

# In Vitro Studies of BB-K8, a New Aminoglycoside Antibiotic

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BB-K8, an aminoglycoside antibiotic which is a derivative of kanamycin, was tested in vitro against 466 clinical bacterial isolates. Over 90% of gram-negative bacilli, except *Proteus* spp., were inhibited by 3.12  $\mu\text{g}$  of BB-K8 per ml. This antibiotic was consistently more active than kanamycin but less active than tobramycin or gentamicin. Unlike kanamycin, BB-K8 was active against *Pseudomonas aeruginosa*. Eleven of 19 isolates resistant to either gentamicin or tobramycin, or both, were susceptible to BB-K8.

Gram-negative bacilli are responsible for an increasing number of the serious infectious complications occurring in hospitalized patients (4). Often these organisms are susceptible to only a few antibiotics. Aminoglycoside antibiotics have been successful for the treatment of many of these infections, but with prolonged usage the emergence of resistant gram-negative bacilli presents a serious problem. A substantial proportion of gram-negative bacilli currently isolated from patients at our institution are resistant to kanamycin. Recently, epidemics of infections caused by gentamicin-resistant organisms have been observed in institutions using topical preparations of this antibiotic (7, 8). Consequently, the evaluation of new aminoglycoside antibiotics is an important avenue of research.

BB-K8 is a new antibiotic which is a chemical derivative of kanamycin sulfate. It has a broad spectrum of activity which includes most *Enterobacteriaceae* and *Pseudomonas aeruginosa* (6). In animals, BB-K8 produces less cochlear toxicity than either kanamycin A or gentamicin. It is slightly less nephrotoxic than kanamycin A and considerably less nephrotoxic than gentamicin in animals. The pharmacology of this drug in dogs and humans is similar to that of kanamycin. This report presents the results of in vitro susceptibility testing of BB-K8 against 466 bacterial isolates obtained from cancer patients. BB-K8 was found to compare favorably with other aminoglycosides.

## MATERIALS AND METHODS

Susceptibility tests were conducted on 466 clinical isolates of gram-negative bacilli, by use of the broth

dilution technique (5). The organisms were inoculated into Mueller-Hinton broth (Difco) and incubated at 37 C for 18 h. A 0.1-ml sample of a  $10^{-4}$  dilution of this broth (approximately  $10^4$  colony-forming units/ml) was used as the inoculum for susceptibility testing of gram-negative bacilli.

BB-K8 used in this study was supplied as a white powder by Bristol Laboratories, Division of Bristol-Meyers Co., Syracuse, N.Y. Twofold serial dilutions of the antibiotic were made with Mueller-Hinton broth, and the minimal inhibitory concentration (MIC) was determined after incubation at 37 C for 18 h. All tubes containing trace growth or no discernible growth were subcultured on sheep blood agar. A wire loop with an inside diameter of 5 mm was used to transfer the inoculum. BB-K8 was considered to be bactericidal against those isolates which yielded no growth on subculture of the tube containing the MIC. Comparative studies were conducted simultaneously with kanamycin sulfate, gentamicin sulfate, and tobramycin.

All gram-negative bacilli used in this study were cultured from blood specimens obtained from pa-

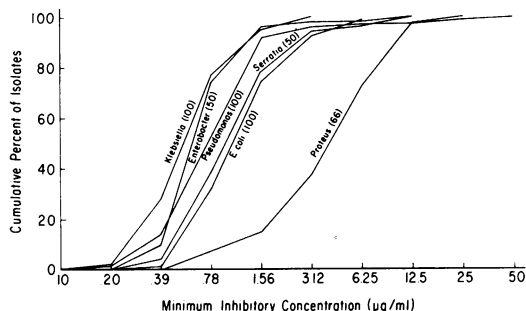


FIG. 1. In vitro activity of BB-K8 against gram-negative bacilli. Numbers in parentheses indicate number of isolates tested.

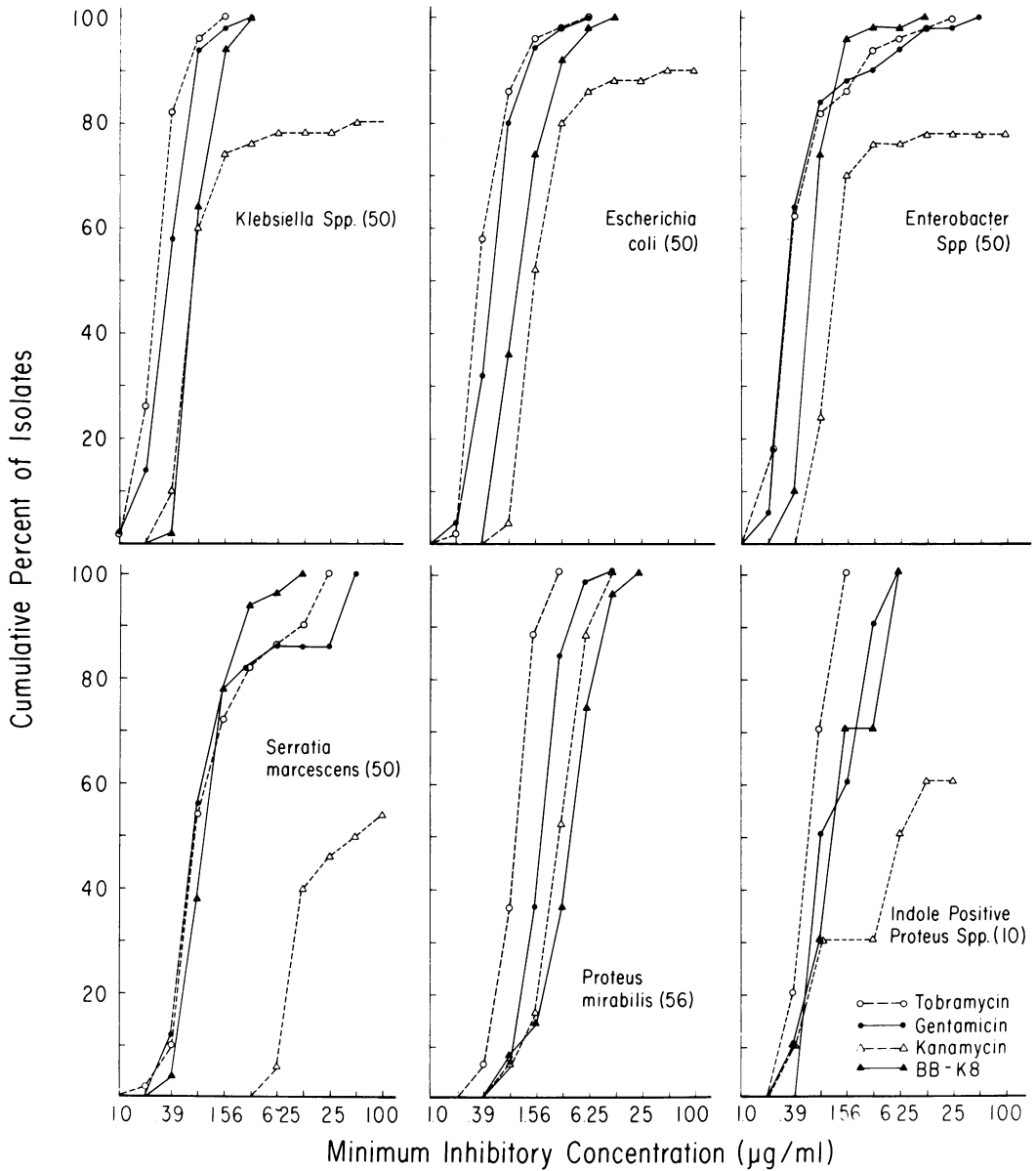


FIG. 2. Comparison of activity of BB-K8 with tobramycin, gentamicin, and kanamycin against Enterobacteriaceae. Numbers in parentheses indicate number of isolates tested.

tients between 1967 and 1972. The majority of the patients were hospitalized at this institution and had underlying malignant disease. A total of 100 isolates each of *P. aeruginosa*, *Escherichia coli*, and *Klebsiella* spp., 66 isolates of *Proteus* spp., 50 isolates of *Enterobacter* spp., and 50 isolates of *Serratia marcescens* were studied.

**RESULTS**

The in vitro activity of BB-K8 against gram-negative bacilli is shown in Fig. 1. At a concen-

tration of 3.12 µg/ml, BB-K8 inhibited over 90% of isolates of all gram-negative bacilli, except *Proteus* spp. This antibiotic inhibited over 90% of *P. aeruginosa* isolates at a concentration of 1.56 µg/ml. The antibiotic was bactericidal against 42% of *Proteus* spp., 40% of *Enterobacter* spp., 30% of *E. coli*, 12% of *Klebsiella* spp., only 3% of *P. aeruginosa* strains, and none of *S. marcescens*.

Figure 2 compares the in vitro activity of

BB-K8 with that of three other aminoglycoside antibiotics, gentamicin sulfate, kanamycin sulfate, and tobramycin, against *Enterobacteriaceae*. BB-K8 was consistently more active than kanamycin sulfate, but less active than tobramycin or gentamicin sulfate. The concentration of gentamicin sulfate and tobramycin which inhibited all isolates of *E. coli* was 6.25  $\mu\text{g/ml}$ , compared with 12.5  $\mu\text{g}$  of BB-K8/ml. Five isolates of *E. coli* were totally resistant to kanamycin sulfate. At a concentration of 3.12  $\mu\text{g/ml}$ , tobramycin, gentamicin sulfate, and BB-K8 inhibited all isolates of *Klebsiella* spp., whereas kanamycin sulfate inhibited only 76%. BB-K8 was only slightly less active than tobramycin and gentamicin sulfate against *Enterobacter* spp. The activity of all three antibiotics was quite similar against *S. marcescens*, and was much greater than the activity of kanamycin sulfate. BB-K8 was more active than kanamycin sulfate and comparable to gentamicin sulfate against indole-positive *Proteus* spp., but was the least active of the four antibiotics against *P. mirabilis*.

The in vitro susceptibility of 50 isolates of *P. aeruginosa* to the four aminoglycoside antibiotics is shown in Fig. 3. Tobramycin was the most active agent, inhibiting 96% of isolates at a concentration of 0.78  $\mu\text{g/ml}$ . At this concentration, gentamicin sulfate inhibited 56% of the isolates and BB-K8 inhibited only 8%. However, BB-K8 inhibited 98% of isolates at a concentration of 6.25  $\mu\text{g/ml}$ . Kanamycin sulfate was much less active against *P. aeruginosa*, inhibiting only 12% of isolates at a concentration of 6.25  $\mu\text{g/ml}$ .

The effect of media on the activity of the four aminoglycoside antibiotics was determined against 10 isolates each of *E. coli*, *P. aeruginosa*, and *Klebsiella* spp. All of the antibiotics were most active against *E. coli* in nutrient broth (Fig. 4). At a concentration of 0.5  $\mu\text{g/ml}$  in this medium, tobramycin inhibited nine isolates, gentamicin and BB-K8 inhibited seven isolates, and kanamycin inhibited one isolate. In Mueller-Hinton broth, the order of activity was gentamicin, tobramycin, BB-K8, and kanamycin. In brain-heart infusion broth, gentamicin, tobramycin, and BB-K8 had similar activity. Kanamycin was more active than the other three antibiotics against 7 of the 10 isolates. In Trypticase soy broth, kanamycin was less active than the other three antibiotics, whose activity was very similar.

All four antibiotics were most active against *P. aeruginosa* in nutrient broth (Fig. 5). At a concentration of 0.20  $\mu\text{g/ml}$ , gentamicin, tobramycin, and BB-K8 inhibited all 10 isolates

in this medium, but kanamycin inhibited none at this concentration. Tobramycin was the most active antibiotic in this medium, as well as in Mueller-Hinton broth and brain-heart infusion broth. The activity of BB-K8 was similar to that of gentamicin in brain-heart infusion broth. Gentamicin and tobramycin had similar activity in Trypticase soy broth and were more active than BB-K8.

The four antibiotics also were most active against *Klebsiella* spp. in nutrient broth (Fig. 6). BB-K8 was slightly more active in this medium than gentamicin and tobramycin. At a concentration of 0.05  $\mu\text{g/ml}$ , BB-K8 inhibited all 10 isolates, gentamicin inhibited 6, tobramycin inhibited 7, and kanamycin inhibited 5. Gentamicin was the most active antibiotic in Mueller-Hinton broth, followed by tobramycin, BB-K8, and kanamycin. In brain-heart infusion broth, kanamycin was the most active against seven isolates and BB-K8 was the least active.

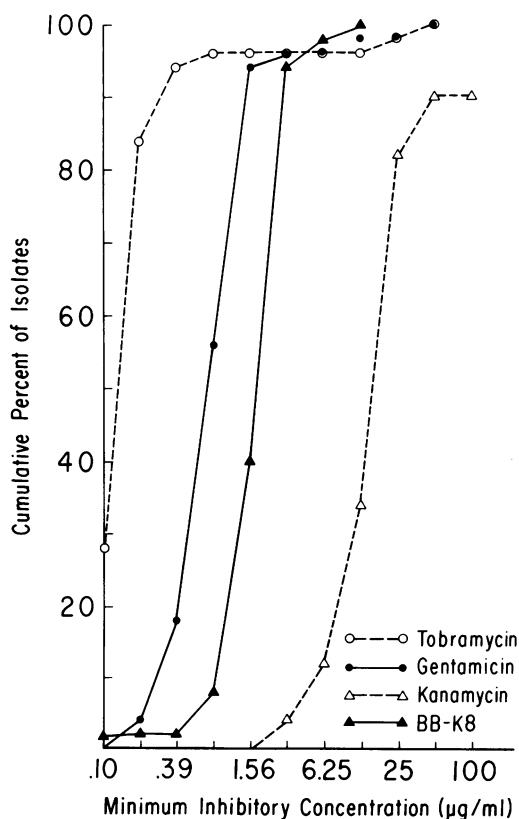


FIG. 3. Comparison of activity of BB-K8 with tobramycin, gentamicin, and kanamycin against *Pseudomonas aeruginosa*. Numbers in parentheses indicate number of isolates tested.

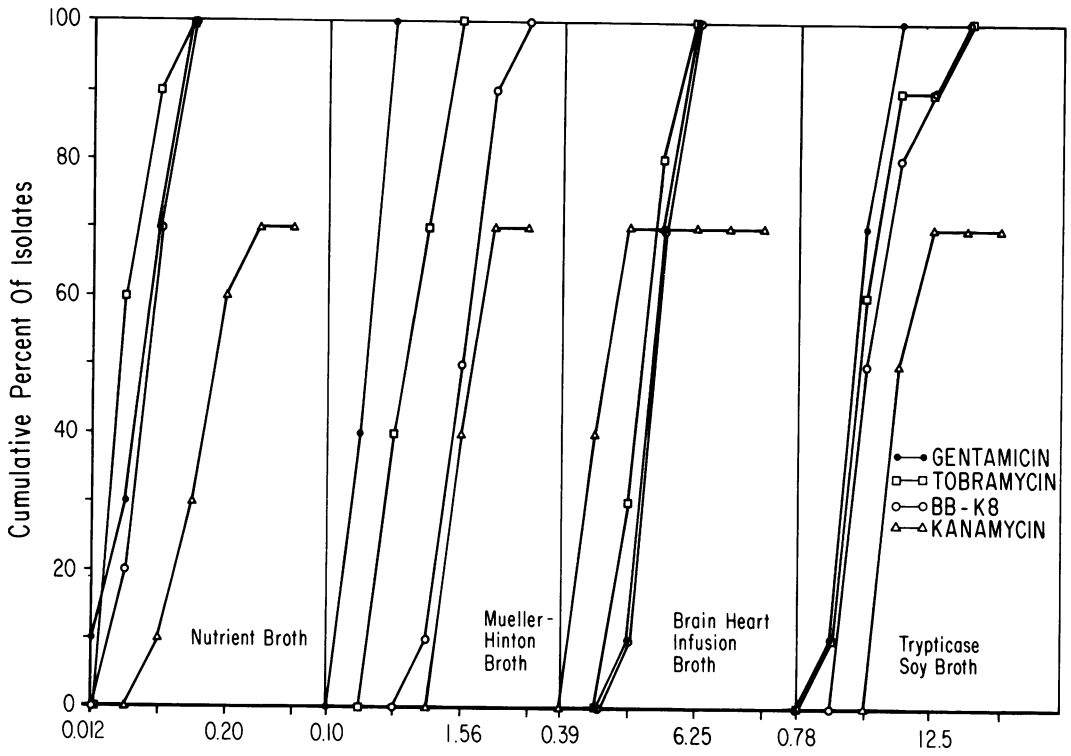


FIG. 4. Effect of media on activity of aminoglycoside antibiotics against 10 isolates of *Escherichia coli*.

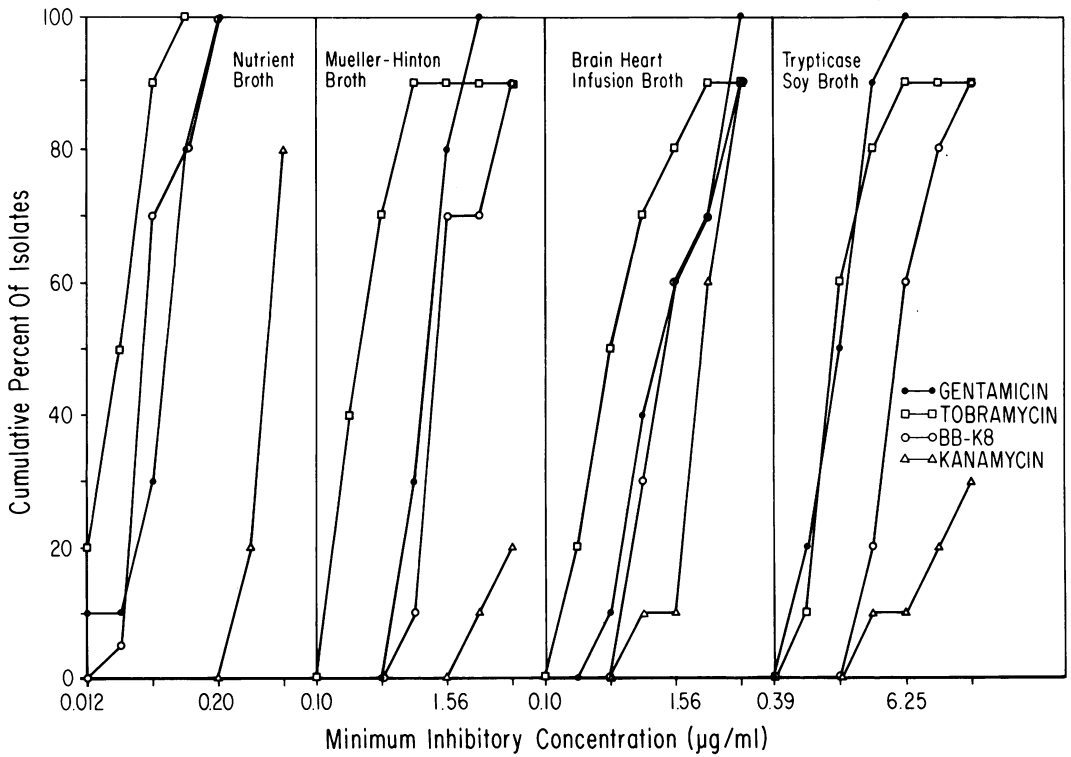


FIG. 5. Effect of media on activity of aminoglycoside antibiotics against 10 isolates of *Pseudomonas aeruginosa*.

BB-K8, gentamicin, and tobramycin were all very active in Trypticase soy broth. In all media, there were two isolates of *Klebsiella* sp. resistant to kanamycin.

The effect of pH on the activity of BB-K8 in Trypticase soy broth was studied against 10 isolates each of *Klebsiella* spp. *E. coli*, *Proteus* spp., and *P. aeruginosa* (Fig. 7). The pH ranges studied were 6.4, 7.2, and 8.0. BB-K8 was most active in the more alkaline pH. The effect of pH was greatest against *Klebsiella* spp. and *E. coli*.

Nineteen isolates of gram-negative bacilli known to be resistant to gentamicin sulfate or tobramycin, or both, were tested for their susceptibility to BB-K8 (Table 1). Of these 19 isolates, 11 were susceptible to BB-K8. All three isolates of *Enterobacter* spp. and all seven isolates of *S. marcescens* were susceptible to BB-K8 but resistant to all of the three other aminoglycosides.

DISCUSSION

BB-K8 was active against most of the isolates of *Enterobacteriaceae* and *P. aeruginosa* tested.

*Proteus* spp. were the least susceptible gram-negative bacilli to this antibiotic. Our results are similar to those reported by Price et al. (6). BB-K8 compared favorably with tobramycin and gentamicin sulfate in vitro, especially against *E. coli* and the *Klebsiella-Enterobacter-Serratia* group (2, 3). *S. marcescens* has caused epidemics of infection in several hospitals during recent years. In our institution, most of the clinical isolates have been susceptible only to gentamicin sulfate (1). However, both new aminoglycosides, tobramycin and BB-K8, have activity against these organisms. Although it is a derivative of kanamycin, which has little activity against *P. aeruginosa*, BB-K8 is active against these organisms. It is considerably less active than tobramycin, but comparable to gentamicin sulfate. A particularly impressive observation was the activity of BB-K8 against gram-negative bacilli which were resistant to tobramycin and gentamicin sulfate. Nearly 50% of the isolates resistant to the latter two antibiotics were inhibited by 1.56 µg or less of BB-K8 per ml. All isolates of *Enterobacter* spp. and *S.*

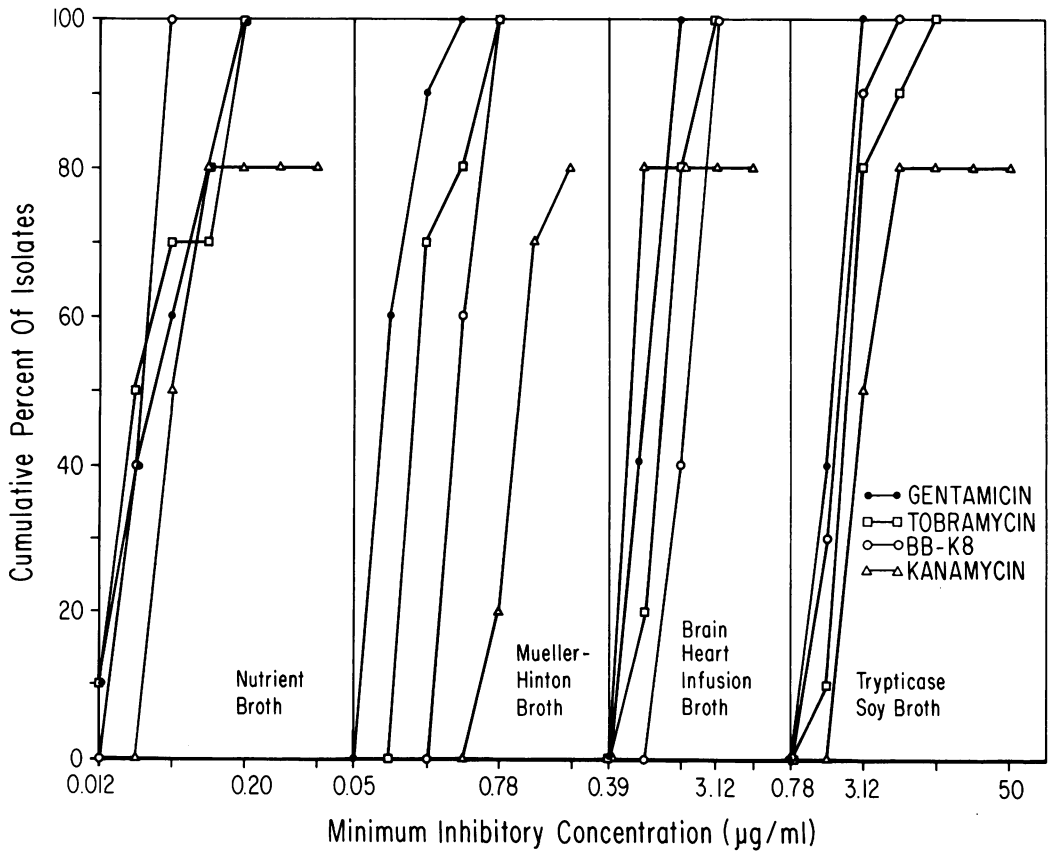


FIG. 6. Effect of media on activity of aminoglycoside antibiotics against 10 isolates of *Klebsiella* sp.

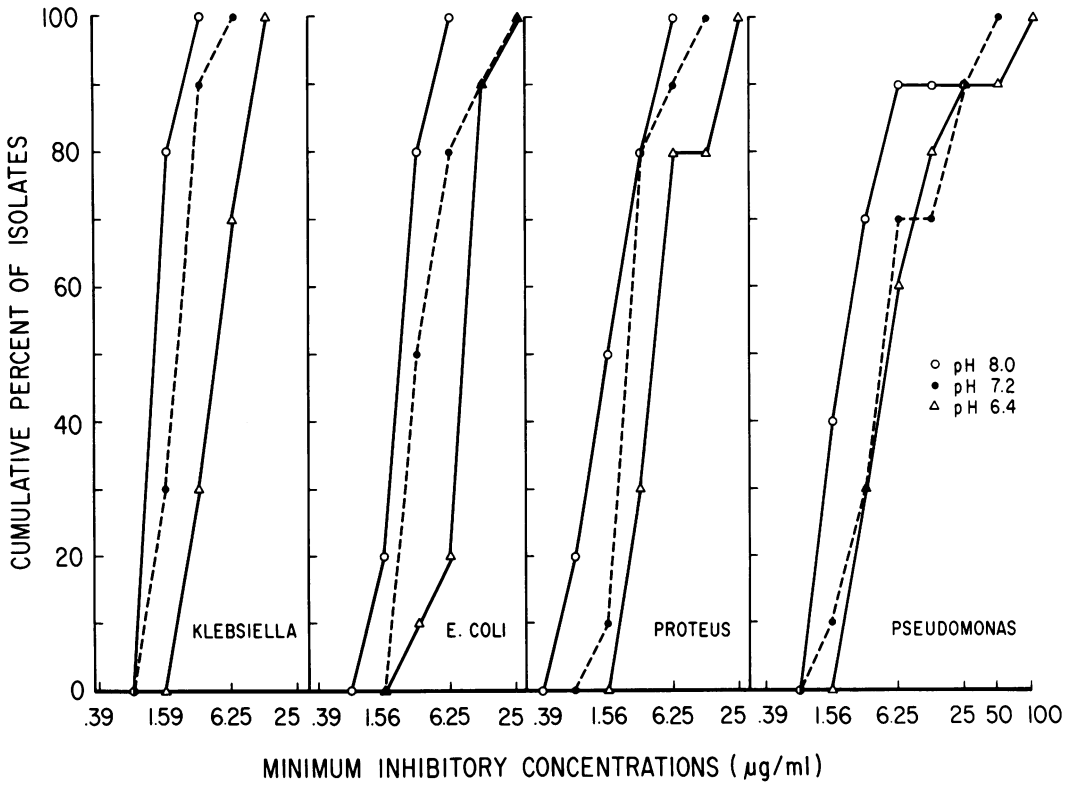


FIG. 7. Effect of pH on the activity of BB-K8 in Trypticase soy broth.

TABLE 1. Activity of BB-K8 against organisms resistant to gentamicin or tobramycin

Organism	Source	Minimal inhibitory concn (µg/ml) <sup>a</sup>			
		BB-K8	Tobramycin	Gentamicin	Kanamycin
<i>Escherichia coli</i> .....	Skin	100	12.5	50	25
<i>Klebsiella</i> sp. ....	Skin	>100	25	25	25
<i>Enterobacter</i> sp. ....	Blood	<i>0.78</i>	12.5	50	>100
<i>Enterobacter</i> sp. ....	Blood	<i>1.56</i>	6.25	12.5	>100
<i>Enterobacter</i> sp. ....	Blood	<i>0.78</i>	25	12.5	12.5
<i>Proteus mirabilis</i> .....	Blood	25	<i>3.12</i>	12.5	12.5
<i>Pseudomonas aeruginosa</i> .....	Blood	>100	50	<i>12.5</i>	100
<i>P. aeruginosa</i> .....	?	<i>6.25</i>	50	>100	>100
<i>P. aeruginosa</i> .....	Skin	100	25	25	25
<i>P. aeruginosa</i> .....	Stool	50	50	>100	25
<i>P. aeruginosa</i> .....	Blood	50	<i>12.5</i>	25	25
<i>P. aeruginosa</i> .....	Throat	100	100	>100	50
<i>Serratia marcescens</i> .....	Blood	<i>1.56</i>	25	50	100
<i>S. marcescens</i> .....	Blood	<i>3.12</i>	25	50	>100
<i>S. marcescens</i> .....	Blood	<i>1.56</i>	25	50	>100
<i>S. marcescens</i> .....	Blood	<i>1.56</i>	12.5	50	>100
<i>S. marcescens</i> .....	Blood	<i>1.56</i>	12.5	50	>100
<i>S. marcescens</i> .....	Blood	<i>1.56</i>	25	50	>100
<i>S. marcescens</i> .....	Skin	<i>1.56</i>	12.5	25	>100

<sup>a</sup> The number in italics indicates the lowest MIC for each isolate.

*marcescens* resistant to the other aminoglycoside antibiotics were susceptible to BB-K8.

This in vitro study suggests that BB-K8 may have potential value for the treatment of infections caused by gram-negative bacilli. Since the autotoxicity of BB-K8 in animals is less than that of gentamicin, it possibly can be administered safely to humans at doses sufficient to provide much higher concentrations in serum than can be achieved with gentamicin. Clinical evaluation of BB-K8 is indicated to ascertain its role for the treatment of infections due to gram-negative bacilli.

#### ACKNOWLEDGMENTS

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#### LITERATURE CITED

1. Bodey, G. P., V. Rodriguez, and J. P. Smith. 1970. *Serratia* species infections in cancer patients. *Cancer* 25:199-205.
2. Bodey, G. P., and D. Stewart. 1972. In vitro studies of tobramycin. *Antimicrob. Ag. Chemother.* 2:110-113.
3. Dienstag, J., and H. C. Neu. 1972. In vitro studies of tobramycin, an aminoglycoside antibiotic. *Antimicrob. Ag. Chemother.* 1:41-45.
4. Dupont, H. L., and W. W. Spink. 1969. Infections due to gram-negative organisms. *Medicine* 48:307-332.
5. Grove, D. A., and W. A. Randall. 1955. Assay methods of antibiotics: a laboratory manual, p. 188-196. Medical Encyclopedia, Inc., New York.
6. Price, K. E., Chisholm, D. R., Misiek, M., Leitner, F., and Tsai, Y. H. 1972. Microbiological evaluation of BB-K8, a new semi-synthetic aminoglycoside. *J. Antibiot.* 25:709-731.
7. Shulman, J. A., P. M. Terry, and C. E. Hough. 1971. Colonization with gentamicin-resistant *Pseudomonas aeruginosa*, pyocine type 5, in a burn unit. *J. Infect. Dis.* 124:S18-S23.
8. Snelling, C. F. T., A. A. Ronald, C. Y. Cates, and W. C. Forsythe. 1971. Resistance of gram-negative bacilli to gentamicin. *J. Infect. Dis.* 124:S264-S270.