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Comparative In Vitro Activity of Three Aminoglycosidic Antibiotics: BB-K8, Kanamycin, and Gentamicin

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Kanamycin, BB-K8, and gentamicin were tested in parallel against 1,037 bacterial strains isolated from clinical material. The activity of BB-K8 at 20 μg/ml was comparable to that of gentamicin at 8 μg/ml against *Pseudomonas aeruginosa* and against *Enterobacteriaceae*, with the exception of gentamicin-resistant strains of *Proteus rettgeri* and *Providencia stuartii*, in which case the activities of BB-K8 and kanamycin were the same. BB-K8 exhibited little or no activity against streptococci. The activity of BB-K8 was affected by pH and inoculum size. A regression graph for inhibition data with a 10-μg disk of BB-K8 was developed, and synergistic activity of penicillin and BB-K8 against *Streptococcus faecalis* was tested.

BB-K8, a new, water-soluble, semisynthetic aminoglycoside antibiotic derived from kanamycin A, has broad antibacterial activity, similar to that of kanamycin (8). Also, it has a high resistance to aminoglycoside-inactivating enzymes and is active against *Pseudomonas aeruginosa* (13). Its ototoxicity and nephrotoxicity have been reported to be slightly less than those of kanamycin and appreciably less than those of gentamicin in animal toxicity studies (14).

In this study, we compared the in vitro activity of BB-K8 with that of kanamycin and gentamicin, and we determined the effects of pH, inoculum size, and combination with penicillin on the activity of BB-K8.

MATERIALS AND METHODS

Minimal inhibitory concentrations (MIC) of BB-K8, kanamycin, and gentamicin were determined in parallel in Mueller-Hinton agar (MHA) by the agar dilution technique (17). The inocula-replicating device of Steers et al. (15) was used, and the concentrations of each of the antibiotics tested were 80, 50, 20, 10, 5, 2.5, 1.2, 0.6, and 0.3 μg/ml. For testing of streptococci other than group D or of *Haemophilus*, 5% whole or heated sheep blood, respectively, was added to the agar. Inoculum size was adjusted so as to deposit 10^5 colony-forming units (CFU) on the surface of the agar. The MIC represented the lowest concentration at which there was no growth, a fine barely visible haze, or no more than three discrete colonies.

The relative activity of BB-K8 in MHA and in Trypticase soy agar (TSA) was tested in parallel in a limited number of instances. The effect of pH on BB-K8 activity was tested by adjusting the pH of MHA to 6.4, 7.4, and 8.4. Bactericidal activity of BB-K8 was determined, by use of procedures described by Otto et al. (12), against inocula of three different sizes (10^3, 10^5, and 10^7 CFU/ml) for each of the following species: 2 strains of *Staphylococcus aureus*, 2 strains of *Proteus mirabilis*, 2 strains of *Escherichia coli*, and 10 strains of *Pseudomonas aeruginosa*. Synergy studies were performed, as described previously (22), between penicillin and BB-K8 with isolates of *Streptococcus faecalis* previously reported not to have been synergistically affected by penicillin and streptomycin (22).

Disk diffusion tests on 111 strains were performed according to the method described by Bauer et al. (1) with the standardization published in the *Federal Register* (37[191]:20525-20529, 1972) and a 10-μg BB-K8 disk. The MIC of each of the 111 test strains was determined by the agar dilution technique, as recommended by Ericsson and Sherris (2) in their report of the World Health Organization-International Collaborative Study (WHO-ICS). In the WHO-ICS agar dilution method, the inoculum is adjusted so as to deposit 10^4 CFU on the surface of the agar. Because this method has been used for most regression graphs relating zone diameters to MIC, it was also used in this study for the sake of uniformity.

RESULTS

At a concentration of 20 μg/ml, BB-K8 had little or no inhibitory activity against streptococci (Table 1), including group D streptococci (Table 2). All strains of *Haemophilus* were inhibited by 20 μg/ml, and 97% were inhibited

by 10 μg/ml. With the exception of one strain of *E. coli* and one of *Providencia stuartii*, BB-K8 inhibited all of the *Enterobacteriaceae* tested at a concentration of 20 μg/ml (Table 2). Furthermore, BB-K8 was highly active against most of the *Enterobacteriaceae* at 5 μg/ml; exceptions were strains of *Proteus mirabilis* and *P. rettgeri*, of which 72 and 65%, respectively, were inhibited by this concentration. At 5 μg/ml, BB-K8 was slightly more active than kanamycin against *E. coli*, *Klebsiella pneumoniae*, *Enterobacter aerogenes*, *E. liquefaciens*, *Serratia marcescens*, salmonellae, and shigellae. With the notable exception of *P. rettgeri* and *P. stuartii*, MIC values of gentamicin against the *Enterobacteriaceae* were generally fourfold lower than those of BB-K8 and kanamycin. Many of the strains of *P. rettgeri* and *P. stuartii* represented gentamicin-resistant (MIC > 10 μg/ml) isolates from a nosocomial outbreak of bacteriuria in a physical medicine and rehabilitation unit.

In contrast to kanamycin, BB-K8 inhibited most isolates of *Pseudomonas aeruginosa* at 20 μg/ml (Table 2). Its activity at 20 μg/ml was equal to that of gentamicin at 8 μg/ml (tested but not included in tabulations). Inhibition of other species of *Pseudomonas* and of other nonfermenting gram-negative bacilli by the three aminoglycosides was variable; however, gentamicin generally was the most active. With the exception of *P. fluorescens*, the activities of BB-K8 and kanamycin against these species were generally equivalent.

All three agents had high activity against staphylococci. Gentamicin inhibited 100% of strains of *S. aureus* and *S. epidermidis* at 1.2 and 0.3 μg/ml, respectively; BB-K8 inhibited 100% of the strains at 5 and 2.5 μg/ml, respectively; kanamycin inhibited 96% of the strains of *S. aureus* and 83% of strains of *S. epidermidis* at 2.5 μg/ml.

In TSA, 50% of the MIC values of BB-K8 against *Pseudomonas* species, 81% of them against *P. aeruginosa*, and 55% of them against staphylococci exceeded those obtained in MHA by at least twofold (Table 3). BB-K8 was more active at an alkaline pH than at an acidic pH (Table 4). In general, the MIC values increased 2-fold with each 100-fold increase in inoculum size; similar increments were observed in those concentrations required to kill 100% of each of the three inocula (Table 5).

A regression graph relating MIC values of BB-K8 to zone diameters is shown in Fig. 1. With the exception of one strain of *Pseudomonas*, all isolates with MIC values of 20 μg or less/ml produced zone diameters in excess of 10 mm.

Against strains of *Streptococcus faecalis* previously reported to be resistant to a combination of penicillin and streptomycin (22), BB-K8 at concentrations of 12.5 and 25 μg/ml acted synergistically with penicillin (Fig. 2). When penicillin was tested with BB-K8 at 6.2 μg/ml, one strain of *S. faecalis* failed to be affected synergistically.

DISCUSSION

According to Price et al. (13), the average peak serum concentrations of BB-K8 and kanamycin in humans after doses of 7.5 mg/kg are approximately 20 μg/ml, which is at least double the maximal safe serum level of gentamicin in man. Furthermore, Reiffenstein and co-workers (14) found, in animal toxicity studies, that the ototoxicity and nephrotoxicity of BB-K8 are slightly less than those of kanamycin and considerably less than those of gentamicin. The clinical significance of these findings is uncertain, because we found gentamicin to be significantly more active than BB-K8 at lower concentrations. There is no published experience with BB-K8 therapy in humans, and the reported incidence of ototoxicity and nephrotoxicity in humans appears to be low with gentamicin therapy (5, 6, 21).

In view of the higher serum concentrations attainable with BB-K8, Price and co-workers

TABLE 1. *Activity of BB-K8 against streptococci (other than group D) and Haemophilus*[a]

Organism	No. of strains	Cumulative % inhibited at each concentration (μg/ml)									
		0.3	0.6	1.2	2.5	5	10	20	40	80	>80
Streptococcus pneumoniae	19								5	74	
Streptococcus, viridans group	9							22	33	89	
Streptococcus, group A	24						4	42	75	96	
Haemophilus[b]	31				10	68	97	100			

[a] Tested in agar supplemented with blood.

[b] Data based on 22 strains of *H. influenzae* and 9 strains of *H. parainfluenzae*.

TABLE 2. *Activity of BB-K8, kanamycin, and gentamicin against various bacteria*

Organism	No. of strains	Antibiotic[a]	Cumulative % inhibited at each concentration (μg/ml)								
			0.3	0.6	1.2	2.5	5	10	20	50	80
Escherichia coli	121	BB-K8			3	44	94	98	99	100	
		Kan				14	72	82	85		
		Gent	1	27	90	98	99		100		
Klebsiella pneumoniae	58	BB-K8	2	3	14	89	100				
		Kan		2	5	52	86		89	91	93
		Gent	10	76	98						
Enterobacter aerogenes	30	BB-K8				59	100				
		Kan				20	96	100			
		Gent		76	100						
E. cloacae	34	BB-K8			9	82	100				
		Kan				53	100				
		Gent	12	76	100						
E. hafniae	20	BB-K8			25	90	100				
		Kan			25	95	100				
		Gent	80	100							
E. liquefaciens	20	BB-K8			45	100					
		Kan			55	70	80		95	100	
		Gent	50	100							
E. agglomerans	20	BB-K8		45	80	100					
		Kan		40	80	100					
		Gent	90	100							
Serratia marcescens	29	BB-K8				14	93	100			
		Kan				10	89	100			
		Gent		41	96	100					
Citrobacter freundii	20	BB-K8			15	80	100				
		Kan				30	85	100			
		Gent	5	85	100						
C. diversus	23	BB-K8			17	87	96	100			
		Kan			4	43	83	100			
		Gent	13	78	100						
Salmonella	31	BB-K8			6	26	97	100			
		Kan				3	81	87			
		Gent	6	55	93	100					
Shigella	29	BB-K8				34	93	100			
		Kan				7	86	97	100		
		Gent		41	93	97	100				
Proteus mirabilis	36	BB-K8				33	72	92	100		
		Kan				25	72	89	100		
		Gent		14	67	92	100				
P. vulgaris	19	BB-K8		5	26	74	100				
		Kan			11	84	95	100			
		Gent	32	63	79	89		100			
P. morganii	19	BB-K8			11	74	89	100			
		Kan			32	74	89	95			
		Gent	16	77	95	100					
P. rettgeri	20	BB-K8		5	10	35	65	85	100		
		Kan			15	30	70	85	95		100
		Gent		15	20	30	35	60	80	85	100
Providencia stuartii	43	BB-K8		7	28	72	93	98			100
		Kan		12	30	67	93	98		100	
		Gent			7	21	32	63	79	93	98
Aeromonas hydrophila	20	BB-K8			5	55	95	100			
		Kan				10	60	90	95		
		Gent	5	55	95	100					
Pasteurella multocida	18	BB-K8		6	17		50	83	100		
		Kan		6	11	44	100				
		Gent	22	28	94	100					
Pseudomonas aeruginosa	59	BB-K8		3	7	49	78	91	93	98	100
		Kan							3	12	31
		Gent	5	10	57	79	90	95	98	100	

TABLE 2—*Continued*

Organism	No. of strains	Anti-biotic[a]	Cumulative % inhibited at each concentration (μg/ml)								
			0.3	0.6	1.2	2.5	5	10	20	50	80
P. maltophilia	17	BB-K8			6		12		18	47	53
		Kan					6	12	35	41	47
		Gent		6		12	29	41	47		71
P. fluorescens	19	BB-K8	5	32	79	100					
		Kan		37	79	84				95	100
		Gent	42	79	100						
P. stutzeri	15	BB-K8		7	20	73	93	100			
		Kan		7	33	60	87	100			
		Gent	47	80	93	100					
P. cepacia	21	BB-K8		5		10	14	19	24	33	43
		Kan		5		10	14	19	29	43	62
		Gent	5	10	14		24	29	33	48	71
Acinetobacter calcoaceticus	30	BB-K8		3	7	67	97				100
		Kan			13	73	97				100
		Gent		33	67	87	93	97			100
Mima polymorpha	20	BB-K8	5	25	35	80	95				100
		Kan	5	20	30	80	90	95			
		Gent	30	85	95				100		
Alcaligenes faecalis	6	BB-K8		17			67	83			100
		Kan		17				33		83	100
		Gent	17		67	83				100	
Staphylococcus aureus	60	BB-K8		3	76	96	100				
		Kan			85	96		98			100
		Gent	96	98	100						
S. epidermidis	52	BB-K8	61	77	86	100					
		Kan	50	71	79	83		86	90		
		Gent	100								
Group D streptococcus	45	BB-K8						2	7		11
		Kan				2		4	7	18	67
		Gent				2	7		36	80	100

[a] Kan = kanamycin; Gent = gentamicin.

TABLE 3. *Relationship between minimal inhibitory concentrations (MIC) of BB-K8 in Mueller-Hinton agar (MHA) and Trypticase soy agar (TSA)*

Organism	No. of strains	MIC range in MHA (μg/ml)	Fold relationship of MIC in TSA[a]				
			−4	−2	1	2	≧4
Enterobacteriaceae							
Escherichia	150	1.2–40		20	105	25	
Salmonella	75	1.2–10	1	10	54	8	2
Klebsiella	213	0.3–10		29	166	17	1
Proteus	141	0.6–80		9	85	44	3
Total	579		1	68	410	94	6
Pseudomonadaceae							
Aeromonas	20	1.2–10		6	14		
Pseudomonas	115	0.3–80	3	19	36	50	7
P. aeruginosa	63	0.6–80		1	11	44	7
Total	198		3	26	61	94	14
Micrococcaceae							
Staphylococcus	112	0.3–5	1	6	43	60	2

[a] Data shown are number of strains.

(13) compared the activity of BB-K8 at 20 μg/ml with that of gentamicin at 8 μg/ml. Among *Enterobacteriaceae*, they found 95.5% of strains to be susceptible to gentamicin and 99.7% to be susceptible to BB-K8. In our study, activities of BB-K8 at 20 μg/ml and of gentamicin at 5 μg/ml were comparable in every respect, except against strains of *P. rettgeri* and *P. stuartii* isolated from a nosocomial outbreak of bacteriuria in patients with chronic indwelling urethral

TABLE 4. *Minimal inhibitory concentrations of BB-K8 against 35 strains of bacteria at three pH levels*

pH of MHA	Cumulative % inhibited at each concentration (μg/ml)								
	0.3	0.6	1.2	2.5	5	10	20	40	80
6.4				6	29	74	97	100	
7.4		9	31	91	100				
8.4		14	71	97	100				

catheters in one unit of a Mayo Clinic-affiliated hospital. Among strains of *Pseudomonas*, of which nearly 97% were *P. aeruginosa*, Price et al. (13) found that BB-K8 had significantly better activity than gentamicin. At a concentration of 8 μg/ml (tested but not included in tabulations), 93% of our strains of *P. aeruginosa* were inhibited by gentamicin, and 93% of these strains were also inhibited by BB-K8 at 20 μg/ml. Our data regarding the activity of the three amino-

TABLE 5. *Effect of inoculum size on bactericidal activity of BB-K8*

Organism	Inoculum size (CFU/ml)	MIC (μg/ml)	Concn resulting in 99.9% killing (μg/ml)	Concn resulting in 100% killing (μg/ml)
S. aureus (ATCC 25923)	2.2×10^3	0.3	2.5	5, 10, 20, 40, 80
	2.2×10^5	2.5	5	10, 20, 40, 80
	2.2×10^7	5	10, 20, 40, 80	
S. aureus G9816	3.9×10^3	0.3	1.2	2.5, 10, 20, 40, 80
	3.9×10^5	5		10, 20, 40, 80
	3.9×10^7	5	20	40, 80
P. mirabilis	4.7×10^3	1.2		2.5, 5, 10, 20, 40, 80
	4.7×10^5	10		20, 40, 80
	4.7×10^7	40		80
P. mirabilis U23862	4×10^3	5		5, 20, 40, 80
	4×10^5	20		20, 40, 80
	4×10^7	40	40, 80	
E. coli (ATCC 25922)	1.5×10^3	1.2		2.5, 5, 10, 20, 40, 80
	1.5×10^5	2.5		5, 10, 20, 40, 80
	1.5×10^7	10		20, 40, 80
E. coli U22988	1.3×10^3	0.6		2.5, 5, 10, 20, 40, 80
	1.3×10^5	2.5	2.5	5, 10, 20, 40, 80
	1.3×10^7	5		10, 20, 40, 80
P. aeruginosa (control)	1.5×10^3	0.6		0.6, 1.2, 2.5, 5, 10, 20, 40, 80
	1.5×10^5	1.2		2.5, 5, 10, 20, 40, 80
	1.5×10^7	5		5, 10, 20, 40, 80
P. aeruginosa Sp4189	2.6×10^3	1.2		2.5, 5, 10, 20, 40, 80
	2.6×10^5	2.5		5, 10, 20, 40, 80
	2.6×10^7	5	10	20, 40, 80
P. aeruginosa G9761	2.9×10^3	1.2		5, 10, 20, 40, 80
	2.9×10^5	1.2		5, 10, 20, 40, 80
	2.9×10^7	2.5	10	20, 40, 80
P. aeruginosa Sp4066	2×10^3	1.2		2.5, 5, 10, 20, 40, 80
	2×10^5	2.5	2.5	5, 10, 20, 40, 80
	2×10^7	5	10, 20	40, 80
P. aeruginosa U23162	5.4×10^3	1.2		2.5, 5, 10, 20, 40, 80
	5.4×10^5	2.5		10, 20, 40, 80
	5.4×10^7	2.5	10, 20	40, 80
P. aeruginosa U22832	4×10^3	2.5		5, 10, 20, 40, 80
	4×10^5	5	20	10, 40, 80
	4×10^7	10		20, 40, 80
P. aeruginosa G9546	5.6×10^3	2.5		5, 10, 20, 40, 80
	5.6×10^5	5		10, 20, 40, 80
	5.6×10^7	10	20	40, 80
P. aeruginosa U23337	3.4×10^3	2.5		2.5, 5, 10, 20, 40, 80
	3.4×10^5	2.5		5, 10, 20, 40, 80
	3.4×10^7	10		20, 40, 80
P. aeruginosa G9847	2.2×10^3	1.2		2.5, 5, 10, 20, 40, 80
	2.2×10^5	1.2	10	2.5, 5, 20, 40, 80
	2.2×10^7	5	20	40, 80
P. aeruginosa G13067	2.1×10^3	2.5	2.5	5, 10, 20, 40, 80
	2.1×10^5	2.5		10, 20, 40, 80
	2.1×10^7	5	10	20, 40, 80

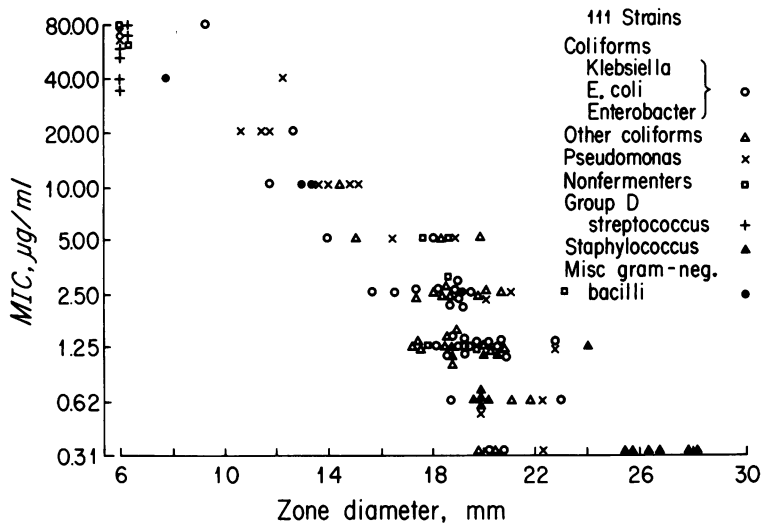

FIG. 1. *Regression graph relating minimal inhibitory concentrations of BB-K8 to zone diameters (10-μg BB-K8 disk).*

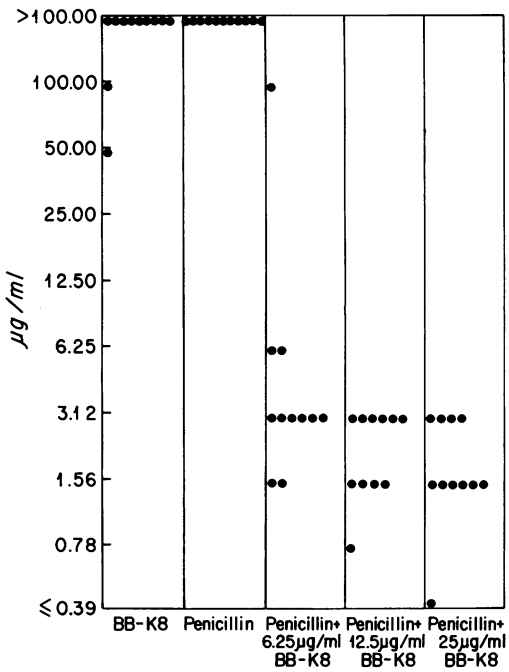

FIG. 2. *Susceptibility of Streptococcus faecalis to penicillin, BB-K8, or penicillin plus BB-K8.*

glycosides against staphylococci corresponded closely with those of Price et al. (13).

BB-K8 has been found to be resistant to bacterial enzymes which inactivate kanamycin and gentamicin (13). However, both kanamycin and BB-K8 were equally active against our gentamicin-resistant isolates of *P. rettgeri* and *P. stuartii*. Focal outbreaks of infections due to gentamicin-resistant *Enterobacteriaceae* have been reported only rarely in the United States (7, 9, 16). Resistance of certain pseudomonads and some other nonfermenting gram-negative bacilli to BB-K8 is due either to as yet unrecognized inactivating enzymes or to nonenzymatic mechanisms.

The influence of the medium on the results of susceptibility testing with BB-K8 against *Pseudomonadaceae* is apparent in Table 3. The higher MIC values in TSA undoubtedly reflect the now well-recognized effects of cation content of media on the activity of tetracycline, aminoglycosides, and polymixin B (2-4, 10, 11, 18-20). The lower activity of BB-K8 at pH 6.4 than at pH 7.4 is similar to data reported for other aminoglycosides (2). Differences between the MIC and minimal bactericidal concentration of BB-K8 varied according to the type of organism being tested and its inoculum size.

Price et al. (13) plotted a regression graph from data with a 30-μg BB-K8 disk and concluded that 15 mm was a satisfactory point for separating resistant strains (MIC > 20 μg/ml) from susceptible strains (MIC ≦ 20 μg/ml). Furthermore, they suggested that, with a 10-μg disk, 13 mm should be used. Our data do not bear out this recommendation because it appears that 10 mm is a better point.

In synergy studies of penicillin and BB-K8 with strains of *S. faecalis* previously isolated from patients with endocarditis and found to be resistant to penicillin and streptomycin (22), synergy was evident in all cases when BB-K8

was present in a concentration of 12.5 or 25 μg/ml and in all but one instance when BB-K8 was present in a concentration of 6.25 μg/ml. As such, the effect of a combination of penicillin and BB-K8 is comparable to that previously found with penicillin and kanamycin and with penicillin and gentamicin (22).

The principal value of BB-K8 seems to be its resistance to gentamicin- and kanamycin-inactivating enzymes. Its clinical application will depend heavily on the extent to which these kinds of resistant bacteria become a problem in causing infection. In our experience, gentamicin-resistant strains of *P. rettgeri* and *P. stuartii*, isolated from a focal outbreak of bacteriuria, were susceptible to kanamycin. Otherwise, taking differences in dosages and mean peak serum concentrations into account, BB-K8 presented no distinct advantages in vitro over gentamicin.

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