We tested the strains by dot blot hybridization for the presence of DNA sequences structurally related to all the genes known to specify an AAD: *aadA* (5), encoding an AAD(3'')(9); *aadE* (13), encoding an AAD(6); and *spc* (10), encoding an AAD(9) (Table 3). DNA fragments were labeled with [α-^{32}P]dCTP by nick translation (9) and single-stranded DNA by the proximal primer method (6). The labeled probes were hybridized to DNA immobilized on nitrocellulose filters. Dot blot hybridization (9) under stringent conditions was in 50% formamide at 42°C for 18 h, followed by three washings in 2× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate)–0.1% sodium dodecyl sulfate at room temperature for 5 min and two washings in 0.1× SSC–0.1% sodium dodecyl sulfate at 65°C for 1 h. With the exception of BM2640 and BM2196, all the streptomycin-resistant strains hybridized to the *aadE* probe. This gene encodes, in gram-positive bacteria, an AAD(6) which confers resistance to streptomycin but not to spectinomycin (13). The gene responsible for resistance to the latter antibiotic in BM2509 and 981 has not been identified. Similarly, the gene conferring streptomycin resistance in BM2640 is apparently of a new type. CLS BM2196 hybridized with the *aadA* probe. This resistance determinant is common in gram-negative bacteria, in which it confers resistance to streptomycin and spectinomycin. There are no probes available for the 3''- or 6-streptomycin phosphotransferases of gram-positive and gram-negative bacteria, and phosphorylation of spectinomycin has not yet been described. The substrate profiles of the enzyme(s) in strain 981 may correspond to the production of a new APH(3'')(9) or to the coexistence in the cells of an APH(3'') or an APH(6) and an undescribed APH(9) enzyme.

The results indicate the spread of a streptomycin resistance determinant, *aadE*, thought to be specific for gram-positive cocci (13) in the genus *Campylobacter*, and the presence in the CLS isolate of *aadA*, conferring cross resistance to streptomycin and spectinomycin in numerous species of gram-negative bacteria (5). On the basis of DNA-annealing studies and nucleotide sequence determination, it has been proposed that the kanamycin (7, 21, 22) and tetracycline (8, 16, 18, 23) resistance genes of *Campylobacter* spp. originated in gram-positive bacteria. The finding of *aadE* in *C. coli* and *C. jejuni* constitutes further evidence for the occurrence of transfer of genetic information from gram-positive to gram-negative bacteria under natural conditions (19, 20). In contrast, and by using the same criteria, CLS BM2196 was previously found to harbor a kanamycin resistance gene of gram-negative origin (12). With the detection of *aadA* in the same bacterium, it therefore appears that *Campylobacter* spp. and *Campylobacter*-like organisms can acquire genes from gram-positive and gram-negative bacteria, respectively. This may result, at least in part, from the close contact of large numbers of these bacteria in the gastrointestinal tract. Resistance by adenylylation to spectinomycin in two strains and to streptomycin in another clinical isolate is apparently due to enzymes of new types, and the spectinomycin resistance by phosphorylation reported here has, to our knowledge, not yet been detected in human pathogens. As already pointed out (18), many aspects of *Campylobacter* resistance to antibiotics remain to be clarified.

TABLE 3. Occurrence of adenylyltransferase genes in *Campylobacter* spp.

Probe	Hybridization[a] of DNA from strain								
	BM2635	BM2509	BM2638	BM2639	BM2640	981	BM2633	BM2634	BM2196
aadE[b]	−	+	+	+	−	+	+	+	−
aadA[c]	−	−	−	−	−	−	−	−	+
spc[d]	−	−	−	−	−	−	−	−	−

[a] +, Positive; −, negative.

[b] Single-stranded M13 recombinant DNA containing a 470-base-pair *Hpa*II DNA fragment intragenic to the streptococcal *aadE* gene (13) was prepared as previously described (6).

[c] Plasmid pSH77 (5) DNA was digested with *Rsa*I, and the 478-base-pair intragenic *aadA* fragment was purified.

[d] Plasmid pEM9698 (10) DNA was digested with *Sty*I and *Pst*I, and the 326-base-pair fragment intragenic to the *spc* gene encoded by the staphylococcal transposon Tn*554* was purified.

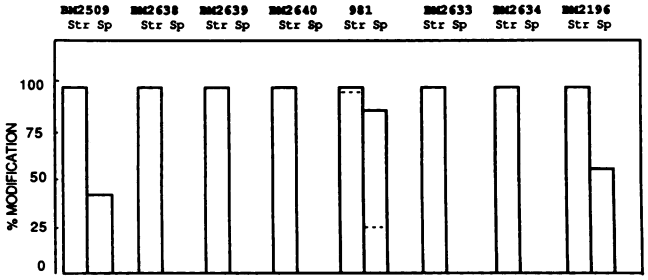

FIG. 1. Substrate profiles of enzymes extracted from *Campylobacter* spp. and CLS. Bacterial extracts were prepared (2) and enzymes were assayed by the phosphocellulose paper-binding technique (4). Adenylylation (——) and phosphorylation (- - -) of spectinomycin (Sp) are expressed relative to streptomycin (Str) values as 100%.

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