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Activity of BB-K8 (Amikacin) Against Clinical Isolates Resistant to One or More Aminoglycoside Antibiotics

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One hundred fifty-two bacterial strains that possess resistance to kanamycin A, gentamicin, or tobramycin, or to more than one of these antibiotics, were collected from various sources in Canada, Europe, Japan, and the United States. This collection was composed of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and members of the *Enterobacteriaceae* family. Their susceptibility to BB-K8 (amikacin), a new broad-spectrum semisynthetic derivative of kanamycin A, and to the other agents, was determined on Mueller-Hinton Medium by the twofold agar dilution method. Test results revealed that 60.5% of the isolates were resistant to 8 μg of tobramycin per ml, 67.1% to 8 μg of gentamicin per ml, 86.2% to 20 μg of kanamycin A per ml, and only 8.6% to 20 μg of amikacin per ml. Of interest is the fact that the amikacin-resistant strains were generally resistant to all of the other aminoglycosides. The broad spectrum of amikacin was not totally unexpected, because the compound has been shown to be a poor substrate for most enzymes that inactivate other aminoglycosides through *O*-phosphorylation, *O*-adenylylation, or *N*-acetylation. A number of susceptibility profiles were obtained when the organisms were tested against a series of nine aminoglycosides. The majority of these profiles resembled those found for organisms that possess known mechanisms of enzymatic inactivation.

Gentamicin has proven to be active against most kanamycin-resistant strains of *Staphylococcus* and *Enterobacteriaceae* and, in addition, has been found markedly inhibitory for most strains of *Pseudomonas aeruginosa*, a species totally refractory to kanamycin (M. J. Weinstein, First Int. Symp., Paris, p. 9-18, 1967). Although reports of gentamicin resistance among clinical isolates were rare during the first several years of its use, such resistance is now being encountered frequently, particularly among strains of *Providencia*, *Proteus*, and *Pseudomonas* sp. (1, 8, 14, 20, 21, 31, 36).

The discovery of tobramycin in the fermentation broths of *Streptomyces tenebrarius* provided an antibiotic that is as active as gentamicin against most strains of *Enterobacteriaceae* and has activity at least twofold greater against *P. aeruginosa*. Interestingly, there have already been reports describing clinical isolates that are resistant to tobramycin (6, 8). However, not all of these strains have been found to be resistant to gentamicin (13, 15, 27). This is not too surprising since it is now known that some gram-negative bacilli produce an R-factor-mediated enzyme (10) that inactivates gentamicin but not tobramycin, although at least three other enzymes (2, 3, 38) readily inactivate both antibiotics.

A recently synthesized kanamycin derivative, BB-K8 (19), is active against a high percentage of gentamicin-resistant strains of *P. aeruginosa* and *Enterobacteriaceae* (30). This antibiotic, which now has the generic name "amikacin," either does not serve as a substrate or is only a poor one for three of the four enzymes that inactivate gentamicin and for two of the three that affect tobramycin. Furthermore, growth of some species of organisms producing the one enzyme that does affect amikacin may still be inhibited by relatively low concentrations of the antibiotic. Thus, on the basis of its unique ability to resist enzymatic attack, one would expect amikacin to have significant activity against many gentamicin- and tobramycin-resistant microbial strains.

The present study was undertaken to explore this suggestion and to attempt to gain insight into the relative incidence of *O*-phosphorylating, *O*-adenylylating, and *N*-acetylating enzymes produced by a collection of clinical strains of *Staphylococcus aureus*, *P. aeruginosa*,

and *Enterobacteriaceae* species, each member of which is resistant to one or more aminoglycoside antibiotics.

MATERIALS AND METHODS

Antibiotics. The aminoglycoside antibiotics utilized and their sources are as follows: kanamycin A sulfate, amikacin base, butirosin A base (Bristol Laboratories); lividomycin A sulfate (Kowa Laboratories, Tokyo, Japan); gentamicin sulfate, a mixture of gentamicins C_1, C_{1a}, and C_2 (Schering Corp.); dideoxykanamycin B base (S. Umezawa, Keio University, Yokohama, Japan); tobramycin base (Eli Lilly & Co.); and neomycin B sulfate (Mann Research Laboratories).

The quantities of the antibiotics employed in both in vitro and in vivo experiments were corrected so that final concentrations of all were in terms of the pure free base.

Microorganisms. The 152 strains of aminoglycoside-resistant organisms used in these studies were predominantly of clinical origin and included members of the following species: *S. aureus*, *P. aeruginosa*, *Escherichia coli*, *Enterobacter cloacae*, *E. aerogenes*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *P. rettgeri*, *Providencia stuartii*, *Serratia marcescens*, and several species of *Salmonella*. Since *P. aeruginosa* strains generally lack susceptibility to kanamycin A, only those resistant to at least one other aminoglycoside (gentamicin, tobramycin, or amikacin) were included in this study.

Organisms were obtained from the Bristol Laboratories culture collection or were acquired through the generosity of the following investigators: J. F. Acar, Hôpital St.-Joseph, Paris, France; J. Addonizio, La Habra, Calif.; J. M. Andronaco, New York, N.Y.; M. Biddle, USC Medical Center, Los Angeles, Calif.; G. P. Bodey, M.D. Anderson Hospital, Houston, Tex.; M. Brook, West Haven VA Hospital, West Haven, Conn.; Y. Chabbert, Pasteur Institute, Paris, France; P. K. Clark, Salt Lake City, Utah; S. Cohen, Michael Reese Hospital, Chicago, Ill.; C. E. Cox, Univ. of Tenn., Memphis; J. Davies, Univ. of Wisconsin, Madison; J. S. Davis, Jr., St. Luke's Hospital, New York, N.Y.; L. J. Griffith, VA Hospital, Batavia, N.Y.; W. L. Hewitt and T. F. Keys, UCLA Medical Center, Los Angeles, Calif.; J. O. Lindsey, CDC, Atlanta, Ga.; S. M. Luchs, New York, N.Y.: R. R. Martin, Baylor College of Medicine, Houston, Tex.; Z. McGee, Vanderbilt Univ., Nashville, Tenn.; S. W. B. Newsom, Papworth Hospital, Cambridge, England; L. A. Orkin, New York, N.Y.; D. J. Pohlod, Henry Ford Hospital, Detroit, Mich.; J. G. Raffensberger, Cook County Children's Hospital, Chicago, Ill.; H. G. Robson, Royal Victoria Hospital, Montreal, Canada; A. R. Ronald, Winnipeg General Hospital, Winnipeg, Canada; L. P. Russell, Presbyterian Medical Center, New York, N.Y.; L. D. Sabath, Boston City Hospital, Boston, Mass.; E. W. Sanders, Creighton Univ., Omaha, Neb.; H. C. Standiford, VA Hospital, Baltimore, Md.; H. H. Stone, Emory School of Medicine, Atlanta, Ga.; M. Turck, Harborview Medical Center, Seattle, Wash.; H. Umezawa, Institute of Microbiology and Chemistry, Tokyo, Japan; U.S. Naval Hospital, Memphis, Tenn.; J. A. Waitz, Schering Corp., Bloomfield, N.J.; H. Wallick, Merck & Co., Rahway, N.J.; J. A. Washington, Mayo Clinic, Rochester, Minn.; J. N. Wilfert, Univ. of Utah, Salt Lake City; L. S. Young, UCLA Medical Center, Los Angeles, Calif.; and M. Yow, Baylor Univ., Houston, Tex.

In vitro antibacterial activity. Minimal inhibitory concentrations (MIC) of the nine antibiotics studied were determined by means of an agar dilution procedure in which standardized bacterial inocula were deposited on the surface of agar plates with the multiple inoculator device described by Steers, Foltz, and Graves (32). Inocula were prepared by diluting 18-h cultures in Mueller-Hinton Broth (Difco) to an optical density of 0.6 at 560 nm with a Spectronic-20 colorimeter (Bausch & Lomb) using 13 by 100 mm cuvettes. The resulting cell suspensions, which contained approximately 5×10^8 viable bacterial cells per ml, were then diluted 5- and 50-fold in Mueller-Hinton Broth. Application of these dilutions in 0.0025-ml amounts to the surface of plates containing Mueller-Hinton Medium (Difco) resulted in final inoculum sizes of approximately 2.5×10^6 and 2.5×10^4 cells, respectively. Antibiotics had previously been added to the inoculated plates in twofold concentration increments. The MIC was considered to be the lowest concentration which permitted the growth at the site of inoculation of no more than five individual colonies during overnight incubation at 37 C. In each case, the final MIC was the geometric mean of the MIC values obtained in five independent determinations.

Demonstration of enzymatic activity. Cell-free preparations of selected organisms were prepared either by osmotic shocking procedures as described by Benveniste, Yamada, and Davies (4) or by ultrasonic treatment (J. Davies, personal communication). Radioactive assays for detection of adenylylating activity were carried out as described by Brzezinska et al. (10) using [^{14}C]adenosine triphosphate (ATP) as the labeled substrate. Phosphorylating activity using [^{32}P]ATP and acetylating activity utilizing [^{14}C]acetyl coenzyme A were determined by the methods of Benveniste and Davies (2). The labeled substrates were obtained from Amersham-Searle. Radioactivity was determined with a Tri-Carb liquid scintillation spectrometer (Packard Instrument Co.).

Therapeutic effectiveness of aminoglycoside antibiotics. Selected strains resistant to aminoglycosides by virtue of known or suspected enzymatic inactivation mechanisms were used to produce experimental infections in mice. Organisms making up the challenge dose were suspended in 4% mucin and administered in 0.5-ml volume by the intraperitoneal route to male Swiss-Webster mice having individual weights of 20 ± 2 g. The number of cells administered was sufficient to kill all untreated mice within 48 h. Antibiotic treatment was administered by the intramuscular (i.m.) route at 1 and 4 h postchallenge or, in the case of several therapeutically refractive *P. aeruginosa* infections, at 0, 2, 4, and 6 h postchallenge. In all cases, dose responses were determined with five to six drug dosages that varied by fourfold increments and with five animals per dosage level. At the

conclusion of the experiment (usually day 5), the total number of surviving mice was recorded and the PD_{50} (the dose in milligram per kilogram per treatment which protected 50% of the animals) was estimated by means of a log-probit plot.

RESULTS

Susceptibility to aminoglycoside antibiotics. Initially, 152 aminoglycoside-resistant bacterial strains were tested by the agar dilution susceptibility test method against the following antibiotics: kanamycin, gentamicin, tobramycin, and amikacin. The criteria of susceptibility chosen for this study were based on the mean peak serum concentrations achievable in humans after i.m. administration of doses recommended for routine therapeutic use. This procedure has been used previously by others to assign clinical strains to susceptible or resistant categories (20, 41).

Kanamycin, when given in a standard dose of 7.5 mg/kg, produces an average peak serum level of about 20 μg/ml (20, 24), whereas gentamicin, at the commonly used dose of 1.6 mg/kg, gives a peak of approximately 8 μg/ml (5). Standard dosage levels of tobramycin and amikacin have not yet been unequivocally established; however, preliminary reports suggest that the recommended dosage level of amikacin and the peak serum concentration achievable after its administration (12; W. W. King, and C. E. Cox, Prog. Abstr. Intersci. Conf. Antimicrob. Ag. Chemother. 13th, Abstr. no. 126, 1973) may be similar to those of kanamycin, whereas those of tobramycin (25, 26; W. W. King and C. E. Cox, Prog. Abstr. Intersci. Conf. Antimicrob. Ag. Chemother. 12th, Abstr. no. 32, 1973) may be generally comparable to those of gentamicin. Thus, for the purposes of the present study, organisms were considered susceptible to kanamycin and amikacin if they were inhibited at a 20 μg/ml concentration. In the case of gentamicin and tobramycin, only those organisms whose growth was inhibited at 8 μg/ml were designated susceptible. The comparative activity of these four antibiotics against wild-type antibiotic-susceptible bacteria were discussed previously by Bodey and Stewart (7) and by Karney et al. (18).

Table 1 shows the number of strains of each species examined in agar dilution tests that were found resistant to kanamycin, gentamicin, tobramycin, or amikacin. Two different inocula of each strain were used; one was a 5-fold dilution of cultures standardized to contain approximately 5×10^8 cells/ml, the other was a 50-fold dilution of the same standardized cultures. Since the MICs for the two inocula were not significantly different, only results obtained with the higher one (1×10^8 cells/ml) are reported here. Also listed in Table 1 is the number of sources from which members of each species were acquired. It seems likely that, the greater the number of sources, the greater the probability that the organisms utilized are truly different strains rather than different isolates of the same strain.

All but one of 36 *E. coli* and *Enterobacter* strains studied were resistant to kanamycin; 18 were resistant to gentamicin and 19 were resistant to tobramycin. Only 7 of the 36 strains were resistant to amikacin. The pattern found for *K. pneumoniae* strains was similar, although the number of strains resistant to gentamicin and tobramycin was slightly higher. All were susceptible to amikacin and all resistant to kanamycin.

Gentamicin and tobramycin failed to inhibit the growth of the majority of *Proteus* and *Providencia* strains. Kanamycin, however, in contrast to what had been found for the previously discussed species, inhibited many of these isolates, and all but one of the 41 strains in these two groups were susceptible to amikacin.

An interesting variation in the pattern of resistance to aminoglycoside antibiotics was observed in strains of *S. marcescens*. With this species, the percentage of strains resistant to both kanamycin and tobramycin was quite high, whereas gentamicin, in sharp contrast, inhibited 12 of the 18 strains tested. None of the 18 *Serratia* strains was resistant to amikacin.

Single strains of *Salmonella derby*, *S. panama*, and *S. orienburg* were each found resistant to kanamycin but not to the other antibiotics. An interesting resistant profile was also noted for the 34 strains of *P. aeruginosa*. The finding that all isolates were resistant to kanamycin was not surprising since it is an antibiotic that appears to be devoid of activity against this species. However, the fact that only 16 strains were refractory to tobramycin whereas 34 were resistant to gentamicin was somewhat unexpected. Amikacin was even more effective than tobramycin, since only five isolates were resistant to it. The seven staphylococcal strains were resistant to kanamycin but susceptible to the other aminoglycosides.

Of the 152 strains tested that were resistant to each of the antibiotics, 131 (86.2%) were resistant to kanamycin, 102 (67.1%) to gentamicin, 92 (60.5%) to tobramycin, and only 13 (8.6%) were resistant to amikacin.

Patterns of cross-resistance among four aminoglycoside antibiotics. It was also considered of interest to determine the extent of

cross-resistance between kanamycin, gentamicin, tobramycin, and amikacin. Table 2 shows the various resistance patterns observed with the 152 aminoglycoside-resistant bacterial strains studied.

When resistance to a single antibiotic was observed, the drug most commonly involved was kanamycin (K). Resistance to kanamycin was observed frequently and among most of the species tested. The incidence of single antibiotic resistance to gentamicin (G) and tobramycin (T) was extremely low, and no strains showed resistance to amikacin (A) alone.

Among organisms that were resistant to two of the four antibiotics, resistance to KG and GT was most frequently detected, i.e., it was detected in 19 and 17 strains, respectively, of the 152 isolates tested. Twelve strains had the KT pattern, and there were no strains with KA, GA, or TA patterns.

Profiles of strains resistant to three antibiotics varied widely in their incidence. For example, 49 strains showed resistance to KGT. This was, therefore, the most common profile identi-

TABLE 1. *Number of bacterial strains resistant to kanamycin, gentamicin, tobramycin, and amikacin*

Organism	No. of sources	No. of strains	No. of aminoglycoside-resistant organisms			
			Kanamycin	Gentamicin	Tobramycin	Amikacin
Escherichia coli	13	21	20	11	12	3
Enterobacter sp.	8	15	15	7	7	4
Klebsiella pneumoniae	6	13	13	9	10	0
Proteus sp.	8	17	11	17	16	1
Providencia stuartii	6	24	10	18	16	0
Serratia marcescens	9	18	18	6	15	0
Salmonella sp.	1	3	3	0	0	0
Pseudomonas aeruginosa	11	34	34	34	16	5
Staphylococcus aureus	3	7	7	0	0	0
Total (%)		152 (100)	131 (86.2)	102 (67.1)	92 (60.5)	13 (8.6)

TABLE 2. *Patterns observed in susceptibility tests with 152 aminoglycoside-resistant bacterial strains*

Resistance patterns	No. of resistant strains							Total no. of resistant strains (152)[c]
	E. coli (21)[a]	*Enterobacter* sp. (15)	*K. pneumoniae* (13)	*Proteus* sp. (17)	*S. marcescens* (18)	*P. stuartii* (24)	*P. aeruginosa*[b] (34)	
Kanamycin (K)	8	8	3		3	6		28
Gentamicin (G)	1					1		2
Tobramycin (T)						2		2
Amikacin (A)								0
KG				1		1	17	19
KT	2		1		9			12
KA								0
GT				6		11		17
GA								0
TA								0
KGT	7	3	9	9	6	3	12	49
KGA							1	1
KTA								0
GTA								0
KGTA	3	4		1			4	12

[a] Numbers in parentheses indicate number of strains tested.

[b] *Pseudomonas aeruginosa* strains were selected so that representative isolates possessing resistance to kanamycin only were excluded from the study.

[c] Includes seven strains of *Staphylococcus aureus* and three strains of *Salmonella* species which were also resistant to kanamycin.

fied in the entire study. In contrast, only one strain showed resistance to KGA. No KTA or GTA patterns were found among any of the isolates. Resistance to all four antibiotics (pattern KGTA) was detected in 12 instances and occurred primarily in *E. coli*, *Enterobacter*, and *P. aeruginosa* strains.

Some of the patterns (K and KGT) were widely distributed among members of almost all species examined, whereas other patterns were more restricted in their distribution. For example, T was encountered only in strains of *P. stuartii*, and KG almost exclusively (17 of 19 times) in strains of *P. aeruginosa*. Similarly, the distribution of GT was also restricted and was observed only in *Proteus* and *Providencia* sp.

Resistance profiles obtained with nine aminoglycoside antibiotics. Since amikacin acts as a poor substrate for most aminoglycoside-inactivating enzymes (30), it seems probable that the low incidence of resistance to the compound noted in the preceding experiments may be attributed to this property. However, because of the obvious difficulties presented by an effort to examine the specific enzyme-producing capability of each member of this large group of 152 bacterial strains, an alternate approach was employed in an effort to gain some information in this regard. Previous studies at Bristol Laboratories have shown that organisms possessing a known inactivating enzyme(s) give a characteristic "resistance profile" when tested against nine selected aminoglycoside antibiotics. It seemed of interest, therefore, to test the present group of clinical isolates against this same series of nine aminoglycosides to determine whether the resistance profiles found were all similar to those previously observed or whether new profiles could be detected.

Table 3 shows 10 resistance profiles that would be expected for organisms which produce the enzymes listed in the last column. For each of 10 enzymes or combinations of enzymes listed, a characteristic profile was obtained. These profiles were deduced from our present knowledge of enzymatic inactivation in resistant strains; it is possible, of course, that an as yet undescribed inactivating mechanism could give rise to similar profiles. Also included in Table 3 is an additional group of six profiles whose characteristics differ from those attributable to organisms with a known enzyme complement. It has not yet been determined whether all organisms displaying such profiles actually produce aminoglycoside-inactivating enzymes.

Patterns 1, 2, and 3 occurred as a result of the action of the plasmid-mediated enzymes, neomycin-kanamycin phosphotransferases I and II (NPT_I and NPT_{II}), which phosphorylate the 3'-OH of antibiotics having this function present in the 4-substituent of 2-deoxystreptamine (2-DOS), or the 5''-OH of the ribose moiety of lividomycins (9, 16, 17, 22, 23, 27, 34, 39).

The next profile (no. 4) is attributable to the transferable R-factor-mediated enzyme, gentamicin adenylylate synthetase (GAS), that expresses itself through the mechanism of *O*-adenylylation at the 2''-OH group of aminohexoses glycosidically linked at the 6-position of 2-DOS (2, 3, 30, 37). Profiles 5 and 6 were obtained with combinations of GAS with NPT_I and with NPT_{II}, respectively, and gave the indicated patterns of resistance.

Pattern 7 occurred with strains that produce gentamicin acetyltransferase (GAT_I), a highly specific enzyme that acetylates the C-3 amino group of the 2-DOS moiety of gentamicins (10). The enzyme appears to be plasmid-mediated in some strains of *Enterobacteriaceae* (35).

Pattern 8 was observed with strains that produce GAT_I and a low level of phosphorylating enzymes. Studies measuring uptake of ^{32}P from ^{32}P-ATP indicated that both NPT_I and NPT_{II} may be present in cell-free preparations of these strains.

The next pattern (no. 9) occurred as a result of inactivation produced by gentamicin acetyltransferase II (Gat_{II}) which acetylates the 2'-amino group of the aminoglucose moiety of neamine and neamine-like components of this group of antibiotics (3). Thus far, no information is available as to whether this type of resistance is associated with the bacterial chromosome or with a transferable plasmid.

Pattern 10 occurred with isolates that produce kanamycin acetyltransferase (KAT). This plasmid-mediated enzyme is transferable and has as its site of action the 6'-amino group that is present in the aminoglucose portion of the antibiotics' neamine or neamine-related moieties (28, 33, 40).

The enzyme complement of strains producing the remaining patterns is unknown. Pattern 11 is characteristic of strains that are resistant to kanamycin and tobramycin, but susceptible to gentamicin and amikacin. Pattern 12 is similar except that the test strains are also susceptible to all pentose-containing antibiotics other than butirosin. Of interest is the fact that all six strains which displayed this pattern were *S. marcescens* strains that possess acetylating activity (K. Price, unpublished data).

Patterns 13 and 14 were obtained with isolates that were resistant to kanamycin and

TABLE 3. *Profiles obtained with bacterial strains that are resistant to various aminoglycoside antibiotics*

Profile no.	Resistance profile									Enzyme(s) responsible for profile[a]
	Kanamycin	Gentamicin	Tobramycin	Amikacin	Dideoxykanamicin B	Butirosin	Lividomycin	Paromomycin	Neomycin	
1	R[b]						R	R	R	NPT_I
2	R					R		R	R	NPT_{II}
3	R					R	R	R	R	NPT_{I+II}
4	R	R	R		R					GAS
5	R	R	R		R		R	R	R	GAS + NPT_I
6	R	R	R		R	R		R	R	GAS + NPT_{II}
7		R								GAT_I
8	R	R				R	R	R	R	GAT_I + NPT_{I+II}
9		R	R		R	R	R	R	R	GAT_{II}
10	R	R[c]	R	R[c]	R	R			R	KAT
11	R		R		R	R	R	R	R	Unknown
12	R		R		R	R				Unknown
13	R	R			R	R	R	R	R	Unknown
14	R	R		R		R	R	R	R	Unknown
15	R	R	R		R	R	R	R	R	Unknown
16	R	R	R	R	R	R	R	R	R	Unknown

[a] NPT_I, Neomycin-kanamycin phosphotransferase I; NPT_{II}, neomycin-kanamycin phosphotransferase II; GAS, gentamicin adenylylate synthetase; GAT_I, gentamicin acetyltransferase I; GAT_{II} gentamicin acetyltransferase II; KAT, kanamycin acetyltransferase.

[b] R, Resistant to kanamycin, amikacin, butirosin, lividomycin, or paromomycin at 20 μg/ml, or to gentamicin, tobramycin, dideoxykanamycin B, or neomycin at 8 μg/ml.

[c] The aminoglycoside acts as a substrate for the enzyme, but some organisms may still show susceptibility to it.

gentamicin but susceptible to tobramycin. They differ from each other in that, in the first (pattern 13), the organisms were resistant to dideoxykanamycin B (DKB) whereas, in the second (no. 14), DKB was active but amikacin was not.

Pattern 15 was found for strains that were susceptible to amikacin only, whereas organisms having pattern 16 were resistant to all nine of the aminoglycosides.

Overall, in the case of the 95 strains with known resistance patterns (1 to 10), and therefore thought to be enzyme producers, kanamycin was the antibiotic most susceptible to inactivation, for it could be phosphorylated, adenylylated, or acetylated. The pentose-containing aminoglycosides, on the other hand, could also be phosphorylated and acetylated but were not subject to adenylylation. Tobramycin, gentamicin, and DKB can be inactivated either through adenylylation or acetylation, while amikacin activity is affected by only one type of acetyltransferase (KAT).

Categorization of strains according to species and type of resistance profile. Table 4 shows the number of strains, among the 152 investigated in this study, which could appropriately be assigned on the basis of their resistance pattern to one of the 16 profiles shown in Table 3. Eighteen of the test organisms had a resistance profile identical to that observed in bacterial strains which produce NPT_I. This profile is widely distributed and was found among representatives of five different genera of *Enterobacteriaceae*. The distribution of profile 2, which is typical of that obtained with NPT_{II}-producing strains, was more restricted and occurred in only two species (*E. coli* and *E. cloacae*). The incidence of these strains was lower than that of strains producing NPT_I. Interestingly, the pattern produced by the combination of NPT_I and NPT_{II} did not occur in any of the same species in which a pattern produced by a single phosphorylating enzyme was found. The combination pattern (no. 3) was equally distributed among strains of *Providencia* and *Staphylococcus* sp. It is likely, that, if wild-type *P. aeruginosa* strains, i.e., those that are resistant to kanamycin but susceptible to gentamicin, tobramycin, and amikacin, had been included in the study, a high percentage would have been found to have this same resistance profile.

The profile (no. 4) of strains that produce GAS alone was observed in only 3 of 152 species. However, patterns like those produced when GAS is present in combination with a phosphorylating enzyme (no. 5 and 6) were observed more often. Overall, 17 strains had resistance profiles which suggested that a combination of

this general type was present. Of these 17 strains, 9 were *K. pneumoniae* isolates, the majority of which have been unequivocally shown by use of labeled substrates to produce GAS concurrently with a phosphorylating enzyme (J. Davies, personal communication; K. E. Price, unpublished data).

GAT_I alone produces a characteristic pattern (no. 7) which was only rarely encountered in the present series of 152 isolates. However, the profile observed with the combination of GAT_I and phosphorylating enzymes (no. 8) occurred relatively frequently, but exclusively, in strains of *P. aeruginosa*. The presence of GAT_I in this species could thus account for the rather high incidence of gentamicin-resistant, tobramycin-sensitive *P. aeruginosa* strains observed in this study and probably for those found in the series of *P. aeruginosa* strains studied by Burger, Sanford, and Zweighaft (11).

Pattern 9, which is characteristic of that obtained with organisms which produce GAT_{II}, was found exclusively in members of the *Proteeae* tribe, specifically *Proteus* sp. and *P. stuartii*. Because kanamycin does not serve as a substrate for GAT_{II} and is therefore inhibitory for the producing organisms in in vitro susceptibility tests, this pattern is readily identifiable.

Pattern 10 is that observed for organisms producing KAT only. The presence of this pattern was observed in only 1 of 152 strains.

The remaining 57 strains, all of which had resistance patterns that differed from those possessed by organisms producing a known enzyme or known combinations of enzymes, had profile numbers 11 through 16. These profiles occurred principally in isolates of *E. coli* (8 strains), *Proteus* sp. (11 strains), *S. marcescens* (12 strains), and *P. aeruginosa* (17 strains). The major pattern observed was no. 15 (28 isolates), which is characterized by the fact that amikacin is the only antibiotic with significant activity. This type of profile could have resulted from the presence of a new type of inactivating enzyme or from certain combinations of enzymes such as GAT_{II} and NPT_I and/or NPT_{II}, or GAS + NPT_I + NPT_{II}. Additional laboratory studies will be required to establish the true enzyme complement of such strains.

Profile 16 was also observed frequently; 12 strains were resistant to the action of all 9 of the aminoglycosides studied. Such resistance, if mediated by enzymatic mechanisms, could be obtained when KAT was found in conjunction with NPT_I, or when an as yet undetermined enzyme is present. There is also the possibility that other nonspecific resistance mechanisms may be operative. For example, cells of these strains could have reduced permeability to this class of antibiotics or have altered ribosomes that are not susceptible to the action of aminoglycoside antibiotics.

Response to therapy of experimental infections caused by enzyme-producing organisms. To determine the effect of enzymatic inactivation of aminoglycoside antibi-

TABLE 4. *Aminoglycoside resistance profiles found among 152 bacterial isolates*

Profile no.	No. of organisms with indicated profile									Total no. of strains (152)	Possible inactivating enzyme(s)[b]
	E. coli (21)[a]	*Enterobacter* sp. (15)	*K. pneumoniae* (13)	*Proteus* sp. (17)	*Salmonella* sp. (3)	*S. marcescens* (18)	*P. stuartii* (24)	*P. aeruginosa* (34)	*S. aureus* (7)		
1	4	5	3		3	3				18	NPT_I
2	4	3								7	NPT_{II}
3							6		7	13	NPT_{I+II}
4	1	2								3	GAS
5		1	2			3		1		7	GAS + NPT_I
6	2		7					1		10	GAS + NPT_{II}
7	1						1			2	GAT_I
8								15		15	GAT_I + NPT_{I+II}
9				6			13			19	GAT_{II}
10	1									1	KAT
11				1			1	2		4	Unknown
12						6				6	Unknown
13	2		1			3				6	Unknown
14								1		1	Unknown
15	3			9		3	3	10		28	Unknown
16	3	4		1				4		12	Unknown

[a] Numbers in parentheses indicate number of strains tested.
[b] See footnote *a*, Table 3, for enzyme identification.

otics on their therapeutic efficacy, a number of microbial strains known to produce inactivating enzymes were used to produce experimental systemic infections in mice. The methods used were described previously. Amikacin, gentamicin, tobramycin, and kanamycin were utilized as therapeutic agents in these experiments.

It is apparent (Table 5) that the protective effects obtained with the various antibiotics were generally consistent with those that would be expected on the basis of the known enzyme complement of the challenge organisms. Particularly interesting were the results obtained in infections caused by the KAT-producing organisms. None of the antibiotics was particularly effective against the *P. aeruginosa* strain (A20897), whereas all were reasonably efficacious against *E. coli* A21218, although it, too, is a producer of KAT. These results do not conflict with those obtained in in vitro tests since, as previously noted, this plasmid-associated resistance may be expressed at different levels in these two organisms.

DISCUSSION

It is clear from results obtained in these studies that the term "cross-resistance" must be used with care when discussing aminoglycoside antibiotics. It is possible to find, when one examines only a limited number of resistant strains from a single source, that all strains are uniformly resistant to several different aminoglycosides, whereas co-resistance may be absent among strains from another source. Such findings are to be expected in view of the variety of substrate profiles produced by the many enzymes now known to have aminoglycoside-inactivating properties.

Observations made in the present investigation regarding the relative percentage of strains resistant to given antibiotics obviously relate to this particular collection of strains. There is little doubt that the results would have been different if there had been changes in the number of representatives of any one of several of the species studied. For example, inclusion of more *P. aeruginosa* isolates undoubtedly would

TABLE 5. *Efficacy of aminoglycosides in experimental infections of mice caused by bacteria that produce antibiotic inactivating enzymes*

Challenge organism	Enzyme[a] complement (antibiotics inactivated)	Challenge (no. of organisms/mouse)	Protective dose, 50% (mg/kg/treatment)			
			Amikacin	Gentamicin	Tobramycin	Kanamycin
Enterobacter cloacae A20960	$GAS + NPT_I$ (G, T, K)	1×10^5	1.5	18	12	>200
		2×10^5	2	9	16	>100
Klebsiella pneumoniae A20636	$GAS + NPT_{II}$ (G, T, K)	4×10^6	3	9	15	140
		6×10^6	6	18	18	>200
Pseudomonas aeruginosa A20717	$GAT_I + NPT_{I+II}$ (G, K)	4×10^5	9	35	4	90
		8×10^5	6	30	3.7	90
P. aeruginosa A20718		9×10^6	29	>100	12	>100
		1×10^7	18	>200	6	>200
Providencia stuartii A20922		1×10^7	3.6	18	30	9
		2×10^7	4	18	18	9
P. stuartii A20894	GAT_{II} (G, T)	2×10^6	5	18	18	2
		6×10^6	4	15	29	5
Proteus rettgeri A21207		2×10^6	4	9	18	7.4
		2×10^6	2	15	45	9
P. aeruginosa A20741	$GAT^c + NPT_{II}$ (G, T, K)	1×10^7 [b]	6	>100	>100	96
		4×10^8	29	>100	>100	>200
Serratia marcescens A20945		2×10^5	4	45	66	>200
		2×10^5	3.6	32	40	>200
P. aeruginosa A20897	$KAT + NPT_{I+II}$ (G, T, K, A)	5×10^6 [b]	45	>100	>100	>100
		4×10^7	70	>100	>100	>200
Escherichia coli A21218	KAT (G, T, K, A)[d]	4×10^7	3.8	1.3	5	18
		6×10^7	6.8	1	3.5	8

[a] See footnote *a*, Table 3, for enzyme identification.

[b] Animals received treatment at 0, 2, 4, and 6 h postchallenge; all others were treated at 1 and 4 h postchallenge.

[c] The characteristics of this acetyltransferase are not yet known.

[d] Minimally inactivated.

have caused an increase in the number of gentamicin-resistant, tobramycin-sensitive strains, whereas additional *S. marcescens* strains would have produced the opposite result. Similarly, inclusion of a greater number of *Proteus* and *Providencia* isolates probably would have increased significantly the number of strains susceptible to kanamycin, but resistant to gentamicin and tobramycin. These predictions are based on the observation that certain species are more likely than others to possess genes that mediate specific inactivating enzymes.

Although it is recognized that the nature of the strains examined could have a marked influence on the type of results obtained, it is likely that the distribution of resistance profiles observed in this study has some significance. The mere fact that there was a relatively wide variation in the number of strains of each species available for incorporation into the study may be meaningful in itself, since it gives some indication as to the relative frequency of aminoglycoside resistance among strains which, at least with respect to their species classification, were collected on a random basis. Those species with the greatest representation in this collection probably are the ones with the highest overall incidence of resistance to aminoglycosides in the clinical environment. The data obtained in this study were not merely the result of conditions that exist within a localized setting, since the isolates examined were obtained from many and diverse sources. In fact, on average, only about two resistant strains were obtained from each source.

Amikacin, as predicted, displayed remarkable effectiveness against strains resistant to other aminoglycosides. Only 13 of the 102 gentamicin-resistant strains and 12 of the 92 tobramycin-resistant strains were refractory to this antibiotic. Particularly noteworthy is the fact that all but one of the amikacin-resistant strains were commonly resistant to gentamicin, tobramycin, and kanamycin.

Thus, among aminoglycoside-resistant organisms, the spectrum of amikacin appears to be broader than that of the other three aminoglycoside antibiotics. When resistance to amikacin occurs, the probability is high that resistance to other aminoglycosides will also be present.

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