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Activity of Five Aminoglycoside Antibiotics In Vitro Against Gram-Negative Bacilli and *Staphylococcus aureus*

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The in vitro susceptibility to BB-K8, butirosin, gentamicin, sisomicin, and tobramycin of seven groups of clinically significant gram-negative bacilli and *Staphylococcus aureus* was assessed by using the International Collaborative Study-World Health Organization criteria. The activity of gentamicin, sisomicin, and tobramycin generally paralleled each other. Sisomicin was the most potent compound by weight and usually demonstrated the most rapid rate of killing. BB-K8 and butirosin were less potent, but higher serum levels may be achieved with these agents. BB-K8 generally showed the greatest ratio between achieveable mean peak serum levels and concentrations needed to inhibit ⅔ of each group of organisms tested. Additionally, BB-K8 was active against six of seven highly gentamicin-resistant strains. All of these antibiotics showed diminished activity at pH 6.4 but only gentamicin and sisomicin showed occasionally enhanced activity at pH 8.4.

With the isolation of the gentamicin complex over a decade ago, the first of a new group of aminoglycoside antibiotics became available, which were characterized by their activity against *Staphylococcus aureus* and most clinically significant aerobic gram-negative bacilli, including *Pseudomonas aeruginosa* and members of the family *Enterobacteriaceae* (20). It has become apparent, however, that the following considerations may affect the continued widespread use of gentamicin. First, the peak serum levels of gentamicin after administration of 1 to 2 mg/kg (considered the upper range of nontoxic doses) may just exceed the minimal inhibitory concentration (MIC) of many clinically significant organisms (21). Secondly, there are increasing reports of bacterial resistance to gentamicin either on the basis of selection of naturally occurring resistant strains (17) or epidemic spread of highly resistant organisms which possess resistance transfer factors (12). For these reasons, the search continues for new antimicrobial agents with a similar anti-gram-negative bacillary spectrum but with increased potency and an improved therapeutic ratio.

Four new aminoglycoside antibiotics are now being evaluated in vitro and in human trials. These include BB-K8, a semisynthetic aminoglycoside produced by acylation of kanamycin A (11); butirosin, a complex of two antibiotics (A and B isomers) obtained from fermentation filtrates of *Bacillus circulans* (9); sisomicin, a fermentation product of *Micromonospora inyoensis* (4); and tobramycin, formed by hydrolysis of the fermentation product of *Streptomyces tenebrarius* (14). In this report we present the results of in vitro antimicrobial susceptibility tests of these five antibiotics against 50 strains each of seven groups of clinically significant gram-negative bacilli and *S. aureus*. The agar dilution method was used principally, but for purposes of comparison a limited number of broth dilution susceptibility test results have also been included. Additionally, information will be presented regarding the rapidity of killing certain strains by these agents and the pH conditions affecting their antimicrobial activity.

Because many studies of in vitro antimicrobial susceptibility differ in their methodology, we have carried out these comparisons according to the recent recommendations of the International Collaborative Study (ICS) on antimicrobial sensitivity testing sponsored by the World Health Organization (5). The advantages of such an approach are that the results may be

compared with those of other laboratories using a widely accepted, standardized technique. In addition, susceptibility data obtained by following ICS criteria will be presented for a number of organisms now commonly used as standard reference strains during antibiotic susceptibility testing. These include the strains recommended by Bauer et al. (1) and some of the reference strains proposed by the ICS Committee. Thus, the results of these studies can be compared readily to data from other laboratories which use similar techniques and reference strains.

MATERIALS AND METHODS

In vitro susceptibility of isolates. In vitro susceptibility of 50 isolates each of *Escherichia coli*, *Enterobacter* sp., *Klebsiella pneumoniae*, *Proteus mirabilis*, indole-positive *Proteus species*, *P. aeruginosa*, *Serratia marcescens*, and *S. aureus* against BB-K8, butirosin, gentamicin, sisomicin, and tobramycin was assessed by using the agar dilution method exactly as recommended by the ICS (5). These organisms were isolated from clinical material since January 1971, either in the Microbiology Laboratory of the Memorial Sloan-Kettering Cancer Center or the Clinical Laboratory of the University of California at Los Angeles (UCLA) Medical Center and identified by standard criteria (2). Mueller-Hinton agar prepared from Mueller-Hinton broth (Difco lot control no. 573075) solidified with 1.5% agar (Difco lot control no. 580019) and supplemented with 5% defibrinated sheep blood (vol/vol) was prepared to contain twofold increments of each antibiotic. Five per cent agar was used for testing of *Proteus* sp. to suppress swarming. Gentamicin and sisomicin were obtained in the sulfate form from George Arcieri of the Schering Corporation; BB-K8 in the sulfate form was from Edward Yevak of Bristol Laboratories; butirosin in the sulfate form was from M. W. Fisher of Parke-Davis & Co.; and tobramycin as the base was from R. S. Griffith of Eli Lilly & Co. An inoculum of approximately 10^4 to 2×10^4 freshly grown organisms, prepared by a thousandfold dilution from a turbid solution matched to a barium sulfate standard, was delivered by the inoculum replicating device of Steers et al. (18) to the surface of the agar plates containing antibiotics. The magnesium content of this sheeps' blood-enriched agar was determined and ranged between 2.4 and 2.6 mg% by freeze-thaw extraction of the liquid contained in the agar, followed by measurement in an atomic absorption spectrophotometer. Broth dilution susceptibility tests were performed with the same Mueller-Hinton broth media, whose magnesium content by the same method was found to range between 0.4 and 0.7 mg%. An inoculum of approximately 5×10^5 organisms was used in accordance with ICS guidelines. Several strains commonly used as reference strains of known, consistent antimicrobial susceptibility were tested in parallel with agar and broth dilution susceptibility tests. These included *S. aureus*, ATCC 25923; *E. coli* ATCC 25922; and three strains recommended for standardized susceptibility testing by the ICS study: *E. coli*, *K. pneumoniae*, and *P. aeruginosa* from the collection of the Pasteur Institute (no. 54.127, 53.153, and 58.38, respectively), kindly provided by Yves Chabbert.

Additionally, several highly gentamicin-resistant strains isolated at Grady Memorial Hospital, Atlanta, Ga., were obtained through the courtesy of Harlan Stone of that Hospital and M. W. Fisher, Parke-Davis and Co., Detroit, Mich. Four other highly gentamicin-resistant strains were isolated at UCLA Medical Center. (All of these gentamicin-resistant isolates are not included in data summarized for agar dilution tests.)

In vitro killing curves in broth medium. The bactericidal activity of these antibiotics as a function of time (rate of killing curves) was assessed against two strains of each of the eight major groups of organisms studied. An inoculum of approximately 5×10^5 organisms in the early logarithmic phase of growth was exposed to the minimal bactericidal concentrations (MBC) of each antibiotic in Mueller-Hinton broth. At 1, 2, and 4 h standing suspensions were sampled, and 0.1-ml volumes of serial 10-fold dilutions in Mueller-Hinton broth were incorporated into Trypticase soy agar (BBL) plates to enumerate surviving bacteria. The original broth tube and each serial dilution were also examined for turbidity after 5 ml of Mueller-Hinton broth was added, and incubated for 18 h. Visual examination of these broth tubes, whose final concentration of antibiotic was always less than the MIC for each antibiotic, was used as a confirmatory test for completeness of killing.

Effect of pH on antibacterial activity. The effect of pH on the MIC of test organisms to the five aminoglycoside antibiotics was determined by suspending Mueller-Hinton broth powder in phosphate-buffered saline, buffered at pH 6.4 and 8.4. Conditions for evaluation of the test were the same as for standard broth dilution methods.

Method of analysis. Organisms were considered susceptible if they were inhibited at or below the peak serum levels usually achieved during administration of these antibiotics to hospitalized patients or normal volunteers. For this laboratory, these peak levels are 4 μg/ml with 1.0 to 1.5 mg of gentamicin or tobramycin per ml, and 32 μg/ml with 7.5 to 10 mg of BB-K8 per ml. Data made available from the Schering Corporation and Parke-Davis and Co., indicate that peak serum levels achieved after administration of 1 mg of sisomicin per ml and 10 mg of butirosin per ml are approximately 4 and 16 μg/ml, respectively. The ratio between peak serum level and the MIC for inhibition of ⅔ of each group of bacteria tested was calculated and designated as the inhibitory index.

RESULTS

Susceptibility of standard reference strains. As determined by the broth dilution method, the MIC and MBC for five standard reference strains are summarized in Table 1. The MBC was usually one additional dilution beyond the MIC. All MICs and MBCs were in the susceptible range for each of the five

TABLE 1. *Comparative activity of five aminoglycoside antibiotics against standard reference strains*[a]

Organism	BBK-8		Butirosin		Gentamicin		Sisomicin		Tobramycin	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Escherichia coli (ATCC 25922)	2	4	4	4	1	1	1	2	1	2
E. coli (Collection of the Pasteur Institute, 54.127)	2	4	2	2	0.5	1	0.25	0.5	1	1
Staphylococcus aureus ATCC 25923	2	4	2	4	0.5	0.5	0.06	0.25	1	1
Klebsiella pneumoniae (Collection of the Pasteur Institute, 53.153)	1	1	0.5	0.5	0.25	0.5	0.12	0.25	0.25	0.50
Pseudomonas aeruginosa (Collection of the Pasteur Institute, 58.38)	2	4	4	16	0.5	1	0.25	0.5	0.12	0.5

[a] Micrograms per milliliter.

aminoglycosides. Furthermore, on repetitive testing these values differed by no more than one dilution.

Susceptibility of clinical isolates. In vitro susceptibility of organisms tested by the agar dilution method is summarized in Fig. 1 through 8. In general, a fairly consistent pattern in the in vitro activities of the five antibiotics was observed. Susceptibilities to gentamicin, sisomicin, and tobramycin closely paralleled each other for most groups of organisms tested, and butirosin and BB-K8 were usually inhibitory at higher concentrations. Sisomicin was the most consistently active of the aminoglycoside agents tested and was the most potent agent against seven of the eight groups tested. The only exception to this finding was that, for 72% of strains of *S. marcescens*, gentamicin was slightly more active than sisomicin. At 4 μg/ml, 100% of *P. aeruginosa* strains were inhibited by sisomicin, as contrasted with 98% by tobramycin and 68% by gentamicin. At 16 μg/ml, 84% of these recent clinical isolates of *P. aeruginosa* were inhibited by BB-K8 but only 40% by butirosin. At 4 μg/ml, 94% of *S. marcescens* were inhibited by sisomicin, 92% by gentamicin, 86% by BB-K8, 84% by to-

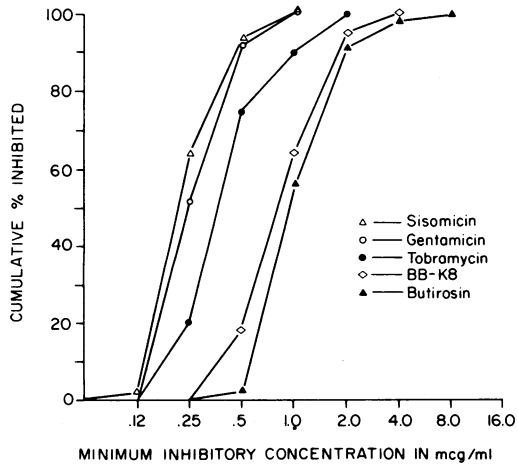

FIG. 1. *Activity of five aminoglycoside antibiotics against Enterobacter species (50 isolates).*

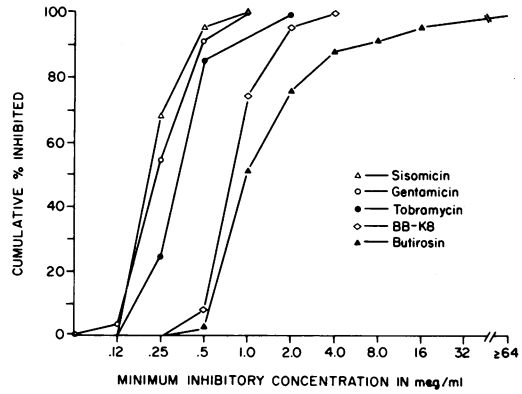

FIG. 2. *Activity of five aminoglycoside antibiotics against Escherichia coli (50 isolates).*

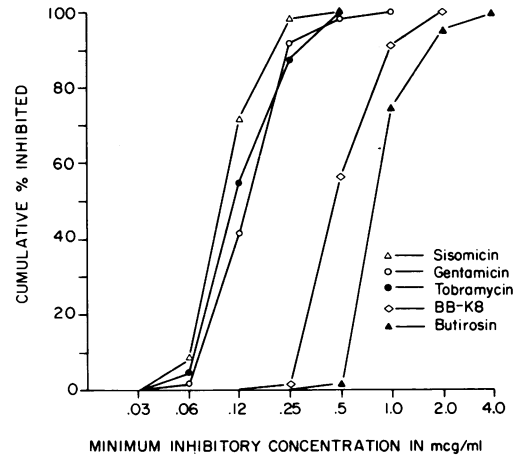

FIG. 3. *Activity of five aminoglycoside antibiotics against Klebsiella pneumoniae (50 isolates).*

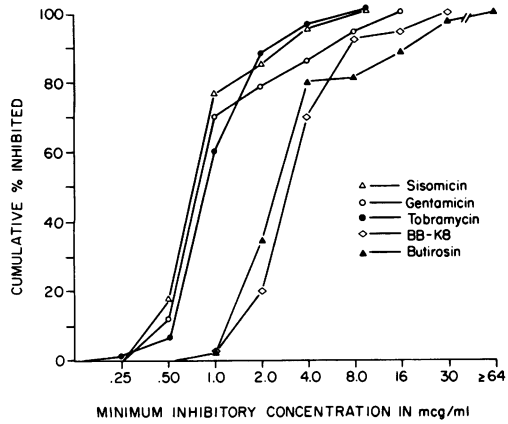

FIG. 4. *Activity of five aminoglycoside antibiotics against Proteus mirabilis (50 isolates).*

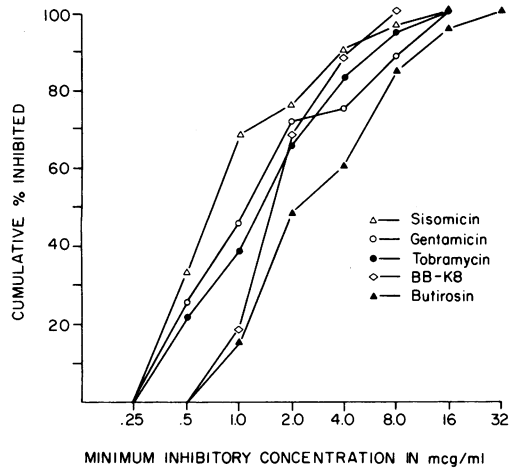

FIG. 5. *Activity of five aminoglycoside antibiotics against indole-positive Proteus species (50 isolates).*

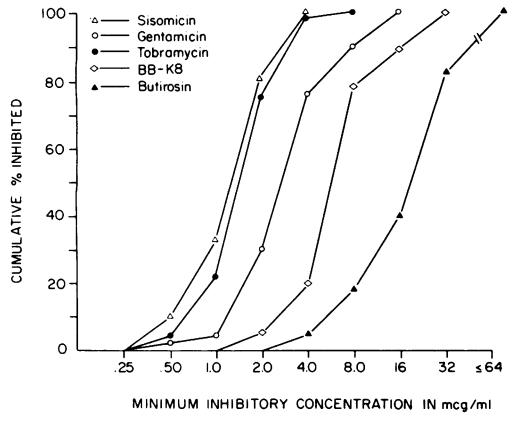

FIG. 6. *Activity of five aminoglycoside antibiotics against Pseudomonas aeruginosa (50 isolates).*

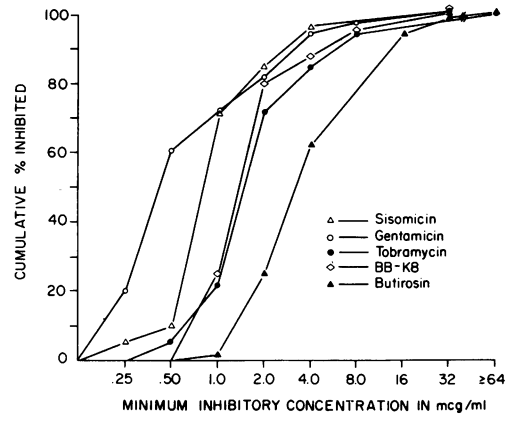

FIG. 7. *Activity of five aminoglycoside antibiotics against Serratia marcescens (50 isolates).*

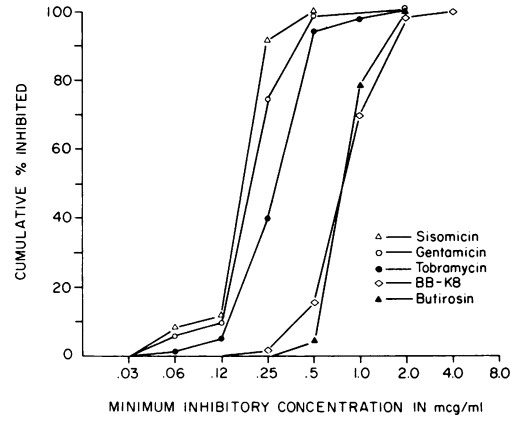

FIG. 8. *Activity of five aminoglycoside antibiotics against Staphylococcus aureus (50 isolates).*

bramycin, but only 62% by butirosin. However, the latter compound inhibited 93% of *Serratia* isolates at 16 μg/ml, which seems to be an achievable blood level in vivo. Better than 90% of *E. coli*, *K. pneumoniae*, *Enterobacter* sp., and *S. aureus* were inhibited at or below concentrations of 4 μg of all five antibiotics per ml.

Comparison of the MICs obtained with Mueller-Hinton broth and agar supplemented with 5% sheep's blood showed no significant differences except for *P. aeruginosa* (Table 2). For this organism, MICs were two- to eightfold higher in agar dilution tests as compared to the broth dilution method. In contrast, agar and broth dilution MICs were within one dilution of each other in 66 of the 70 comparative tests performed simultaneously on seven other groups of organisms. Furthermore, when the results differed by even one dilution, the broth dilution MICs were usually higher.

Results of studies with highly gentamicin-resistant strains. Three distinct serotypes (6) of *P. aeruginosa* highly resistant to gentamicin, isolated from a burn unit at Grady Memorial Hospital, were found to have varying susceptibility to tobramycin and butirosin but were consistently inhibited by BB-K8 (Table 3). Susceptibility to BB-K8 and butirosin was noted in one strain of *S. marcescens* which was highly resistant to the three other aminoglycosides. No strains were found to be resistant to BB-K8 but sensitive to another aminoglycoside. However, one mucoid strain of *P. aeruginosa* was resistant to all five aminoglycosides. This was isolated from a patient with cystic fibrosis, who had been treated with gentamicin and tobramycin.

Comparison of inhibitory indexes. The relative potency by weight of antimicrobial agents, considered by itself, is likely to have little clinical significance inasmuch as the pharmacokinetics of these agents may differ significantly. For each group of organisms and each aminoglycoside antibiotic, the ratios between mean peak levels and the MICs required to inhibit ⅔ of each group of organisms were calculated. If the concentration required to inhibit ⅔ of each group of organisms did not correspond exactly with one of the concentrations used, the higher of the two adjacent MICs was used for these calculations. The results (Table 4) show no clearcut advantages for any of these antibiotics against *P. aeruginosa*. With this exception, BB-K8 consistently showed the greatest inhibitory index, followed by sisomicin. All antibiotics tested showed a high index against *S. aureus* and *K. pneumoniae* and comparable activity against *Enterobacter* sp. and probably *E. coli*. Against *S. marcescens* and indole-positive *Proteus* sp., BB-K8 appeared to have a significant advantage over the other compounds.

Results of in vitro killing curves. Bactericidal activity was demonstrable as a function of time in standing broth suspensions containing the MBC of each antibiotic against two strains each of the eight groups of bacteria tested.

Greater than 99.9% killing was observed against *S. aureus*, *K. pneumoniae*, *E. coli*, and indole-positive *Proteus* within 4 h for all five agents. However, the rapidity of killing appeared to be significantly less against *P. aeruginosa* and *S. marcescens*. The most rapid killing was observed with sisomicin against 9 of the 16 organisms tested, followed by gentamicin, which most rapidly killed 5 test strains. Representative killing curves for two organisms are shown in Fig. 9 and 10. The activities (assesed by rate) for butirosin, BB-K8, and tobramycin was no better than third (in terms of order) for 9 of the 16 organisms tested.

Effect of pH. Table 5 summarizes these results, expressed in the number of dilutions by which MICs changed under acid or alkaline conditions. Acidification of Mueller-Hinton broth from pH 7.4 to 6.4 resulted in a more than an eightfold increase in BB-K8 and butirosin MICs. Similar, though less marked, reduction in inhibitory activity was observed with the other aminoglycoside antibiotics. Interestingly, BB-K8, butirosin, and tobramycin also demon-

TABLE 2. *Comparisons of agar dilution and broth dilution MIC as determined by ICS recommended methods*[a]

Organism	BB-K8		Butirosin		Gentamicin		Sisomicin		Tobramycin	
	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth	Agar	Broth
Pseudomonas aeruginosa 2628	8	2	32	8	1	0.5	0.5	0.25	1	0.25
P. aeruginosa 1930	8	2	32	16	4	2	2	0.5	4	0.5
Serratia marcescens 2311	4	8	32	32	4	2	4	8	8	8
S. marcescens 831	2	4	8	16	1	1	2	1	2	4
Escherichia coli 36	2	4	2	4	0.5	0.5	0.25	0.5	0.5	0.5
E. coli 3011	1	4	2	4	0.5	1	0.5	1	0.5	1
Enterobacter sp. M2673	1	1	1	2	0.25	0.5	0.25	0.50	0.5	0.5
Enterobacter sp. 333	1	2	4	8	1	2	0.5	1.0	0.5	2
Klebsiella pneumoniae 188	0.5	1	0.5	1	0.12	0.12	0.12	0.25	0.25	0.25
K. pneumoniae 166	1	1	1	2	0.25	0.12	0.25	0.25	0.25	0.5
Staphylococcus aureus 344	0.5	1	1	4	0.12	0.12	0.12	0.06	0.25	0.12
S. aureus 418	1	2	1	2	0.12	0.12	0.12	0.25	0.12	0.5
Proteus vulgaris 468	16	32	16	16	4	4	4	4	4	8
P. mirabilis 396	16	32	16	16	2	4	2	4	8	8
P. mirabilis 206	16	16	16	16	4	4	4	4	4	4
P. morgani 233	1	2	2	4	0.5	1	0.5	1	0.5	1

[a] Results in micrograms per milliliter.

TABLE 3. *Comparative activity of five aminoglycoside antibiotics against highly gentamicin-resistant clinical isolates*[a]

Organism	Identification	BB-K8		Butirosin		Gentamicin		Sisomicin		Tobramycin	
		MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Proteus inconstans	UCLA 120, XVI	16	16	256	512	32	128	16	16	32	128
P. rettgeri	UCLA 104	16	32	256	512	256	512	64	128	64	128
Pseudomonas aeruginosa	630-PD, serotype I[a]	2	8	32	128	512	1,024	512	1,024	512	1,024
P. aeruginosa	71-PD, serotype II[b]	4	8	16	64	64	256	32	128	64	128
P. aeruginosa	G no. 64-PD, serotype III[b]	2	8	128	512	1,024	2,048	512	1,024	1	2
P. aeruginosa	UCLA 4184	512	2,048	512	2,048	1,024	4,096	1,024	2,048	1,024	2,048
Serratia marcescens	UCLA 1484	4	8	16	16	512	2,048	256	512	128	512

[a] Serotyping performed according to Fisher et al. (6).
[b] Results in micrograms per milliliter.

TABLE 4. *Inhibitory indexes: ratio between the mean peak serum levels and concentration required to inhibit two-thirds of each species*

Drug	Mean peak[a] levels	*E. coli*	*Enterobacter*	*Klebsiella*	*Proteus mirabilis*	*Proteus* indole positive	*Pseudomonas aeruginosa*	*Serratia marcescens*	*S. aureus*
BB-K8	32	32	16	32	8	16	2	16	32
Butirosin	16	8	8	16	4	2	0.5	2	16
Gentamicin	4	8	8	16	4	2	1	4	16
Sisomicin	4	16	16	16	4	4	2	4	32
Tobramycin	4	8	8	8	2	2	2	2	16

[a] Micrograms per milliliter.

strated some reduction in antibacterial activity at pH 8.4. Gentamicin was the least affected by alkaline pH, and against 6 out of the 14 strains tested the organisms became more sensitive at alkaline pH by twofold or more dilutions. No organisms were more resistant to gentamicin at alkaline pH. Four out of the 14 organisms became more resistant to sisomicin at alkaline pH, but the majority of isolates showed no change in MICs or a two- to eightfold reduction. Thus, it appears that in an acid milieu all of these aminoglycoside antibiotics became significantly less active. Optimal activities for BB-K8, butirosin, and tobramycin were exhibited in the neutral range. Alkalinization either had no effect or slightly increased the in vitro activity of gentamicin.

DISCUSSION

The aminoglycoside antibiotics evaluated in this study had in vitro activity which placed them into two groups. The first group consisted of gentamicin, sisomicin, and tobramycin, among which sisomicin was the most consistently potent by weight, followed by gentamicin and tobramycin. The overall differences appeared slight, but it is possible that a twofold increase in activity (assuming equivalent serum levels are achieved) might make a difference in infections caused by organisms such as *P. aeruginosa*, since peak serum levels attained with gentamicin are just adequate to inhibit most *Pseudomonas* strains (21). In general, these orders of activity are comparable to those contained in previous reports (10, 19), although simultaneous comparisons between BB-K8, butirosin, and sisomicin have not been made.

The second group of agents, which included BB-K8 and butirosin, was less active by weight, but this apparent disadvantage appears to be offset by evidence that significantly higher serum levels can be achieved in animals and human volunteers with doses which have not produced ototoxicity or nephrotoxicity (3; M. W. Fisher, private communication). One advantage of BB-K8 is its ability to inhibit and kill highly gentamicin-resistant strains, such as have been found to colonize patients treated in a burn unit where administration of topical

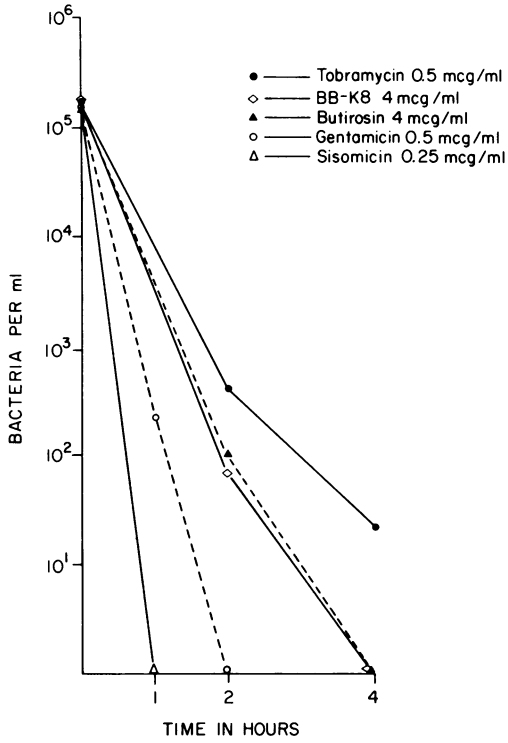

FIG. 9. *Rate of killing of Staphylococcus aureus (ATCC 25923). Viability of organisms was measured in Mueller-Hinton broth containing the MBC of each aminoglycoside antibiotic.*

gentamicin was prevalent (17). This pattern of BB-K8 susceptibility and a high degree of resistance to gentamicin, sisomicin, and tobramycin have also been observed in four clinical isolates obtained at the UCLA Hospital during the last six months (Table 3). One organism, an isolate of *P. aeruginosa* from a patient treated with gentamicin and tobramycin, has been found resistant to all 5 aminoglycosides. Susceptibility to BB-K8 appears to be related to the antibiotic's resistance to enzymatic, R-factor-mediated phosphorylation or adenylation. The latter mechanism is known to inactivate gentamicin, sisomicin, and tobramycin, but not butirosin (unpublished data). It should be indicated, however, that BB-K8-resistant strains from various clinical sources have been detected, and an acetylating mechanism of BB-K8 inactivation has been described (15). Our data are in agreement with the finding that gentamicin-resistant strains are often susceptible to BB-K8, but BB-K8-resistant organisms are also gentamicin-resistant (15).

To a limited extent, butirosin resembles BB-K8 in activity and pharmacokinetics, but it is not as potent. Some organisms, particularly *S. marcescens*, show a wide variation in susceptibility to this agent. An R-factor enzymatic mechanism has already been described which inactivates this antibiotic (22).

The only organism for which MIC data by agar dilution methods should be interpreted with caution is *P. aeruginosa*. It is now established firmly that the high magnesium content of some lots of Mueller-Hinton agar result in higher MICs than obtained with broth dilution tests (8, 23). It has been suggested that a practical solution to this problem is to prepare the agar by adding pure agar to Mueller-Hinton broth. Unfortunately, the ICS recommendation to supplement Mueller-Hinton medium with sheep's blood increases the magnesium content of this specially prepared agar. On the basis of studies with tobramycin (13) and sisomicin (23), broth dilution tests will result in significantly lower MICs against *P. aeruginosa*. However, the magnesium content effect on aminoglycoside antibiotics seems restricted to *Pseudomonas* organisms (23). Thus, there is a clear cut need for a readily available agar media

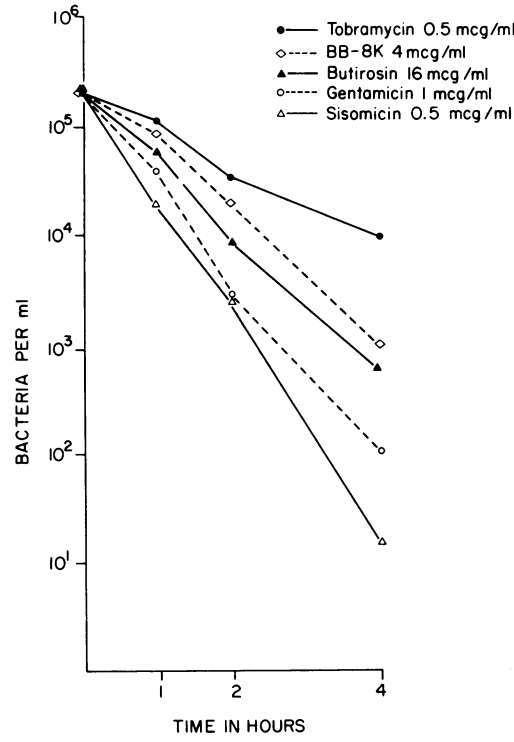

FIG. 10. *Rate of killing of Pseudomonas aeruginosa (Collection Pasteur Institute 58.38). Viability of organisms was measured in Mueller-Hinton broth containing the MBC of each aminoglycoside antibiotic.*

TABLE 5. *Change in MIC of antibiotics for different organisms at various pH values*

Organism	BB-K8		Butirosin		Gentamicin		Sisomicin		Tobramycin	
	6.6	8.4	6.4	8.4	6.4	8.4	6.4	8.4	6.4	8.4
Escherichia coli CIP 54.127	+8	0	+4	−4	+8	−2	+16	+2	+4	0
E. coli ATCC 25922	+8	+2	+8	0	+8	−2	+4	−2	+4	0
Staphylococcus aureus 418	+32	+4	+64	+4	+16	−2	+8	0	+16	+4
S. aureus ATCC 25933	+16	+8	+32	+4	+4	0	+16	+4	+4	+2
Pseudomonas aeruginosa CIP 58.38	+4	0	+8	+2	+2	0	+2	−2	+8	+2
P. aeruginosa 1930	+8	+2	+4	−2	+4	0	+8	+4	+4	+2
Klebsiella pneumoniae CIP 53.153	+8	0	+32	+2	0	−8	0	−8	+8	0
K. pneumoniae 166	+4	+2	+4	−2	+2	−2	+2	−2	+4	0
Enterobacter 800-3	+16	+8	+8	−2	+4	0	+4	0	+32	+4
Enterobacter M2673	+2	+2	+2	+2	0	0	+2	0	+4	+2
Serratia marcescens 187-1	+8	+2	+4	+2	+4	−2	+2	0	+4	+2
S. marcescens 831	+4	+2	+8	0	+8	0	+16	+4	+8	+4
Proteus morgani 233	+32	+8	+16	0	+8	0	+8	0	+8	+2
P. mirabilis 657	+8	0	+8	−2	+8	0	+8	0	+8	+2

[a] The number indicates dilutions by which MIC increased (+) or decreased (−).

with a standardized magnesium content for sensitivity testing of *P. aeruginosa*.

The peak serum level after gentamicin administration has been the commonly used criterion for separating susceptible from resistant strains, even though such peak levels are maintained for only 1 to 2 h after drug administration. Applying this principle further, we have calculated the ratio between peak serum levels at 1 h after intramuscular administration of each drug and the antibiotic concentration which will inhibit ⅔ of each species tested. These inhibitory indexes have no established predictive clinical value. However, the data do suggest that, for most clinically significant gram-negative bacilli tested, BB-K8 shows the largest ratio between peak levels and inhibitory levels. Although criteria for drug sensitivity and achievable blood levels differ from the original report of Price et al. (15) and Cabana and Taggart (3), such discrepancies are probably due to difference in methodology, and the conclusions reached by the former report are supported by this study.

Of the other variables which might affect aminoglycoside activity, we have investigated rate of killing and the effects of pH. All of these agents showed prompt bactericidal activity when killing curves at the MBC of each of 14 test strains was performed, with killing usually exceeding 99% at 4 h. It is interesting that the slope of the killing curve was less marked for the *Pseudomonas* strains tested than for all seven other species tested, confirming a previous report when gentamicin was studied alone (16). Perhaps more significant is that sisomicin and gentamicin consistently demonstrated the most rapid rate of killing, followed by butirosin, BB-K8, and tobramycin. With in vivo tests, sisomicin at doses comparable to gentamicin and tobramycin showed greater activity in experimental mouse infections (19). Although several reasons have been implicated to explain these results, a more rapid rate of bacterial killing could also have been a factor. We tested only a few representative strains from each species for rapidity of killing against these five antibiotics, but this parameter of antibiotic activity clearly deserves further study with these and other antibiotics.

The older aminoglycoside antibiotics, including streptomycin and kanamycin, were noted for their ineffectiveness in the acid pH range and enhanced activity under alkaline conditions (7). We have verified the adverse effect of an acid milieu on the activity of each of these new aminoglycosides, but activity of these compounds is quite variable when the pH is alkaline. Only gentamicin and sisomicin showed increased activity in the alkaline pH range. For the other agents, there is either no change or diminished activity.

Of the aminoglycoside antibiotics which have been developed since gentamicin, only clinical experience will decide which, if any, may affect the role of gentamicin. This clinical experience will probably focus on two related trends: first, whether there is increasing incidence of clinical and bacteriological resistance to gentamicin; and second, whether any of the newer agents demonstrate safer, more potent in vivo activity. Although sisomicin seems more potent than gentamicin and is more active than gentamicin against gentamicin-sensitive strains, there is

usually consistent cross-resistance between these two agents (19). BB-K8 appears particularly promising in that it is active against many gentamicin-resistant strains (15) and has the largest inhibitory index for most classes of bacteria which we have studied.

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