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Transfer of Amikacin Resistance by Closely Related Plasmids in Members of the Family *Enterobacteriaceae* Isolated in Chile

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During a 9-month period when amikacin was the sole aminoglycoside used clinically in a hospital in Santiago, Chile, resistance to amikacin and other antibiotics was encountered in 42 strains of the family *Enterobacteriaceae*, including *Escherichia coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Enterobacter cloacae*, *Serratia marcescens*, and *Serratia liquefaciens*. Amikacin resistance was transferable by conjugation and carried by IncM plasmids ranging in size from ca. 48.4 to 58.1 kilobase pairs. The plasmids had ca. 70 to 80% of their structure in common, as judged after digestion with restriction endonucleases. The resistance was mediated by a 6′ aminoglycoside acetyltransferase. We conclude that selective pressure has favored the dissemination of a wide-host-range amikacin resistance plasmid and its derivatives.

Amikacin, a semisynthetic derivative of kanamycin (9), is modified inefficiently or not at all by the majority of aminoglycoside-modifying enzymes (5) and therefore remains active against a number of otherwise aminoglycoside-resistant bacteria. Its use has been generally restricted to the treatment of serious infections caused by multiresistant bacteria.

The incidence of amikacin resistance, which in gram-negative bacteria depends essentially on the synthesis of an aminoglycoside acetyltransferase, AAC(6′) (8), has remained low (1, 18, 21; R. S. Hare and G. H. Miller, Antimicrob. Newsl. 1:77–84, 1984). It appears from studies limited in each case to one institution that the substitution of amikacin for all other aminoglycosides is not necessarily followed by a concomitant increase in resistance to amikacin and that its predominant or exclusive use may, in fact, result in the general reduction of aminoglycoside-resistant isolates (2, 16). Recently, however, a correlation was observed between the occurrence of enzyme-mediated amikacin resistance in gram-negative bacteria and the use of this antibiotic (11).

During a 9-month period (1982 to 1983) when amikacin was the only aminoglycoside in use, 42 amikacin-resistant strains of *Enterobacteriaceae* belonging to five genera were isolated at the San Juan de Dios hospital in Santiago, Chile. This prompted us to study the mechanism of the resistance and the mode of its dissemination.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The 42 amikacin-resistant strains of *Enterobacteriaceae* were clinical isolates, mainly obtained from urinary tract infections. They were isolated during the period and at the hospital stated above from patients in the general medicine, surgery, and intensive care units, in which amikacin was the only aminoglycoside in use. The strains belonged to five genera and were multiply antibiotic resistant (Table 1). Only one isolate of a species from a given patient was included in this study. The recipient strain for conjugational transfer was *Escherichia coli* BM694 (F^- Lac^+ *gyrA* Nal^r; restrictionless, modificationless) (10). *E. coli* K-12 J5 (F^- Pro^- Azi^r Rif^r) containing IncM plasmid R135 (Gen^r Str^r Sul^r Tet^r) was used for incompatibility testing. The strains were grown in Mueller-Hinton broth at 37°C (Institut Pasteur Production) unless otherwise stated.

Susceptibility testing. The antibiotic resistance phenotypes were determined by disk susceptibility testing. The MICs of aminoglycosides were determined on Mueller-Hinton agar by the twofold agar dilution method with a Steers-type replicator.

Conjugational plasmid transfer and incompatibility group testing. Transfer of plasmids to *E. coli* was carried out on Drigalski agar plates (Institut Pasteur Production) containing 20 μg of nalidixic acid and 25 μg of kanamycin per ml. Incompatibility testing was carried out as described previously (3, 4).

Assay of aminoglycoside-modifying enzymes. Cells from 100-ml cultures in the mid-logarithmic phase of growth were subjected to sonic disruption in 2 ml of buffer on ice, and supernatants (S100) were obtained for assay after centrifugation at 100,000 × *g* at 4°C. The enzyme activities were assayed immediately with radioactive cofactors (see below), and the products were bound to Whatman P81 phosphocellulose filters for radioactivity counting as described by Haas and Dowding (7). Supernatants were prepared, and enzyme assays were carried out in the same buffer (0.05 M Tris, 0.1 M NH_4Cl, 0.01 M $MgCl_2$, 0.01 M β-mercaptoethanol; pH 7.4). The reaction volume was 60 μl containing 2 μl of S100, and the amounts of antibiotic and cofactor were as described previously (7). The assay was carried out at 30°C for 20 min.

Preparation and analysis of plasmid DNA. Plasmid DNA was prepared after alkaline lysis according to either of two procedures (12, 17). Extraction of plasmid DNA solutions with phenol-chloroform, equilibrium density gradient centrifugation in cesium chloride-ethidium bromide, and agarose gel electrophoresis were carried out by standard methods (12). Restriction endonucleases were used according to the instructions of the supplier. Plasmid DNA prepared by rapid isolation was dialyzed for 18 h against TE buffer (12) before digestion.

Chemicals. [1-^{14}C]Acetyl coenzyme A (1.85 to 2.2 GBq/mmol), [γ-^{32}P]ATP (110 TBq/mmol; triethylammonium salt), and [U-^{14}C]ATP (18.5 to 22 GBq/mmol; ammonium salt)

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TABLE 1. Other antibiotic resistances associated with amikacin-resistant clinical isolates[a]

Antibiotic resistances[b]					No. of strains				
Cm^r	Gm^r	Su^r	Tc^r	Tp^r	*Klebsiella pneumoniae*	*Escherichia coli*	*Serratia marcescens*	*Citrobacter freundii*	*Enterobacter cloacae*
+	+	+	+	+	3	3	5	3	1
+	+	+	−	+	2	1	2	0	0
−	+	+	+	+	0	0	0	2	0
+	−	+	+	+	5	1	0	0	0
+	−	+	−	+	0	1	0	0	0
−	−	+	+	+	0	0	0	1	0
−	−	+	−	+	5	0	0	1	2
−	−	−	−	−	0	1	0	0	3

[a] All strains were resistant to ampicillin, kanamycin, netilmicin, sisomicin, streptomycin, and tobramycin.
[b] +, Resistance to chloramphenicol (Cm), gentamicin (Gm), sulfonamide (Su), tetracycline (Tc), and trimethoprim (Tp).

were obtained from Amersham France SA. Restriction endonucleases were obtained from Boehringer Mannheim Biochemicals. Antibiotics were provided by the following laboratories: amikacin, kanamycin, kanamycin B, and BB K311 by Bristol Laboratories; lividomycin and dibekacin by Laboratoire Roger Bellon; apramycin, hygromycin, and tobramycin by Eli Lilly & Co.; neomycin B, neamine, and spectinomycin by The Upjohn Co.; butirosin and paromomycin by Warner-Lambert; gentamicin, G-418, netilmicin, sisomicin, Sch 21561, and Sch 21562 by Schering Corp.; streptomycin by Specia; and fortimicin A by Abbott Laboratories. The antibiotic disks used for disk susceptibility testing were from Institut Pasteur Production.

RESULTS

Susceptibility to antibiotics and plasmid content of the clinical isolates. All strains were resistant or had a reduced susceptibility to amikacin, kanamycin, netilmicin, sisomicin, streptomycin, and ampicillin, whereas some were also resistant, in various combinations, to chloramphenicol, gentamicin, sulfonamide, tetracycline, and trimethoprim (Table 1). The MIC ranges for aminoglycosides are shown in Table 2. The MICs of kanamycin, netilmicin, tobramycin, and particularly sisomicin, but not amikacin, tended to be higher in the gentamicin-resistant strains (Table 2). Most of the strains carried multiple plasmids, and all had at least one large plasmid of ca. 40 megadaltons (MDa) (Fig. 1), as determined by their mobilities relative to those of plasmids of known size.

In vitro transfer of amikacin resistance. Conjugational transfer of amikacin resistance to *E. coli* after selection on amikacin (5 μg/ml) or kanamycin (20 μg/ml), was obtained with 36 of the 42 isolates of *Enterobacteriaceae* listed in Table 2. The remaining six strains belonged to three genera. All of these contained at least one plasmid, but the localization of amikacin resistance gene(s) was not studied. The plasmid content of the transconjugants was monitored by the method of Portnoy et al. (17). The amikacin-resistant transconjugants carried one large plasmid of ca. 40 MDa and were of two resistance phenotypes (Table 3). Both types displayed resistance to kanamycin, tobramycin, and netilmicin and to streptomycin and ampicillin, but not to gentamicin. Transconjugants of type I, which were predominant, were additionally resistant to sulfonamide and trimethoprim (Table 3). The MICs of aminoglycosides for the transconjugants were similar to those for the *E. coli* clinical isolates (Table 2). All of the plasmids conferring resistance to amikacin were found to belong to the incompatibility group M. In eight transconjugants, the cotransfer of a small plasmid, apparently unrelated to the resistance phenotype, was observed.

Aminoglycoside-modifying enzymes. Assays for the modification of aminoglycosides were performed with a set of S100 preparations from the transconjugants representing each donor genus and each resistance phenotype. Acetylation of amikacin was found to occur in every case, but there

TABLE 2. Susceptibility of clinical isolates and transconjugants to aminoglycosides

Bacterial strains	No. of strains	MICs (μg/ml)					
		Amikacin	Gentamicin	Kanamycin	Netilmicin	Sisomicin	Tobramycin
Clinical isolates							
K. pneumoniae (Gm^r)	5	8–32	32–64	128–>256	32–128	32–128	16–32
K. pneumoniae (Gm^s)	10	8–32	0.12–0.5	32–128	8–128	4–16	4–16
E. coli (Gm^r)	4	4–64	128–>256	64–256	64–256	256–>256	32–128
E. coli (Gm^s)	3	64–128	1–2	128–>256	64–128	32	32
S. marcescens (Gm^r)	7	16–64	64–256	128–>256	32–>256	128–>256	16–64
S. marcescens (Gm^s)	0						
C. freundii (Gm^r)	5	16–32	64–>256	32–>256	32–>256	128–>256	16–64
C. freundii (Gm^s)	2	16	0.25–0.5	64	64	16	8–16
E. cloacae (Gm^r)	1	32	64	>256	128	256	32
E. cloacae (Gm^s)	5	8–16	0.5	32–64	32–64	8–16	8
Transconjugants: *E. coli* BM694 Gm^s	36	32–64	1–2	128–256	128–256	32–64	32–64
Recipient: *E. coli* BM694	1	1	0.25	2	0.25	0.25	0.5

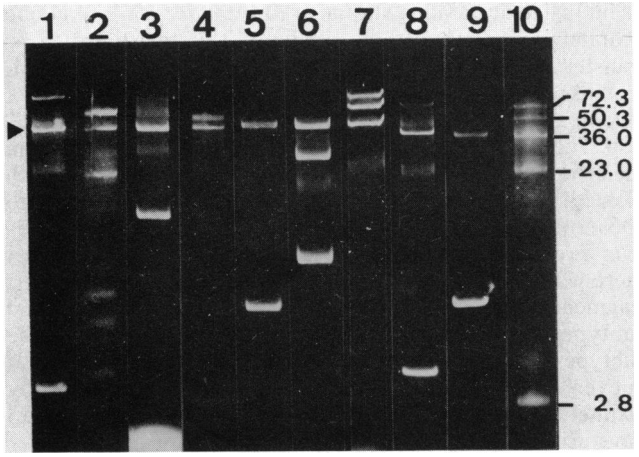

FIG. 1. Plasmid content of clinical isolates which transferred amikacin resistance. Plasmid DNA was prepared according to the procedure of Portnoy et al. (17). Lanes: 1 and 8, *E. cloacae*; 2, 3, 4, and 6, *Citrobacter freundii*; 5 and 9, *E. coli*; 7, *S. marcescens*; 10, *E. coli* containing plasmids R64 (72.3 MDa), R135 (50.3 MDa), RP4 (36 MDa), RS-a (23 MDa), and pBR322 (2.8 MDa). The area to which the ca. 40-MDa plasmids migrated is indicated on the left.

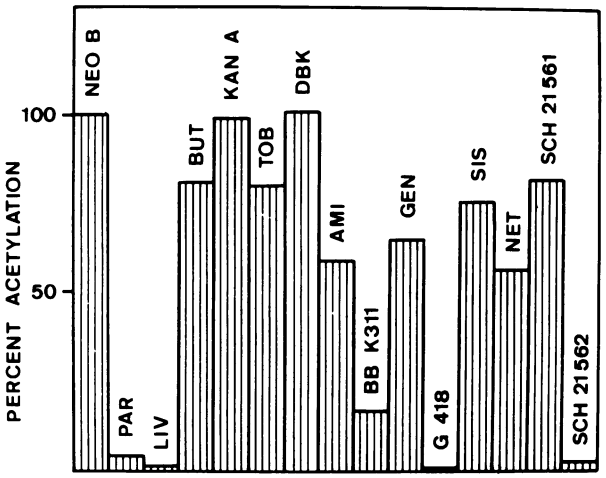

FIG. 2. Substrate profile of the aminoglycoside acetyltransferase from one amikacin-resistant *E. coli* transconjugant. The 100% value corresponds to approximately 6,500 cpm. Abbreviations: NEO B, neomycin B; PAR, paromomycin; LIV, lividomycin; BUT, butirosin; KAN A, kanamycin A; TOB, tobramycin; DBK, dibekacin; AMI, amikacin; BB K311, 4′-deoxy-6′-*N*-methylamikacin; GEN, gentamicin complex; SIS, sisomicin; NET, netilmicin; SCH 21561, 2′-*N*-ethylnetilmicin; SCH 21562, 6′-*N*-ethylnetilmicin. Modification of hygromycin, apramicin, fortimicin A, streptomycin, and spectinomycin was less than 2%.

was no adenylylation or phosphorylation. The substrate profiles of the acetyltransferases resembled each other closely, and a typical profile is shown in Fig. 2. The substantial acetylation of amikacin as opposed to the weak modification of 4′-deoxy-6′-*N*-methylamikacin (BB-K311) and the acetylation of 2′-*N*-ethylnetilmicin (Sch 21561), but not of 6′-*N*-ethylnetilmicin (Sch 21562), was indicative of an AAC(6′), and the full profile (Fig. 2) was consistent with this interpretation. The resistance profiles, as determined by the disk diffusion method, were similar for all transconjugants.

We have not studied systematically the mechanisms of resistance to gentamicin and streptomycin. However, after gel filtration as described elsewhere (20) of an S100 preparation from one amikacin-, gentamicin-, and streptomycin-resistant transconjugant strain, obtained from a *Serratia marcescens* donor, two enzyme activities were revealed in addition to that of the AAC(6′): a gentamicin acetyltransferase, AAC(3)-II, and a streptomycin adenyltransferase, probably AAD(3″) (9). This particular transconjugant, which is not included in Tables 2 and 3, was selected on gentamicin (4 μg/ml) and contained a plasmid ca. 8 kilobase pairs (kbp) larger than that found in the gentamicin-susceptible transconjugant selected on amikacin and obtained from the same donor (data not shown). The association of AAC(6′) and AAC(3) in strains isolated in Chile has been observed previously (R. S. Hare and G. H. Miller, unpublished data, quoted in Hare and Miller, 1984) and might explain the higher MICs for the gentamicin-resistant strains shown in Table 2.

Analysis of plasmid DNA after digestion with restriction endonucleases. Plasmid DNA was prepared from all amikacin-resistant transconjugants by rapid extraction, and a number of restriction endonucleases were screened for the generation of fragment patterns, allowing an estimation of the relatedness of the plasmids. *Pvu*II generated approximately 25 fragments (Fig. 3), whereas *Eco*RI, *Sal*I, *Hin*dIII, and *Bam*HI appeared to recognize very few sites, and digestion with *Hae*III yielded fragments only below ca. 0.8 kbp (data not shown). The fragment patterns of all plasmids, after digestion with *Pvu*II, were very similar, and differences were restricted to the mobility of three fragments (Fig. 3). On the basis of these differences, the plasmids were assigned subtypes. While the presence or absence of a fragment of ca. 1.8 kbp was characteristic of types I and II, respectively, the subtypes were characterized by variations in the sizes of the two largest fragments, which ranged between ca. 4.9 and 9.5 kbp (Fig. 3). Twenty-eight plasmids could be assigned to subtypes A through D of type I or to subtypes A and B of type II, and their distribution among the clinical isolates is shown in Table 4. This distribution did not appear to be totally random, in that type IA was predominant or frequent in all strains except *Enterobacter cloacae*, which, for its

TABLE 3. Transfer of amikacin resistance to *E. coli* by conjugation[a]

Type	Resistance phenotype transferred[b]				No. of donor strains				
	Ak^r	GM^r	Su^r	Tp^r	*K. pneumoniae*	*E. coli*	*S. marcescens*	*C. freundii*	*E. cloacae*
I	+	−	+	+	14	3	7	3	3
II	+	−	−	−	0	3	0	0	3

[a] Transfer was obtained from 36 of the 42 strains listed in Table 1.
[b] All transconjugants were also resistant to kanamycin, netilmicin, sisomicin, and tobramycin and to ampicillin and streptomycin. See Table 1 footnote *b* for an explanation of abbreviations.

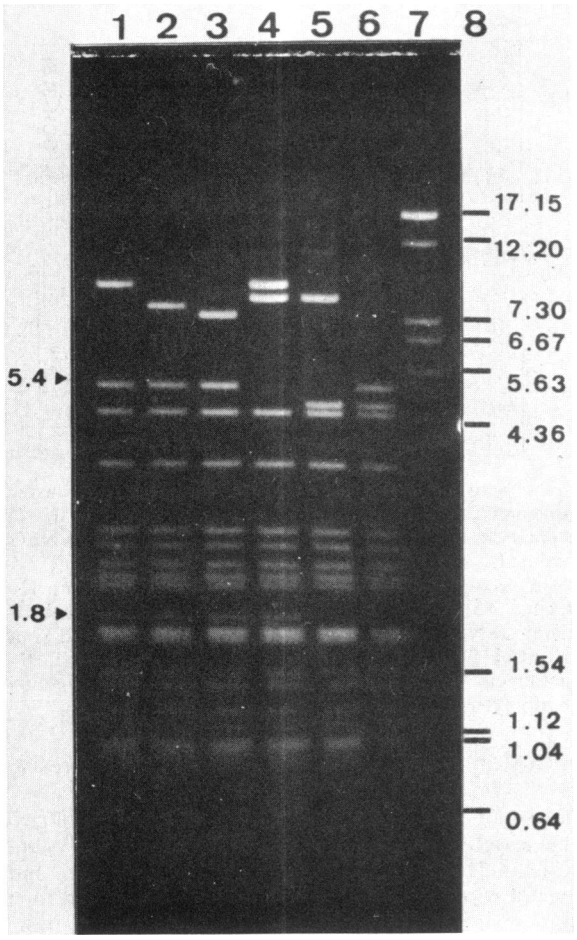

FIG. 3. Fragment patterns of amikacin resistance plasmids. Plasmid DNA was obtained after alkaline lysis and equilibrium density gradient centrifugation in cesium chloride-ethidium bromide (12), and then digested with *Pvu*II. Lanes: 1, plasmid of type IA; 2, type IB; 3, type IC; 4, type ID; 5, type IIA; 6, type IIB; 7, λDNA digested with *Bam*HI; 8, position and size (kbp) of the λDNA and pBR322 fragments obtained after digestion with *Pvu*II or with *Pvu*II, *Bam*HI, *Eco*RI, and *Ava*I.

part, contained the otherwise infrequent types IC and IIB. Eight plasmids were not assigned unambiguously to a subtype due to the presence of additional large fragments derived from the apparently cryptic plasmids (see above); however, the majority, if not all, of the fragments smaller than ca. 4.9 kbp comigrated with those shown in Fig. 3. We conclude from this that all of the amikacin resistance plasmids analyzed have a core structure in common. The size of the amikacin resistance plasmids, computed from the size of their fragments, ranged from ca. 48.4 to 58.1 kbp.

DISCUSSION

Low incidence has been a salient feature of amikacin resistance, particularly in strains of *Enterobacteriaceae* (1, 18, 21; Hare and Miller, 1984) despite the fact that enzyme-mediated resistance may be transferable by conjugation (22) and encoded by a multiresistance transposon (14) and despite the possibility that selection for this resistance could occur with other frequently used aminoglycosides such as tobramycin, dibekacin, or netilmicin.

While studying a sudden and rapid dissemination of amikacin resistance among strains of *Enterobacteriaceae* in one hospital in Santiago, Chile, we found this resistance to be transferable to *E. coli* from ca. 85% of the isolates obtained. In these cases, resistance was associated with plasmids of incompatibility group M, ranging in size from ca. 48.4 to 58.1 kbp. These plasmids were remarkably similar as judged from their fragment patterns after digestion with *Pvu*II, which yielded an adequate number of fragments. There was no apparent variation in the distribution of at least 23 *Pvu*II sites over a region representing ca. 70 to 80% of the plasmids, but there was some variation in the size of the two largest *Pvu*II fragments, which allowed us to distinguish six plasmid subtypes (Fig. 3). Although the distribution of these subtypes among the clinical isolates appeared somewhat uneven (Table 4), they were not obviously phenotypically distinct. The variations among the subtypes would appear to have arisen during plasmid dissemination.

Not all resistance genes of the transconjugants (Table 3) have been localized on individual *Pvu*II fragments, but trimethoprim and sulfonamide resistance was associated with the presence of the 1.8-kbp fragment, and the *aac*(6′) and *aad* genes were found, in one case, to be carried on a 5.4-kb fragment (Fig. 3) after cloning into pBR322 (G. Tran Van Nhieu, unpublished observation).

Amikacin resistance, where tested (see Results), was mediated by a 6′-acetyltransferase. Synthesis of AAC(6′) is the most frequent cause of amikacin resistance in gram-negative clinical isolates (8, 18), while reduced uptake (18; Hare and Miller, 1984) may also be responsible. In the absence of a detailed biochemical characterization of the enzyme found in this study, and with several nomenclatures proposed (6, 13, 15; Hare and Miller, 1984), we have not ascribed this enzyme to one particular type. Its substrate profile would fit that of type 4 of the classification of Mitsuhashi and Kawabe (15); however, after preliminary chromatographic and electrophoretic analyses (data not shown), we found no indication of the existence of multiple active forms or of the particular tetrameric protein structure reported for an AAC(6′)-4 (19), and we did not use additional substrates which would allow a further distinction between amikacin-acetylating enzymes (T. Morohashi, T. Toriaya, Y. Shiritani, S. Yokoiyama, K. Fujimoto, and K. Hayano, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 949, 1984).

In an analysis of over 300 gram-negative aminoglycoside-resistant bacterial strains collected in Chile from 1980 to 1983, no amikacin-acetylating enzyme was detected (Hare

TABLE 4. Distribution of plasmid subtypes among clinical isolates

Plasmid type and subtype[a]	No. of strains				
	K. pneumoniae	*E. coli*	*S. marcescens*	*C. freundii*	*E. cloacae*
I					
A	8	2	4	1	0
B	2	0	0	0	0
C	0	0	0	1	3
D	1	0	1	0	0
II					
A	0	1	0	0	0
B	0	1	0	0	3

[a] For the definition of type see Table 3; for the definition of subtype, see the text.

and Miller, 1984). The results of the present study thus imply that selective pressure, exerted by predominant if not exclusive usage of amikacin in a restricted area, has favored the dissemination of an apparently wide-host-range plasmid and its derivatives, carrying the *aac*(6′) gene. A similar spread of amikacin resistance, also due to AAC(6′) but carried by an IncC plasmid, has been observed recently in a hospital in Paris, France (A. Buré, C. Carlier, L. Slim, B. Pangon, E. Rouveix, and P. Courvalin, Program Abstr. 14th Internatl. Congr. Chemother., Kyoto, Japan, abstr. no. P-42-20, 1985). These observations may be the reflection of a trend (11) not previously seen in the evolution of amikacin resistance. It is an open question whether such factors as the daily dose, frequency of administration, or combination with other drugs may have played a role in the selection of the amikacin resistance plasmids.

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LITERATURE CITED

1. **Acar, J. F., J. L. Witchitz, F. Goldstein, J. N. Talbot, and F. Le Goffic.** 1976. Susceptibility of aminoglycoside-resistant gram-negative bacilli to amikacin: delineation of individual resistance patterns. J. Infect. Dis. **134**:S280–S285.
2. **Betts, R. F., W. M. Valenti, S. W. Chapman, T. Chonmaitree, G. Mowrer, P. Pincus, M. Messner, and R. Robertson.** 1984. Five-year surveillance of aminoglycoside usage in a university hospital. Ann. Intern. Med. **100**:219–222.
3. **Chabbert, Y. A., M. R. Scavizzi, J. L. Witchitz, G. R. Gerbaud, and D. H. Bouanchaud.** 1972. Incompatibility groups and the classification of *fi*⁻ resistance factors. J. Bacteriol. **112**:666–675.
4. **Datta, N., and R. W. Hedges.** 1971. Compatibility groups among *fi*⁻R factors. Nature (London) **234**:222–223.
5. **Davies, J., and D. I. Smith.** 1978. Plasmid-determined resistance to antimicrobial agents. Annu. Rev. Microbiol. **32**:469–518.
6. **Foster, T. J.** 1983. Plasmid-determined resistance to antimicrobial drugs and toxic metal ions in bacteria. Microbiol. Rev. **47**:361–409.
7. **Haas, M., and J. R. Dowding.** 1975. Aminoglycoside-modifying enzymes. Methods Enzymol. **43**:611–628.
8. **Kawabe, H., T. Naito, and S. Mitsuhashi.** 1975. Acetylation of amikacin, a new semisynthetic antibiotic, by *Pseudomonas aeruginosa* carrying an R factor. Antimicrob. Agents Chemother. **7**:50–54.
9. **Kawaguchi, H., T. Naito, S. Nakagawa, and K. Fujisawa.** 1972. BB-K8, a new semisynthetic aminoglycoside antibiotic. J. Antibiot. **25**:695–708.
10. **Labigne-Roussel, A., G. Gerbaud, and P. Courvalin.** 1981. Translocation of sequences encoding antibiotic resistance from the chromosome to a receptor plasmid in *Salmonella ordonez*. Mol. Gen. Genet. **182**:390–408.
11. **Levine, J. F., M. J. Maslow, R. E. Leibowitz, A. A. Pollock, B. A. Hanna, S. Schaefler, M. S. Simberkoff, and J. J. Rahal, Jr.** 1985. Amikacin-resistant gram-negative bacilli: correlation of occurrence with amikacin use. J. Infect. Dis. **151**:295–300.
12. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
13. **Meyer, J. F., and B. Wiedemann.** 1985. Characterization of aminoglycoside 6′-*N*-acetyltransferases (AAC(6′)) from gram-negative bacteria and *Streptomyces kanamyceticus*. J. Antimicrob. Chemother. **15**:271–282.
14. **Meyer, J. F., B. A. Nies, and B. Wiedemann.** 1983. Amikacin resistance mediated by multiresistance transposon Tn*2424*. J. Bacteriol. **155**:755–760.
15. **Mitsuhashi, S., and H. Kawabe.** 1982. Aminoglycoside antibiotic resistance in bacteria, p. 97–122. *In* A. Whelton and H. C. Neu (ed.), The aminoglycosides. Marcel Dekker, Inc., New York.
16. **Moody, M., C. A. de Jongh, S. C. Schimpff, and G. L. Tillman.** 1982. Long-term amikacin use: effects on aminoglycoside susceptibility patterns of gram-negative bacilli. J. Am. Med. Assoc. **248**:1199–1202.
17. **Portnoy, D. A., S. L. Moseley, and S. Falkow.** 1981. Characterization of plasmids and plasmid-associated determinants of *Yersinia enterocolitica* pathogenesis. Infect. Immun. **31**:775–782.
18. **Price, K. E., P. A. Kresel, L. A. Farchione, S. B. Siskin, and S. A. Karpow.** 1981. Epidemiological studies of aminoglycoside resistance in the U.S.A. J. Antimicrob. Chemother. **8(Suppl. A)**:89–105.
19. **Radika, K., and D. B. Northrop.** 1984. Purification of two forms of kanamycin acetyltransferase from *Escherichia coli*. Arch. Biochem. Biophys. **233**:272–285.
20. **Sanson-Le Pors, M.-J., I. M. Casin, and E. Collatz.** 1985. Plasmid-mediated aminoglycoside phosphotransferases in *Haemophilus ducreyi*. Antimicrob. Agents. Chemother. **28**:315–319.
21. **Witchitz, J. L.** 1981. Epidemiological aspects of aminoglycoside resistance in France. J. Antimicrob. Chemother. **8(Suppl. A)**:71–82.
22. **Witchitz, J. L., F. W. Goldstein, B. Pagnon, D. Christol, and J. F. Acar.** 1979. Phénotypes de résistance aux aminosides et sensibilité à l'amikacine en mileu hospitalier. Nouv. Presse Med. **8**:3417–3420.