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Comparison of Five Aminocyclitol Antibiotics In Vitro Against *Enterobacteriaceae* and *Pseudomonas*

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The in vitro susceptibility of 163 strains of *Enterobacteriaceae* and 23 isolates of *Pseudomonas aeruginosa* to various concentrations of gentamicin, kanamycin, spectinomycin, tobramycin, and BB-K8, a new semisynthetic aminoglycoside antibiotic, was determined. Studies were performed in Mueller-Hinton agar and broth, and two different sizes of bacterial inocula were used. On a weight basis, gentamicin and tobramycin demonstrated comparable activity in vitro and were the most active of the five drugs tested against *Escherichia coli*, *Klebsiella*, *Enterobacter*, and *Proteus* species. All of these organisms were inhibited by gentamicin or tobramycin at concentrations of 5.0 μg or less/ml in agar with both inocula of bacterial cells. In addition, tobramycin was the most active drug against isolates of *P. aeruginosa* and gentamicin was the most active against *Salmonella* and *Shigella* species. Although kanamycin and BB-K8 demonstrated a high degree of activity against most *Enterobacteriaceae*, they were not the most active agents tested for any genus. Spectinomycin was the least active compound, and many isolates grew in concentrations higher than those readily attainable in serum.

The response of gram-negative bacillary infections to antimicrobial therapy frequently has been unsatisfactory. Although the results of therapy may be determined by factors other than the therapeutic regimen employed, antimicrobial agents remain the cornerstone of therapy, and there has been a continued search for effective new agents. The purposes of the present study were to assess the in vitro effectiveness of two new semisynthetic aminocyclitol antibiotics, tobramycin and BB-K8, and to compare their activity against *Enterobacteriaceae* and *Pseudomonas aeruginosa* with that of three clinically available aminocyclitols, kanamycin, gentamicin, and spectinomycin.

BB-K8, which is derived from the acylation of the deoxystreptamine moiety of kanamycin A, has been reported to have a high degree of resistance to aminoglycoside-inactivating enzymes (K. E. Price et al., J. Antibiot. [Tokyo], in press). Unlike kanamycin, BB-K8 appears to be effective against a majority of *P. aeruginosa* strains. Tobramycin is derived from nebramycin, an antibiotic complex produced by *Streptomyces tenebrarius* (2, 3).

MATERIALS AND METHODS

Minimal inhibitory concentrations (MIC) of BB-K8, tobramycin, gentamicin, kanamycin, and spectinomycin were determined with 163 strains of *Enterobacteriaceae* and 23 isolates of *P. aeruginosa*. Studies were performed in Mueller-Hinton agar at a pH of 7.4 by use of a Steers replicator device (7). For each isolate, both 10^{-4} and 10^{-3} dilutions of an overnight broth culture of bacterial cells, containing approximately 10^5 and 10^7 organisms/ml, respectively, were tested.

The MIC for each strain was defined as the lowest concentration of antibiotic in which three or fewer colonies grew in the inoculated area of the agar plates after overnight incubation at 37 C. Twenty-five isolates of *Enterobacteriaceae* and five of *P. aeruginosa* also were tested by a dilution technique in Mueller-Hinton broth against BB-K8 and tobramycin. In these studies, the MIC was defined as the lowest concentration of antibiotic in which there was macroscopic inhibition of growth after overnight incubation at 37 C, and the bactericidal end point (MBC) was defined as the lowest concentration of antibiotic in which fewer than three viable colonies were recovered when approximately 0.005 ml of broth from each clear tube was subcultured onto antibiotic-free agar. Final concentrations of the antibiotics tested in both broth and agar dilution studies were 100, 50, 25, 20, 15, 12.5, 10, 7.5, 5.0, 2.5, 1.0, and 0.5 μg/ml. Strains of *E. coli* also were tested against tobramycin and gentamicin at a concentration of 0.25 μg/ml.

RESULTS

The antibacterial activity of five aminocyclitol antibiotics against *Enterobacteriaceae* and *P.*

aeruginosa is depicted in Fig. 1–7, which also show the effect of inoculum size on determination of the MIC in agar. It can be seen that against *E. coli* (Fig. 1), with the exception of spectinomycin, there was little difference among the drugs. The lowest MICs were observed with gentamicin and tobramycin; all isolates were inhibited by 1.0 μg of gentamicin/ml and by 2.5 μg of tobramycin/ml, and little effect related to the size of the inoculum was observed in agar. Kanamycin and BB-K8 exerted comparable activity against these isolates of *E. coli*; all strains were inhibited by 5 μg/ml.

Similarly, all 28 isolates of *Klebsiella* (Fig. 2) were inhibited by tobramycin or gentamicin at a concentration of 0.5 μg/ml when the lower inoculum of bacterial cells was used; a few isolates were not inhibited by this concentration when the higher inoculum was used. All *Klebsiella* species also were inhibited by 2.5 μg of BB-K8/ml, but a single isolate grew in concentrations of kanamycin as high as 100 μg/ml. Again, there was little effect of the size of the inoculum on the MIC of these antibiotics in agar against *Klebsiella*.

The results with 23 isolates of *Enterobacter* are summarized in Fig. 3. Once more, on a weight basis, regardless of the size of the inoculum, tobramycin and gentamicin were the most active preparations in vitro; all isolates were inhibited by 1.0 μg of either drug/ml. BB-K8 and kanamycin also demonstrated high degrees of activity, with BB-K8 being slightly more potent at the lower concentrations of antibiotic. Approximately 20% of *Enterobacter* species grew

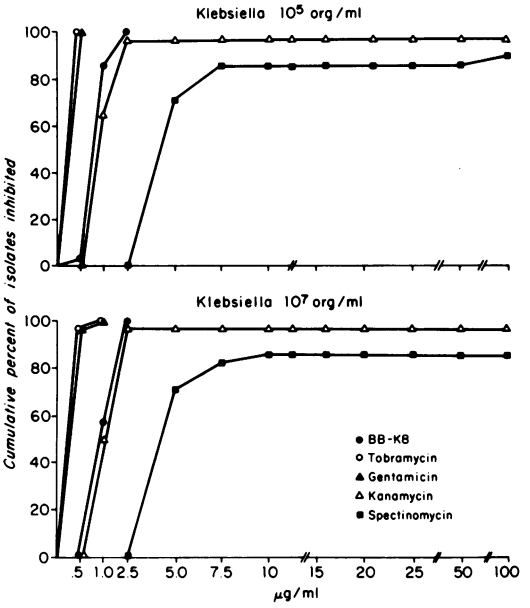

FIG. 2. *Cumulative percentage of 28 isolates of Klebsiella inhibited by increasing concentrations of antibiotic in agar medium with bacterial inocula of two different sizes.*

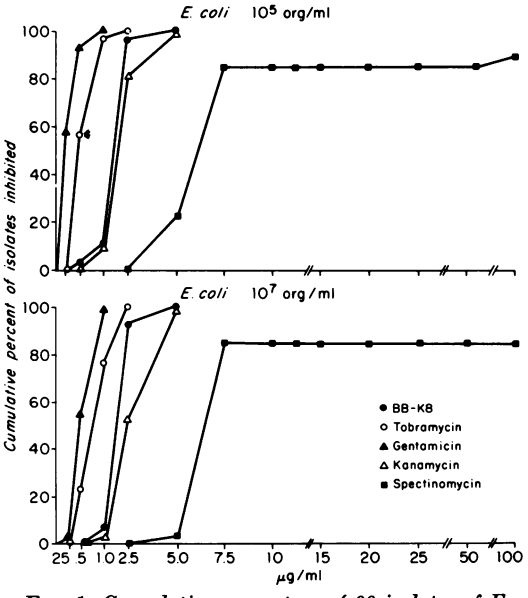

FIG. 1. *Cumulative percentage of 26 isolates of E. coli inhibited by increasing concentrations of antibiotic in agar medium with bacterial inocula of two different sizes.*

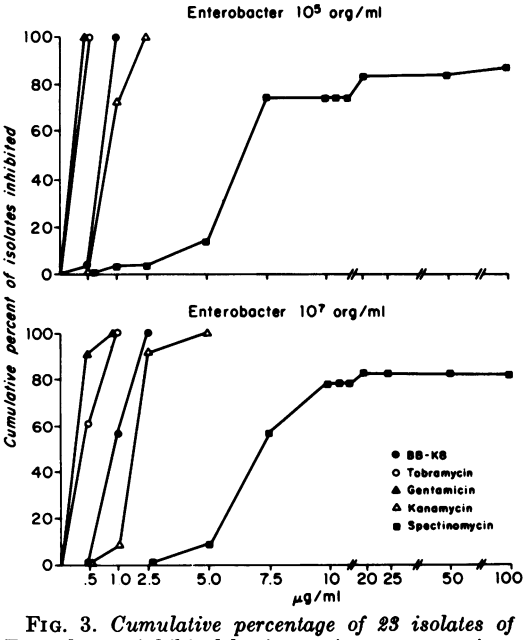

FIG. 3. *Cumulative percentage of 23 isolates of Enterobacter inhibited by increasing concentrations of antibiotic in agar medium with bacterial inocula of two different sizes.*

in spectinomycin concentrations of 50 μg/ml or greater.

With *P. mirabilis* (Fig. 4), results were similar to those with other *Enterobacteriaceae*; i.e., gentamicin and tobramycin were the most active agents, and all 30 isolates were inhibited by 5 μg of either antibiotic/ml. Comparable observations were made with 29 isolates of indole-positive *Proteus* species (Fig. 5).

The relative activity of the five antibiotics against 15 strains of *Salmonella* and 12 strains of *Shigella* is depicted in Fig. 6. As demonstrated previously with other *Enterobacteriaceae*, gentamicin and tobramycin were the most active of the five drugs. Note that the effectiveness of kanamycin was decreased only among strains of *Shigella*; isolates of *Salmonella* were inhibited by 2.5 μg of kanamycin/ml.

The activity of the five antimicrobials against 23 isolates of *P. aeruginosa* is shown in Fig. 7. Tobramycin was the single most active preparation, and even with the higher inoculum of bacterial cells all isolates were inhibited in agar by 5 μg of drug/ml. Gentamicin and BB-K8 had parallel activity against *Pseudomonas*, and kanamycin and spectinomycin were much less effective.

The results of testing BB-K8 and tobramycin against 30 representative organisms in both agar and broth are shown in Fig. 8. There was good agreement between the results of the two types

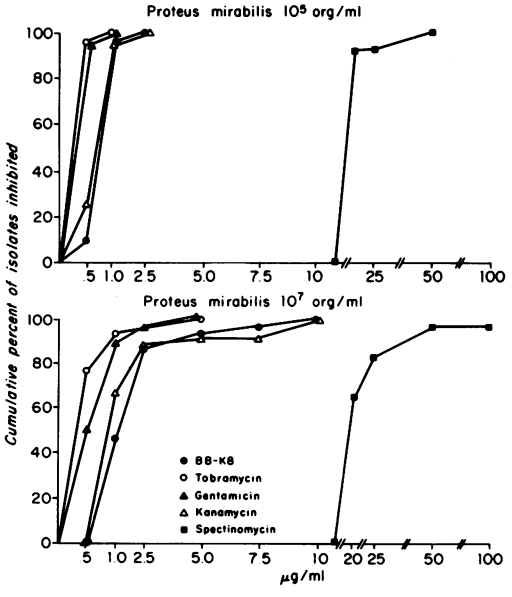

FIG. 4. *Cumulative percentage of 30 isolates of P. mirabilis inhibited by increasing concentrations of antibiotic in agar medium with bacterial inocula of two different sizes.*

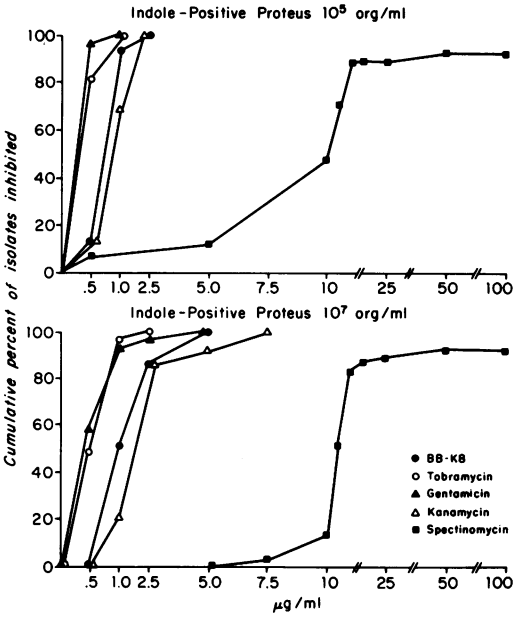

FIG. 5. *Cumulative percentage of 29 isolates of indole-positive Proteus species inhibited by increasing concentrations of antibiotic in agar medium with bacterial inocula of two different sizes.*

of in vitro susceptibility testing with the lower inoculum of organisms. However, with the larger inoculum, there was a greater diminution of activity in broth than in agar. Even then, all 30 isolates were inhibited by 15 μg of either drug/ml when tested in broth medium with the higher inoculum of bacterial cells.

The cumulative percentage of 30 representative organisms inhibited (MIC) or killed (MBC) by increasing concentrations of BB-K8 or tobramycin is shown in Fig. 9, which also summarizes the effects of inoculum size on the antibacterial activity of these antibiotics in broth. In general, with a given inoculum of organisms, there was a close parallel between inhibition and killing with either drug. However, at all concentrations of antibiotic, the MBC was slightly higher than the MIC.

DISCUSSION

During the past 5 years, there have been several reports dealing with inactivation of aminoglycoside antibiotics by bacteria possessing transferable R-factor enzyme systems. The effectiveness of new agents such as gentamicin, tobramycin, and BB-K8 against microorganisms resistant to other drugs of the same family is believed to be related to structural changes in the antibiotic molecule which interfere with enzymatic acetylation or phosphorylation (1, 5).

Obviously, when interpreting the potential clinical significance of comparative in vitro results such as those reported herein, one must take into account other properties of the anti-

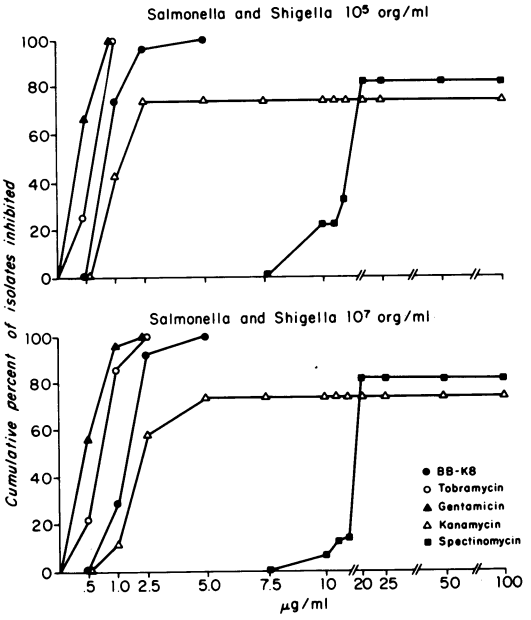

FIG. 6. *Cumulative percentage of 15 isolates of Salmonella and 12 isolates of Shigella inhibited by increasing concentrations of antibiotic in agar medium with bacterial inocula of two different sizes.*

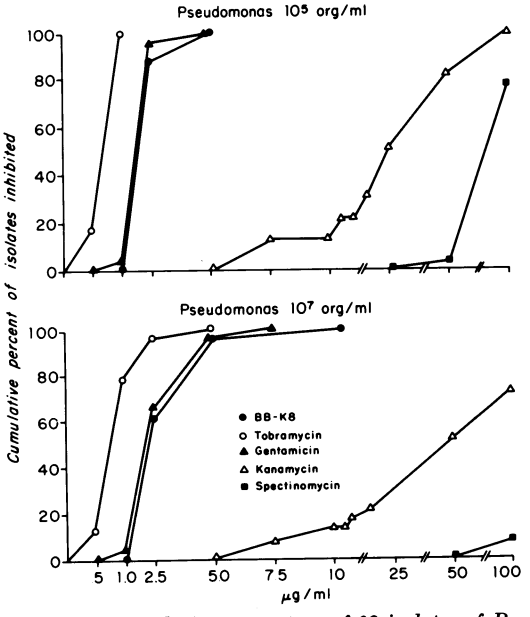

FIG. 7. *Cumulative percentage of 23 isolates of P. aeruginosa inhibited by increasing concentrations of antibiotic in agar medium with bacterial inocula of two different sizes.*

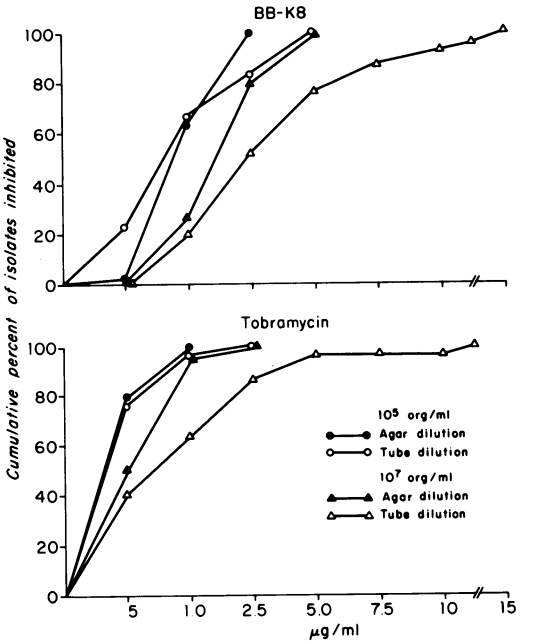

FIG. 8. *Cumulative percentage of 25 isolates of Enterobacteriaceae and 5 isolates of P. aeruginosa inhibited by increasing concentrations of antibiotic in agar or broth with bacterial inocula of two different sizes.*

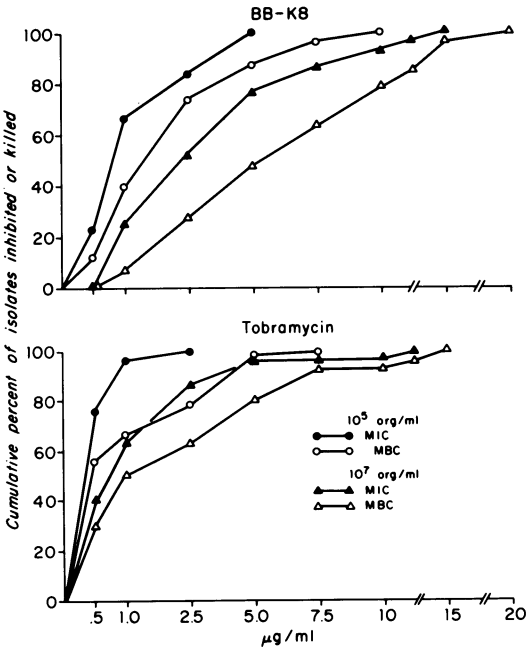

FIG. 9. *Cumulative percentage of 25 isolates of Enterobacteriaceae and 5 isolates of P. aeruginosa inhibited (MIC) or killed (MBC) by increasing concentrations of antibiotic in broth with bacterial inocula of two different sizes.*

microbial agents, such as feasible blood levels, toxicity, and toleration, to mention a few factors. Suffice it to say that, at levels anticipated to be achieved in man, there was little to choose from gentamicin, tobramycin, or BB-K8. Against most *Enterobacteriaceae*, kanamycin also demonstrated a high degree of activity, but it was inferior to the other agents against *Pseudomonas*. In addition, some strains of *Enterobacteriaceae* that were resistant to feasible levels of kanamycin, i.e., 20 μg/ml, were inhibited by the newer agents.

Spectinomycin was the least active drug tested against all genera. Spectinomycin was isolated in 1960, and spectinomycin sulfate appeared to be effective in the treatment of gonorrhea in early clinical trials (8). The more soluble dihydrochloride salt of spectinomycin (spectinomycin hydrochloride) has been recently made available for single-dose parenteral treatment of gonorrhea in patients allergic to penicillin (6). Although spectinomycin appears to be effective clinically against *Neisseria gonorrhoeae*, there is evidence that its usefulness against *Enterobacteriaceae* is severely restricted by the emergence of resistance during therapy (4). For this reason, spectinomycin should be restricted to the treatment of gonorrhea.

In conclusion, both tobramycin and BB-K8 appear highly active in vitro and warrant further studies. Whether they will possess any advantage in man over gentamicin is presently unclear.

ACKNOWLEDGMENTS

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