Epidemiol. Infect. (1993), **111**, 229–238

Replicon typing characterization of plasmids encoding resistance to gentamicin and apramycin in *Escherichia coli* and *Salmonella typhimurium* isolated from human and animal sources in Belgium

P. POHL[1], Y. GLUPCZYNSKI[2], M. MARIN[1], G. VAN ROBAEYS[1], P. LINTERMANS[1] AND M. COUTURIER[3]

[1] *Institut National de Recherches Vétérinaires, Groeselenberg 99, 1180 Brussels, Belgium*

[2] *Service de Microbiologie, Hôpital Universitaire Brugmann, 4 Place A. van Gehuchten, 1020 Brussels, Belgium*

[3] *Service de Génétique, Université Libre de Bruxelles, 67 rue des Chevaux, 1640 Rhode-St-Genèse, Belgium.*

(*Accepted 2 April 1993*)

SUMMARY

Escherichia coli and salmonella strains with plasmids conferring resistance to gentamicin and apramycin have been isolated with increasing frequency both from cattle and hospital patients in Belgium. The apramycin-gentamicin resistance plasmids were characterized in recipient strains by their profiles and molecular weights using agarose gel electrophoresis, by their antimicrobial resistance patterns and by replicon typing using a series of DNA probes specific for the genes controlling their systems of replication. Overall, most of the plasmids differed in their DNA electrophoretic patterns. Seventeen different antimicrobial resistance profiles were observed, and there were six different types of replicons. However, two replication genes predominated and had a preferential distribution in different bacterial species. The rep FIC.a plus rep Q multireplicon was found mainly in plasmids recovered from gentamicin- and apramycin-resistant *E. coli* while replicon of the type rep FIC.b largely prevailed in *S. typhimurium*. Identical replication genes were found in most animal and human strains, hence suggesting a high homology between apramycin- gentamicin plasmids in these communities. Finally, our results indicate that the rapid spread of apramycin-gentamicin-resistance in several species of Enterobacteriaceae isolated from animals and from humans in Belgium is not due to a single plasmid, but rather that the gene encoding AAC(3)-IV is carried by various replicons.

INTRODUCTION

The aminoglycoside antibiotics apramycin and gentamicin have been used extensively in veterinary medicine in different countries in Europe since their first introduction for this purpose in the early 1980s. The first strains of *Salmonella typhimurium* and of *Escherichia coli* resistant to apramycin and gentamicin were

Table 1. *Properties of apramycin-gentamicin resistant strains and plasmids*

Clinical isolates	Origin	Resistance pattern	Resistance transferred	M.W. of plasmid (MDal)	Replicons identified by hybridization with rep probes
E. coli					
748S88	Human urine	Tp Apr Gm	Tp Apr Gm	65	Q+FIC.a
749	Human bronchus	Tp Apr Gm	Tp Apr Gm	65	Q+FIC.a
750	Human vulva	Su Sm Tc Cm Km Ap Tp Apr Gm	Tp Apr Gm	65	Q+FIC.a
751	Human blood	Su Sm Tc Cm Km Ap Tp Apr Gm	Su Sm Ap Apr Gm	65	Q+FIC.a
752	Human urine	Su Sm Tc Ap Tp Apr Gm	Su Sm Ap Tp Apr Gm	65	Q+FIC.a
753	Human skin	Su Sm Tc Cm Km Ap Tp Apr Gm	Apr Gm	65	FIC.a
754	Human urine	Su Sm Cm Km Ap Tp Apr Gm	Su Sm Ap Apr Gm	64	Q+FIC.a
755	Human urine	Su Sm Tc Cm Km Tp Apr Gm	Tp Apr Gm	65	Q+FIC.a
756	Human stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Apr Gm	65	Q+FIC.a
757	Human 'catheter'	Sm Tp Apr Gm	Sm Apr Gm	65	FIC.a
758	Human urine	Su Sm Tc Cm Ap Tp Apr Gm	Su Sm Tc Tp Apr Gm	71	P+FIC.b
759	Human stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Su Sm Ap Apr Gm	65	Q+FIC.a
760	Human stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Tp Apr Gm	71	Q+FIC.a
761	Human stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Sm Apr Gm	69	Q+FIC.a
762	Human 'catheter'	Su Ap Apr Gm	Apr Gm	71	Q+FIC.a
763	Human wound	Su Sm Tc Km Ap Tp Apr Gm	Su Sm Ap Tp Apr Gm	57	Q+FIC.a
764	Human urine	Su Sm Tc Tp Apr Gm	Sm Apr Gm	69	FIC.b
765	Human urine	Su Sm Tc Apr Gm	Apr Gm	57	Q+FIC.a
766	Human skin	Su Sm Tc Km Ap Tp Ctn Apr Gm	Km Tp Apr Gm	72	Q+FIC.b
658Ani88	Bovine intestine +lung	Su Sm Tc Cm Km Ap Tp Nal Apr Gm	Tp Apr Gm	74	Q+FIC.a
807S88	Bovine stools	Su Sm Tc Cm Ap Nal Apr Gm	Su Sm Ap Apr Gm	73	Q+FIC.a
809	Bovine stools	Su Sm Tc Km Ap Tp Apr Gm	Tp Apr Gm	73	Q+FIC.a
2072S86	Bovine stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Tp Apr Gm	74	Q+FIC.a
152Ani89	Bovine stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Sm Ap Tp Apr Gm	81	Q+FIC.a
10Auto89	Bovine spleen	Su Sm Tc Cm Km Ap Tp Apr Gm	Sm Ap Tp Apr Gm	69	Q+FIC.a
262Ani89	Bovine stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Tp Apr Gm	69	Q+FIC.a
191S89	Bovine stools	Su Sm Tc Cm Km Ap Aug Tp Apr Gm	Sm Tp Ap Apr Gm	74	Q+FIC.a
194S89	Bovine stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Tp Apr Gm	82	Q+FIC.a
192S89	Bovine stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Sm Ap Tp Apr Gm	71	Q+FIC.a
428S89	Bovine stools	Su Sm Tc Cm Km Ap Tp Apr Gm	Tp Apr Gm	69	Q+FIC.a
608Ani89	Bovine stools	Su Sm Ap Tp Agr Gm	Su Ap Tp Apr Gm	69	Q+FIC.a
546S89	Bovine lung	Su Sm Tc Cm Km Ap Tp Apr Gm	Sm Ap Tp Apr Gm	69	Q+FIC.a
595S89	Bovine foetus	Su Sm Tc Ap Tp Nal Apr Gm	Sm Ap Apr Gm	69	Q+FIC.a
598S89	Bovine intestine	Su Sm Tc Cm Km Ap Tp Nal Apr Gm	Sm Ap Apr Gm	69	Q+FIC.a
614S89	Bovine intestine	Su Sm Tc Cm Km Ap Tp Apr Gm	Sm Ap Apr Gm	71	Q

851S89	Bovine spleen	Su Sm Te Cm Ap Tp Aug Nal Apr Gm	Sm Ap Tp Apr Gm	81	Q + FIC.a
975S89	Bovine stools	Su Sm Te Cm Km Ap Tp Apr Gm	Sm Ap Apr Gm	59	Q + FIC.a
155Auto89	Bovine intestine	Su Sm Te Cm Km Ap Nali Ena Apr Gm	Sm Tp Apr Gm	79	Q + FIC.a
869Ani89	Bovine intestine	Su Sm Te Cm Km Ap Aug Tp Apr Gm	Sm Ap Apr Gm	69	Q + FIC.a
35Ani89	Bovine stools	Su Sm Te Cm Km Ap Tp Apr Gm	Sm Ap Apr Gm	56	Q + FIC.a
978S88	Bovine stools	Su Sm Te Cm Km Ap Ctn Aug Tp Apr Gm	Apr Gm	73	Q + FIC.a
14KH87	Bovine stools	Su Sm Te Cm Km Ap Tp Apr Gm	Apr Gm	74 + 76	Q + FIC.a
203Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Apr Gm	?	Q + FIC.a
204Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Apr Gm	59	Q + FIC.a
205Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Ap Apr Gm	62	Q + FIC.a
206Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Ap Apr Gm	56	Q + FIC.a
207Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Apr Gm	65	Q + FIC.a
208Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Apr Gm	65	FIC.a
209Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Apr Gm	64	FIC.a
210Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Apr Gm	65	Q + FIC.a
211Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Apr Gm	58	Q + FIC.a
212Anu90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Apr Gm	59	Q + FIC.a
213Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Apr Gm	?	FIC.b
214Ani90	Bovine stools	Su Sm Te Cm Km Ap Nal Enr Apr Gm	Su Sm Apr Gm	59	FIC.b
215Ani90	Bovine stools	Su Sm Te Cm Ap Tp Nal Apr Gm	Su Sm Apr Gm	59	FIC.b
216Ani90	Bovine stools	Su Sm Te Cm Ap Tp Nal Apr Gm	Su Apr Gm	54	FIC.b
788S87	Pig stools	Su Sm Te Ap Tp Apr Gm	Apr Gm	58	None
S. typhimurium					
768S88	Human	Te Apr Gm	Apr Gm	69	FIC.b
361S85	Bovine	Su Sm Te Cm Km Ap Tp Apr Gm	Ap Apr Gm	61	Q
362S85	Bovine	Su Sm Te Cm Km Ap Tp Apr Gm	Su Sm Ap Apr Gm	60	Q
419Auto88	Bovine septicaemia	Su Sm Te Cm Km Ap Tp Aug Apr Gm	Su Km Ap Aug Apr Gm	63	FIC.b
698Ani89	Bovine intestine	Su Sm Te Cm Km Ap Tp Nal Apr Gm	Su Sm Km Tp Apr Gm	60	FIC.b
17Ani90	Bovine	Su Sm Te Cm Km Ap Tp Nal Apr Gm	Sm Km Apr Gm	58 + 66	FIC.b
176Ani90	Bovine/abortion	Su Sm Te Cm Km Ap Tp Nal Apr Gm	Sm Km Apr Gm	66	FIC.b
144Ani90	Bovine intestine	Su Sm Te Cm Km Ap Tp Nal Apr Gm	Sm Km Apr Gm	58 + 66	FIC.b
220Ani90	Bovine intestine	Su Sm Te Cm Km Ap Tp Nal Apr Gm	Sm Km Apr Gm	58 + 66	FIC.b
222Ani90	Bovine	Su Sm Te Cm Km Ap Tp Nal Apr Gm	Sm Km Apr Gm	58	FIC.b
Klebsiella sp.					
767S88	Human	Su Sm Te Cm Ap Cm Tp Apr Gm	None	?	None (clinical isolate)
Citrobacter sp.					
233Ani89	Pig	Su Sm Te Cm Km Ap Tp Apr Gm	None	66	FIC.b (clinical isolate)
231Ani89	Bovine	Su Sm Te Cm Km Ap Tp Apr Gm	None	?	FIC.b (clinical isolate)

observed in England in 1982 [1, 2] and were isolated thereafter from farm animals in various countries [3–6]. The mechanism of resistance to apramycin and to gentamicin is due to N-acetylation by an enzyme of the aminoglycoside acetyltransferase 3 class (AAC(3)IV) [7]. In recent years, a rapid spread of this aminoglycoside resistance pattern has been observed in Belgium in Gram-negative Enterobacteriaceae isolated from calves [8]. In parallel to these findings, the AAC-(3)-IV aminoglycoside modifying enzyme has been reported on several occasions in aminoglycoside-resistant human isolates [5, 9–11]. The true prevalence of this resistance mechanism in isolates from humans is however unknown and likely to be underestimated because apramycin is an antibiotic whose usage is restricted to veterinary therapy, and hence clinical human isolates are not routinely tested for sensitivity to apramycin.

A gene encoding for resistance to apramycin and gentamicin has been cloned and sequenced [12] and found to be carried on various conjugative plasmids [4, 5]. Digestion with several restriction endonucleases has revealed some degree of similarity between plasmids of human and animal origin which confer resistance to apramycin and gentamicin [13]. The aim of this study was to characterize further the plasmids of apramycin-resistant Enterobacteriaeceae recently isolated in Belgium, by hybridization with different replicon-specific probes in order to determine their replicating genes and to assess further the level of genetic homology between the plasmids of human and of animal origin.

Replicon probes are derived from incompatibility loci of plasmids (replication and partition loci). These probes allow an accurate identification of most plasmids of Enterobacteriaceae and of other bacteria [14].

MATERIALS AND METHODS

Strains

Seventy apramycin-resistant clinical strains isolated in Belgium between 1985 and 1990 were studied. The clinical strains of human origin were isolated in an *in vitro* study performed in eight hospitals. The apramycin-resistant isolates were selected among a collection of aminoglycoside resistant isolates obtained during this study [9]. The strains of animal origin were recovered from sick animals during the same time period. Overall there were 57 strains of *E. coli* (19 isolated from hospitalized patients in Belgium, and 38 animal isolates comprising 37 bovine and one porcine isolate), 10 strains of *Salmonella typhimurium* (9 of bovine origin and 1 human isolate), 2 *Citrobacter freundii* (1 porcine and 1 bovine isolate) and 1 *Klebsiella pneumoniae* strain of human origin. Duplicate strains originating from the same patient or from the same animal were excluded. The origins and properties of the strains are listed in Table 1.

Microbiological techniques

Enterobacterial strains were isolated on MacConkey medium or on G2SN Salmonella agar [15] and identified according to standard identification techniques [16]. Antimicrobial resistance was determined by the disk diffusion test on Mueller-Hinton agar [17]. The following antimicrobial agents were tested: ampicillin, disk 10 mcg (Ap); amoxycillin, 20 mcg/clavulanic acid, 10 mcg (Aug);

apramycin, 100 mcg (Apr); cephalothin, 30 mcg (Ctn); chloramphenicol, 30 mcg (Cm); gentamicin, 10 mcg (Gm); kanamycin, 30 mcg (Km); nalidixic acid, 30 mcg (Nal); polymyxin B, 300 I.U. (Po); sulphonamide, 200 mcg (Su); streptomycin, 10 mcg (Sm); tetracycline, 30 mcg (Tc); trimethoprim, 5 mcg (Tp). Minimal inhibition concentrations (MICs) of gentamicin and of apramycin were also determined using the same Mueller-Hinton agar medium [17]. The resistance mechanisms were inferred from the aminoglycoside resistance patterns observed by disk susceptibility testing against ten different aminoglycoside antibiotics, following the method described by Shimizu and colleagues [18]. The aminoglycosides tested included: amikacin, disk 30 mcg; apramycin, 100 mcg; 5-episisomicin (Sch 22591; Schering corp.), 10 mcg; 2′-N-ethyl-netilmicin (Schering corp.), 10 mcg; 6′-N-ethyl-netilmicin (Schering corp.), 10 mcg; fortimicin, 30 mcg; gentamicin, 10 mcg; isepamicin (Sch 21420; Schering corp.), 30 mcg; netilmicin, 30 mcg; tobramycin, 10 mcg.

Conjugation

An overnight conjugation in brain heart infusion broth was performed following a previously described technique [19]. *Escherichia coli* K-12 14R525 [20] and *S. typhimurium* TM123 [21] were used as recipient strains. After transfer to the first recipient strain, the plasmids encoding resistance to apramycin and gentamicin were transferred to a second recipient in order to obtain transconjugants carrying a single plasmid. Apramycin was used at a concentration of 500 micrograms per milliliter for selection of transconjugants.

Plasmid DNA

Preparation of plasmid DNA of the transconjugants and agarose gel electrophoresis were performed following the alkaline lysis technique of Portnoy and colleagues [22]. Size standards were plasmids from strain *E. coli* V517 [23] and *Erwinia uredovora* [24].

Probes and hybridization

The replicon control systems of the different transconjugants were determined by DNA-DNA hybridization using specific rep probes following the replicon typing technique described by Couturier and colleagues [14]. Fifty transconjugants were tested with the following replicon radiolabelled gene probes: repFIA, repFIB, repFIC, repFIIA, rep9, repB/O, repK, rep11, repHI.1, repHI.2, repL/M, repP, repQ, repT, repU, repW, repX and repY.

Hybridization was usually performed on colony filters obtained from the transconjugants. However, with 35 transconjugants hybridization was done on dried agarose gels using the rep9, rep11 and repQ probes. Since no hybridization occurred with a number of rep probes, only the probes specific for repFIB, rep9, rep11 and repL/M were tested on the 17 subsequent transconjugants. In 3 cases (1 *K. pneumoniae* isolate, 2 *C. freundii* isolates) hybridization was performed directly on the donor strains as no transconjugants could be obtained from these strains. Single plasmids isolated in the transconjugants obtained from all *E. coli* strains as well as from the single *K. pneumoniae* strains of human origin and from

7 *E. coli* and 2 *S. typhimurium* strains isolated from cattle hybridized on filters with a probe specific for the gene encoding AAC(3)-IV acetylase [5–13].

RESULTS

All 70 strains were highly resistant to apramycin (MICs $\geqslant$ 1024 μg/ml) and moderately resistant to gentamicin (MICs ranging between 16 and 32 μg/ml) and most were resistant to other antimicrobial agents, irrespective of the source (Table 1). Twenty-five strains were resistant to ten or more antimicrobial agents. All strains were susceptible to polymyxin B; only three were resistant to cephalothin and five to amoxycillin-clavulanic acid (Table 1). Transconjugants carrying a single plasmid that encoded resistance to apramycin and to gentamicin were obtained in 63 strains. The aminoglycoside resistance patterns determined for all the transconjugants were compatible with the presence of the *aac4* gene with resistance to apramycin, gentamicin, netilmicin, 2′-N-ethyl, 6′-N-ethyl netilmicin, tobramycin and susceptibility to amikacin, 5′-episisomicin, fortimicin and isepamicin. In 9 cases, the plasmids only encoded resistance to apramycin and to gentamicin, while 16 others had resistance to 3 antimicrobial agents, 19 to 4 agents, 14 to 5 agents and 5 to 6 agents (Table 2). Seventeen different antimicrobial resistance patterns were observed (Table 2). Resistance to ampicillin, streptomycin, and trimethoprim was frequently found in association with apramycin and gentamicin. In contrast, none of the apramycin-gentamicin resistant transconjugants expressed resistance to cephalothin, chloramphenicol, enrofloxacin, nalidixic acid or polymyxin B.

The molecular weights of the plasmids conferring resistance to apramycin and to gentamicin ranged between 57 MDa and 72 MDa in the *E. coli* strains of human origin, while those found in the bovine strains varied between 54 MDa and 82 MDa (Table 1).

The single apramycin-gentamicin *E. coli* transconjugant obtained from a pig contained a plasmid of approximately 58 MDa. Plasmids found in the apramycin-resistant *S. typhimurium* transconjugants of bovine origin had a molecular weight ranging between 58 and 66 MDa, while the only apramycin-resistant *S. typhimurium* of human origin contained a plasmid of approximately 69 MDa. Apramycin-gentamicin resistance plasmids were not found in the single isolate of *K. pneumoniae* or in one *C. freundii* strain of animal origin. A 66 MDa plasmid was found in another animal strain of *C. freundii*, but it is unknown whether this plasmid encoded resistance to apramycin and to gentamicin since it could not be transferred into recipient strains.

Hybridization results on colony filters with the rep probes are also shown in Table 1 and are summarized in Table 3. Overall, 42 out of 57 (74·7%) apramycin-gentamicin resistant *E. coli* transconjugants carried a double replicon of the types rep FIC.a plus repQ. These two replicons were found at a similar frequency in the resistant strains irrespective of their origin. In addition, six transconjugants (10·5%) hybridized with the rep FIC.a probe alone while one strain (1·7%) hybridized solely with the rep Q probe. The FIC.b replicon alone (five strains: 8·7%) or in association with either the rep P or rep Q types (one strain each; 1·7%) were less frequently encountered in the *E. coli* transconjugants carrying a single apramycin-gentamicin resistance plasmid. The transconjugant obtained from the

Table 2. *Resistance patterns of 63* apramycin-gentamicin resistance plasmids*

Resistance pattern	Origin of plasmids*				
	E. coli			*Salmonella* sp.	
	Human ($n = 19$)	Cattle ($n = 36$)	Pig ($n = 1$)	Human ($n = 1$)	Cattle ($n = 6$)
Apr Gm	4	3	1	1	—
Tp Apr Gm	5	6	—	—	—
Sm Apr Gm	3	—	—	—	—
Su Apr Gm	—	1	—	—	—
Ap Apr Gm	—	—	—	—	1
Km Tp Apr Gm	1	—	—	—	—
Sm Tp Apr Gm	—	1	—	—	—
Sm Ap Apr Gm	—	6	—	—	—
Su Sm Apr Gm	—	9	—	—	—
Sm Km Apr Gm	—	—	—	—	2
Su Sm Ap Apr Gm	3	3	—	—	1
Sm Ap Tp Apr Gm	—	5	—	—	—
Su Ap Tp Apr Gm	—	2	—	—	—
Su Sm Ap Tp Apr Gm	2	—	—	—	—
Su Sm Tc Tp Apr Gm	1	—	—	—	—
Sm Km Ap Aug Apr Gm	—	—	—	—	1
Su Sm Km Tp Apr Gm	—	—	—	—	1

* These 63 transconjugants contained only one plasmid.

Table 3. *Replicon-typing of apramycin-gentamicin resistance plasmids*

Bacterial species	Replicons	Origin			Total
		Human ($n = 19$)	Cattle ($n = 37$)	Pig ($n = 1$)	
E. coli	FIC.a + Q	14	28*	—	42
	FIC.b + P	1	—	—	1
	FIC.b + Q	1	—	—	1
	FIC.a	2	4	—	6
	FIC.b	1	4	—	5
	Q	—	1	—	1
	Unknown	—	—	1	1
		($n = 1$)	($n = 9$)		
S. typhimurium					
	FIC.b	1	7†	—	8
	Q	—	2	—	2
		($n = 1$)			
Klebsiella sp.	Unknown	1‡	—	—	1
			($n = 1$)	($n = 1$)	
Citrobacter sp.	FIC.b	—	1‡	1‡	2

* One of the 28 transconjugants contained two plasmids.
† Three of the seven transconjugants contained two plasmids.
‡ Hybridization with the clinical isolates.

bovine *E. coli* strain 14K87 contained one plasmid of 74 MDa and another of 76 MDa (Table 1). Hybridization on agarose gel showed that rep FIC.a and rep Q were located on the larger plasmid. The resistance plasmid of the single *E. coli* strain of porcine origin did not hybridize with any of the 14 probes used.

https://doi.org/10.1017/S0950268800056934 Published online by Cambridge University Press

In contrast, the replicon of the rep FIC.b type predominated in *S. typhimurium* being found in seven transconjugants from bovine strains and in the single strain of human origin. However, three transconjugants of *S. typhimurium* of animal origin contained two plasmids (58 and 66 MDa) (Table 1) so it remains unknown in these cases whether the FIC.b replicon was located on the apramycin-gentamicin plasmid. The two remaining *S. typhimurium* transconjugants reacted with the rep Q probe alone. The *K. pneumoniae* isolate did not react with any of the probes while the two *C. freundii* carried rep FIC.b, although it was not determined whether this was on the same plasmid that encoded resistance to apramycin and gentamicin.

DISCUSSION

Resistance to apramycin and gentamicin is mediated by a 3-N-acetyltransferase type IV enzyme (AAC(3)-IV) encoded by a gene located on bacterial plasmids [4, 7, 12, 18] which are present in isolates from veterinary and human origin [1–5, 9–11, 25]. Homology between plasmids from isolates of human and animal origins encoding AAC(3)-IV has been suggested previously. Chaslus Dancla and colleagues [5, 13] found a high degree of similarity in the plasmid DNA fingerprint obtained on agarose gels after digestion with several restriction enzymes of apramycin-resistant *E. coli*, isolated from hospital patients in Belgium and strains isolated from cattle in France and Belgium. Salauze and colleagues [25] used specific probes and southern hybridization to show that the AAC(3)-IV gene was always linked with *hphB*, a gene encoding a 4-I phosphotransferase which inactivates hygromycin B, another aminoglycoside antibiotic. These genes formed an operon, which contains insertion sequences (IS*140*) that are similar in apramycin-resistant Enterobacteriaceae isolated from different human and animal sources.

By using probes that are specific for different replicon control systems, we were able to show that the apramycin-gentamicin resistance plasmids found in different Enterobacteriaceae isolated both from human and animal sources frequently carry similar replicons. A double replicon, FIC.a plus repQ, was present in a large majority of the human and animal *E. coli* strains. In contrast, the FIC.b replicon was less frequently present in *E. coli*, but predominated in transconjugants of *S. typhimurium*. The replication system of the plasmid isolated in the single porcine isolate of *E. coli* is unknown as the transconjugant did not hybridize with any of the rep probes. This suggest that other, as yet uncharacterized replicon control systems exist. Our results also confirm that apramycin-gentamicin resistance is limited to a few species of Enterobacteriaceae in both animals and humans (mainly *E. coli* and *S. typhimurium*). Only three apramycin-gentamicin resistant strains were found among two other Gram-negative species (*K. pneumoniae* and *C. freundii*), and transferable plasmids were not found in these bacteria.

This work indicates that the epidemic spread of apramycin and gentamicin resistance observed in *E. coli* and *S. typhimurium* isolated from farm animals was not associated with a single plasmid. Rather, resistance check was associated with a variety of plasmids which also carried resistance determinants to other classes of antimicrobial agents. This suggests that the *aac4* gene is also probably carried on transposable elements. Seventeen different antimicrobial resistance patterns

were found among the apramycin- gentamicin resistant transconjugants (Table 2), and six different replicon control systems were identified (Table 3). However, two types of replication control genes predominated namely, the double replicon FIC.a plus Q and replicon FIC.b. The other replicon types identified may have resulted from genetic rearrangement (deletions/recombinations). The double replicon FIC.a plus Q was almost exclusively found in *E. coli* strains, while the FIC.b type was mainly found in *S. typhimurium* thus indicating that a preferential relation may exist between plasmids and their bacterial hosts. During the course of this study, randomly selected Enterobacteriaceae belonging to species other than *E. coli* or *S. typhimurium* were screened. Resistance to apramycin was not found except in one *K. pneumoniae* strain of human origin and in two *C. freundii* isolates of animal origin (data not shown). The observation that apramycin and gentamicin resistance apparently exists in only a few bacterial species agrees with the observations of others [4, 10], and indicates a possible preferential association between plasmids and their hosts [26]. However, more isolates from other Enterobacteriaceae species should be studied in order to corroborate this impression.

ACKNOWLEDGEMENTS

We are grateful to D. Mathy for excellent technical assistance. Thanks are due to E. Chaslus-Dancla for testing the strains with the AAC(3)-IV specific gene probe. This work has been supported by grants from the Fonds National de la Recherche Scientifique, the Action de la Recherche Concertée, and the Banque Nationale de Belgique.

REFERENCES

1. Sojka WJ, Wray C, Pritchard GC, Hedges RW. Gentamicin resistant salmonella. Vet Rec 1982; **111**: 512.
2. Sojka WJ, Wray C, McLaren I. Survey of drug resistance in Salmonellae isolated from animals in England and Wales from 1979 to 1981. Brit Vet J 1984; **140**: 576–91.
3. Chaslus-Dancla E, Lafont J-P. Resistance to gentamicin and apramycin in *Escherichia coli* from calves in France. Vet Rec 1985; **177**: 90–1.
4. Chaslus-Dancla E, Martel J, Carlier C, Lafont J-P, Courvalin P. Emergence of aminoglycoside 3-N-acetyltransferase IV in *Escherichia coli* and *Salmonella typhimurium* isolated from animals in France. Antimicrob Agents Chemother 1986; **29**: 239–43.
5. Chaslus-Dancla E, Glupczynski Y, Gerbaud G, Lagorce M, Lafont J-P, Courvalin P. Detection of apramycin resistant Enterobacteriaceae in hospital isolates. FEMS Microbiol Lett 1989; **61**: 261–5.
6. Wray C, Beedell YE, McLaren IM. A survey of antimicrobial resistance in *Salmonellae* isolated from animals in England and Wales during 1984–1987. Br Vet J 1991; **147**: 356–69.
7. Davies J, O'Connor, S. Enzymatic modification of aminoglycoside antibiotics: 3-N-acetyltransferase with broad specificity that determines resistance to the novel aminoglycoside apramycin. Antimicrob Agents Chemother 1978; **14**: 69–72.
8. Pohl P, Verlinden M, Lintermans P, Van Robaeys G, Stockmans F. Antibiogrammes des entérobactéries pathogènes pour les animaux d'élevage et les pigeons, isolées en Belgique de 1986 à 1990. Ann Méd Vét 1991; **135**: 101–8.
9. Glupczynski Y, Dewit J, Hare RS, Miller HG, Yourassowsky E. Emergence of aminoglycoside 3-N-Acetyltransferase type IV in *Escherichia coli* isolated from patients in Belgium. *Abstr Ann Meet Am Soc Microbiol* 1988; *A*-82: 14.
10. Gomez-Lus R, Rivera MJ, Gomez-Lus ML, et al. Resistance to apramycin in two enterobacterial clinical isolates: detection of a 3-N-acetyltransferase IV. J Chemother 1990; **12**: 241–3.

11. Gomez-Lus R, Gil J, Gomez-Lus ML, Castillo J, Bouza E, Rubio MC. Resistance to apramycin in *Klebsiella pneumoniae* isolated from patients: detection of 3-N-acetyltransferase IV. 27th Intersc Conf Antimicrob Agents Chemother 1987; abstr. no. 400.
12. Braü B, Pilz U, Piepersberg, W. Genes for gentamicin-(3)-N-acetyltransferases III and IV: I-nucleotide sequence of the AAC(3)-IV gene and possible involvement of an ISI40 element in its expression. Mol Gen Genet 1984; **193**: 179–87.
13. Chaslus-Dancla E, Pohl P, Meurisse M, Marin M, Lafont J-P. High genetic homology between plasmids of human and animal origins conferring resistance to the aminoglycosides gentamicin and apramycin. Antimicrob Agents Chemother 1991; **35**: 590–3.
14. Couturier M, Bex F, Bergquist PL, Maas WK. Identification and classification of bacterial plasmids. Microbiol Rev 1988; **52**: 375–95.
15. Pohl P, Thomas J. Nouveau milieu d'isolement et de détection des Salmonella. Ann Inst Pasteur de Lille 1966; **126A**: 435–48.
16. Ewing WH. Differentiation of *Enterobacteriaceae* by biochemical reactions. In: Ewing WH, ed. Identification of Enterobacteriaceae, 4th ed. New York: Elsevier, 1986. 47–72.
17. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard 1990; Vol. 10, nr. 7. National Committee for Clinical Laboratory Standards, Villanova, Pennsylvania.
18. Shimizu K, Kumada T, Hsieh WC et al. Comparison of aminoglycoside resistance patterns in Japan, Formosa, and Korea, Chile, and the United States. Antimicrob Agents Chemother 1985; **28**: 282–8.
19. Lesage DD, Gerbaud, GB, Chabbert YA. Carte génétique et structure chez *Escherichia coli* K12 d'un plasmide de résistance isolé de *Salmonella ordonez*. Ann Inst Pasteur Microbiol 1975; **126A**: 435–48.
20. Anderson ES, Lewis MJ. Characterization of a transfer factor associated with drug resistance in *Salmonella typhimurium*. Nature 1965; **208**: 843–9.
21. Popoff MY, Myras I, Coynault C, Lasselin C, Pardon P. Molecular relationship between virulence plasmids of *Salmonella* serotypes *typhimurium* and *dublin* and large plasmids of other *Salmonella* serotypes. Ann Inst Pasteur Microbiol 1984; **135A**: 389–98.
22. Portnoy DA, Moseley SL, Falkow S. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect Immun 1981; **31**: 775–82.
23. Macrina FL, Kopecko DJ, Jones KR, Ayers DH, McGowen SM. A multiple plasmid containing *Escherichia coli* strain: convenient source of size reference plasmid molecules. Plasmid 1978; **1**: 417–20.
24. Thiry G. Plasmids of the epiphytic bacterium *Erwinia uredovora*. J Gen Microbiol 1984: **130**, 1623–31.
25. Salauze D, Otal I, Gomez-Lus R, Davies J. Aminoglycoside acetyltransferase 3-IV (*aacC4*) and hygromycin B 4-I phosphotransferase (*hphB*) in bacteria isolated from human and animal sources. Antimicrob Agents Chemother 1990; **34**: 1915–20.
26. Hedges RW, Rodriguez-Lemoine V, Datta N. R-factors from *Serratia marcescens*. J Gen Microbiol 1975; **86**: 88–92.