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High Genetic Homology between Plasmids of Human and Animal Origins Conferring Resistance to the Aminoglycosides Gentamicin and Apramycin

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***Escherichia coli* and *Salmonella* strains resistant to gentamicin and apramycin were isolated from cattle in France and Belgium and from patients in hospitals. Homology between plasmids of both human and animal origins encoding aminoglycoside 3-*N*-acetyltransferase was revealed by digestion with several restriction endonucleases and confirmed by hybridization with different replicon-specific probes.**

Gentamicin and apramycin were introduced into veterinary therapy in the early 1980s in different countries in Europe. We isolated the first strains of *Salmonella typhimurium* and *Escherichia coli* resistant to apramycin and gentamicin in 1984 (6). This cross resistance was due to the production of the aminoglycoside 3-*N*-acetyltransferase type IV [AAC(3)IV]. The dissemination of this resistance mechanism was followed through the Nationwide Monitoring Network of Antibiotic Resistance in Bovine Pathogens (16). In France (3, 4, 7) and in Belgium (unpublished results), we observed a rapid spread of this resistance in gram-negative bacteria from animals, and a similar dissemination in Great Britain was reported (22, 24). Later on, such resistant strains were isolated from patients in several hospitals in Belgium (5).

In order to investigate the probable exchanges of resistance plasmids between bacteria from animal and human sources, we studied the molecular relationship between apramycin and gentamicin resistance plasmids from both origins. Our results show a strong genetic homology between plasmids of animal and of human origins.

Clinical strains. Twelve apramycin-resistant clinical strains isolated in Belgium or France from 1985 to 1988 have been studied. Ten were animal isolates from calves and from one pig, and two were from patients in hospitals in Belgium (5). The origins and properties of the strains are listed in Table 1.

MIC determinations and biotyping. MICs of gentamicin (Sigma Chemical Co., St. Louis, Mo.), apramycin, and hygromycin (Eli Lilly & Co., Indianapolis, Ind.) were determined on Mueller-Hinton agar (Diagnostics Pasteur, Paris, France). Biotyping was as described previously (7).

Conjugation. An overnight conjugation in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) was as described previously (7). *E. coli* K-12 BM21 or *S. typhimurium* TM123 (19) was used as the recipient. Antibiotic concentrations (micrograms per milliliter) for selection of transconjugants were as follows: apramycin, 15; gentamicin, 4; nalidixic acid (Winthrop Laboratories, New York), 40.

Plasmid DNA. Preparation of plasmid DNA and agarose gel electrophoresis were as described previously (15, 21). Size standards were pIP112 (100.5 kb), plasmids from strain V517 (14), and bacteriophage lambda DNA (Boehringer GmbH, Mannheim, Germany). Restriction enzymes *Bam*HI, *Eco*RI, and *Hin*dIII (Appligene, Illkirch, France, and Amersham International, England) were used according to the recommendations of the manufacturers.

Probes and hybridization. Two types of probes were prepared: a probe specific for the gene encoding AAC(3)IV acetylase and probes specific for *rep* genes. The apramycin resistance probe was purified by cesium chloride-ethidium bromide ultracentrifugation (15) and prepared as described previously (1, 11). This probe was labeled by sulfonation (Chemiprobe; PBS-orgenics, Illkirch, France). The total DNA of transconjugants was spotted onto gridded Hybond-N membranes (Amersham). Hybridization was performed in 50% formamide at 42°C as described previously (15). Hybridization signals were developed by the Chemiprobe kit according to the manufacturer's recommendations. Positive controls were *E. coli* BM21(pWP701) (1) and *E. coli* BN8921 (5). *E. coli* K-12 BM14 (*azi met pro*) and *E. coli* HB101 *recA* (15) were used as negative controls.

The rep probes used were specific for different replicon control systems: rep9, repA2, repFIA, repFIIA, repHI.1, repHI.2, repL/M, repP, repQ, repU, repW, and repY. They were prepared and radiolabeled as described previously (10). Hybridization was performed either on colony filters or on dried agarose gels. The filters were saturated with the following solutions: (i) 0.5 M NaOH–1.5 M NaCl for 3 min, (ii) 0.5 M NaOH–1.5 M NaCl for 1 min at 20°C, and (iii) 1 M Tris-HCl (pH 7)–2 M NaCl for 5 min at 20°C. An overnight hybridization was performed at 65°C in a solution (3× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 10× Denhardt solution, 1% salmon sperm DNA, 0.1% sodium dodecyl sulfate [SDS]). Filters were washed three times for 30 min at 65°C in 3× SSC–0.1% SDS and three times in 1× SSC–0.1% SDS, dried, and autoradiographed. For dried gel hybridization assays, plasmid DNA of the transconjugants was extracted by the method of Kado and Liu (12). After overnight electrophoresis at 4°C in 0.5% agarose gels, the DNA was denatured and the gels were dried. Hybridization was then performed under the same conditions as on the filters.

Phenotypes. Eight strains of animal origin were identified as *E. coli* and two were identified as *S. typhimurium*; two hospital isolates were identified as *E. coli* (5). All the strains were resistant to multiple antibiotics, with MICs (micro-

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TABLE 1. Origins and properties of the strains

Yr of isolation	Wild strain	Source	Resistance pattern[a]	Resistance transferred
1986	*Escherichia coli* BN8936	France, calf	Ap Apr Cm Gm Km Sm Tc Tp	Apr Gm Tp
1988	*E. coli* BN8935	Belgium, calf	Ap Apr Cm Gm Km Sm Tc Tp	Apr Gm Tp
1988	*E. coli* BN8934	Belgium, calf	Ap Apr Cm Gm Sm Tc	Ap Apr Gm Sm
1988	*E. coli* BN8933	Belgium, calf	Ap Apr Gm Km Sm Tc Tp	Apr Gm Tp
1988	*E. coli* BN8932	Belgium, calf	Ap Apr Cm Gm Km Sm Tc Tp	Ap Apr Gm Sm
1988	*E. coli* BN8931	Belgium, calf	Ap Apr Cm Gm Sm Tc	Apr Gm Tp
1988	*E. coli* BN8930	Belgium, calf	Ap Apr Cm Gm Sm Tc	Apr Gm Tp
1987	*E. coli* BN8929	Belgium, pig	Ap Apr Gm Sm Tc Tp	Apr Gm Sm
1985	*Salmonella typhimurium* BN8928	Belgium, calf	Ap Apr Cm Gm Km Sm Tc Tp	Ap Apr Gm Sm
1985	*S. typhimurium* BN8927	Belgium, calf	Ap Apr Cm Gm Km Sm Tc Tp	Ap Apr Gm Sm
1986	*Escherichia coli* BN8915	Belgium, hospital patient[b]	Ap Apr Cm Gm Km Sm Tc Tp	Ap Apr Gm Sm
1987	*E. coli* BN8913	Belgium, hospital patient[b]	Apr Gm Sm Tp	Apr Gm Sm Tp

[a] Resistance nomenclature is in accordance with that of Novick et al. (18).
[b] As previously described (5).

grams per milliliter) for apramycin, gentamicin, and hygromycin of ≥256, 4 to 16, and 128 to 256, respectively. Apramycin and gentamicin resistance was always associated with resistance to hygromycin. Such a linkage has already been observed (1) in a plasmid encoding AAC(3)IV. The hygromycin B phosphotransferase and AAC(3)IV genes were expressed as one transcriptional unit from a single promoter located upstream from the AAC(3)IV gene and which seemed to be formed by one end of the element IS*140*. Such a structure suggests a transposon.

Plasmid comparisons. Apramycin- and gentamicin-resistant transconjugants were obtained from each strain (Table 1). When analyzed by agarose gel electrophoresis, seven transconjugants harbored a plasmid of approximately 115 kb. In the three other transconjugants, one or two plasmids of smaller size were revealed (Fig. 1). The 115-kb plasmid DNAs were compared after digestion with different restriction enzymes. The restriction profiles obtained after digestion by *Hin*dIII were identical for plasmids from strains BN8935 and BN8936 and closely related to those of plasmids from strains BN8913 and BN8931. A slightly different profile was obtained for the plasmid from strain BN8933. Another profile was common to plasmids from strains BN8915, BN8932, and BN8934 (Fig. 2). Only minor differences were detected on restriction profiles of plasmids from strains BN8913, BN8935, and BN8936 generated by digestion with *Eco*RI. These results were confirmed by those obtained by digestion with *Bam*HI of plasmids from strains BN8933, BN8935, and BN8936 and from strains BN8915 and BN8934 (data not shown). From these results, two different groups of plasmids could be distinguished: the plasmid from strain BN8913 (hospital patient) was closely related to plasmids from strains BN8931, BN8933, BN8935, and BN8936 (animals), and the plasmid from strain BN8915 (hospital patient) was closely related to plasmids from strains BN8932 and BN8934 (animals).

All the transconjugant DNAs tested hybridized with the AAC(3)IV-specific probe (Fig. 3). Production of the acetylase AAC(3)IV seems to account for all the apramycin-resistant isolates reported in the literature (9, 20), except for

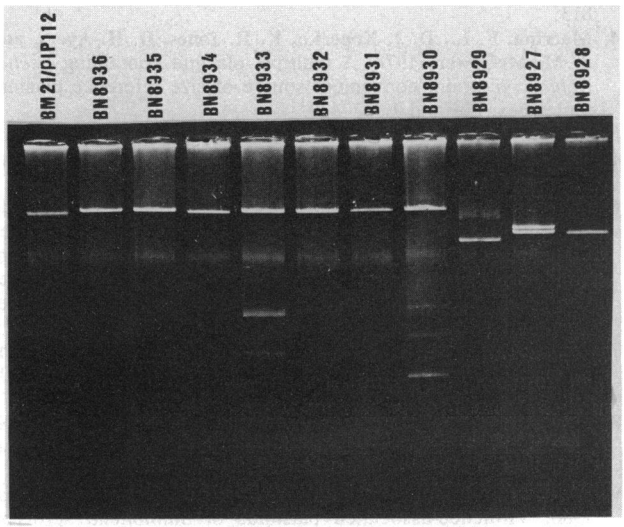

FIG. 1. Agarose gel electrophoresis of plasmid DNAs from transconjugants. *E. coli* BM21 or *S. typhimurium* TM123 was used as the recipient for apramycin and gentamicin transfer from the donor strains indicated above each lane. Electrophoresis was carried out in a 0.8% agarose gel for 3 h and 30 min at 12 V/cm. Plasmid pIP112 (100.5 kb) served as the molecular size standard.

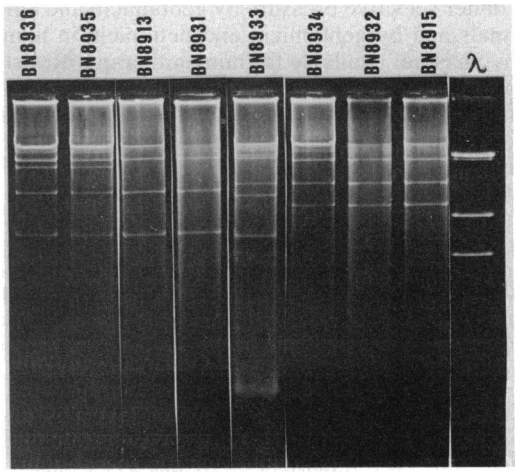

FIG. 2. Analysis of transconjugant plasmid DNAs by agarose gel electrophoresis. Plasmid DNA was digested by *Hin*dIII. Donor strains are indicated above each lane. Fragments obtained by digestion of λ DNA with *Hin*dIII were used as molecular size standards.

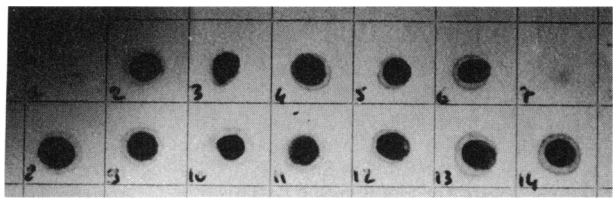

FIG. 3. Analysis of total DNA by dot blot hybridization. Total DNA was transferred to a nitrocellulose sheet and hybridized to a pWP701 754-bp *Sst*I probe modified by sulfonation. Squares: 1, BM14; 7, HB101; 2, BM21(pWP701); 3, BN8921. Squares 4 to 6 and 8 to 14 correspond to the transconjugants from strains 8927 to 8936 in ascending order.

a new mechanism recently described in a single strain (13). This mechanism has not yet been encountered in our field isolates.

Hybridization with both rep9 and repQ probes was observed on gels and filters for all the transconjugants tested (data not shown).

All of these results suggest a plasmid exchange between bacteria of animal and human origins. Similar exchanges have been documented with other resistance genes (2, 17, 23).

Until recently, the production of AAC(3)IV was a mechanism considered to be characteristic of animal isolates (3, 7, 22, 24). However, apramycin- and gentamicin-resistant strains of *S. typhimurium* lysotype 204C have been isolated from humans, presumably contaminated by a bovine source (22). Recent reports of systematic surveys of aminoglycoside resistance in hospital isolates have demonstrated that apramycin resistance was more widespread than was previously thought in several genera of the family *Enterobacteriaceae* (5, 8).

The first study of the molecular structure of the plasmids encoding this resistance in human strains revealed the presence of several unrelated groups (5). This shows that the gene encoding the AAC(3)IV enzyme was already present on many different replicons in the human community, possibly following transposition events. The emergence of this resistance may have occurred simultaneously in the two communities under selective pressure by gentamicin and apramycin in animals and by gentamicin and netilmicin in humans, as apramycin is not available for human therapy. Resistance to apramycin is not routinely tested in human medicine and thus may have been overlooked before the first systematic surveys mentioned above. This stresses the need for antibiotic resistance surveillance involving physicians and veterinarians and pleads for the prudent use of antibiotics in both communities.

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