ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1975, p. 172-178
Vol. 7, No. 2

Printed in U.S.A.

Activity of Three Aminoglycosides and Two Penicillins Against Four Species of Gram-Negative Bacilli

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Received for publication 7 October 1974

Three aminoglycoside antibiotics and two penicillins were compared for their in vitro activity against 60 isolates of *Serratia*, *Pseudomonas*, *Proteus mirabilis*, and indole-positive *Proteus* sp. Testing was done by the agar dilution method using Mueller-Hinton broth solidified with 1.5% agar. The activity of amikacin, aminodeoxybutirosin, and gentamicin against *Proteus* and *Pseudomonas*, as related to their peak blood levels, showed no significant differences. Amikacin was the most active against *Serratia marcescens*. Results using Mueller-Hinton media in broth dilution tests correlated with the agar dilution method except for *Pseudomonas aeruginosa*. The minimal inhibitory concentration for aminoglycosides in agar was considerably greater than the minimal inhibitory concentration in Mueller-Hinton broth, and the disparity was related to the higher divalent cation concentration of agar. BL-P1654 and carbenicillin were similar except that carbenicillin was much more active against indole-positive *Proteus* sp. Additionally, the ratio of bactericidal to bacteriostatic concentrations of BL-P1654 was considerably greater than for carbenicillin.

Gentamicin and carbenicillin, used alone or in combination, are now among the most widely used antibiotics for the treatment of serious, hospital-acquired, gram-negative bacillary infections. Although the activity by weight of gentamicin against *Enterobacteriaceae* and *Pseudomonas aeruginosa* is comparable to other aminoglycosides, several problems have been associated with its clinical use. First, the mean peak blood level obtained with gentamicin at commonly recommended dosages (1.5 mg/kg) is about 4 μg/ml, whereas the trough level is often less than 1 μg/ml. This peak level may barely exceed the minimal inhibitory concentration (MIC) of many gram-negative isolates and it is likely that larger doses will be associated with greater toxicity. Second, clinically significant gentamicin-resistant organisms are being reported with increasing frequency (14, 19). Carbenicillin is the only clinically available penicillin with activity against *P. aeruginosa*, *Serratia*, and indole-positive *Proteus* sp. However, a number of reports have documented the rapid development of carbenicillin resistance during therapy (12, 13). For these reasons, new antimicrobial agents of the aminoglycoside or semisynthetic penicillin classes continue to be investigated for their in vitro activity against those gram-negative bacilli which are difficult to treat. In this report, we have compared the activity of gentamicin to amikacin (BB-K8) and aminodeoxybutirosin (A-butirosin) and carbenicillin to a new semisynthetic penicillin, BL-P1654, against approximately 60 clinical isolates of *Proteus mirabilis*, indole-positive *Proteus*, *P. aeruginosa*, and *Serratia marcescens*.

The in vitro testing was performed in accord with the methods recommended by the International Collaborative Study (ICS) on antimicrobial testing sponsored by the World Health Organization (8). Standard reference strains from the Pasteur Institute Collection (among ghose reviewed by the ICS) were used so that the results of this study can be compared with data from other laboratories using standard methods and these reference strains.

(This paper was presented in part at the 14th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., 11 to 13 September, 1974.)

MATERIALS AND METHODS

In vitro testing. In vitro susceptibility to various agents of approximately 60 clinical isolates each of *P. aeruginosa*, *S. marcescens*, indole-positive *Proteus* sp, and *P. mirabilis* were determined by the agar dilution method as outlined by the ICS (8). The organisms were isolated from clinical specimens since 1971 from the Sloan-Kettering Cancer Center (Memorial Hospital), New York; Univ. of California (CHS), Los Angeles; and Wadsworth VA Hospital, Los Angeles. They were identified by standard criteria (3). Tube

dilution testing was performed in Mueller-Hinton broth (MHB) (Difco, lot no. 075701) and agar dilution on Mueller-Hinton agar (MHA) prepared from MHB solidified with 1.5% agar (Difco, lot no. 014001). MHA for testing *Proteus* sp. contained 4% agar to prevent swarming.

Gentamicin was obtained from George Acieri of the Schering Corp., amikacin and BL-P1654 were from Edward Yevak of Bristol Laboratories, aminodeoxybutirosin was from M. W. Fisher of Parke-Davis and Co., and carbenicillin was from B. Horn-Bostel of Roerig Division, Pfizer Pharmaceuticals.

Plastic petri dishes containing serial twofold dilutions of antibiotics in agar were prepared within 48 h of use. An inoculum of approximately 1×10^4 organisms diluted from an overnight broth culture was used. The organisms were delivered to dry agar surfaces using the inoculum replicating device of Steers et al. (20). The Ca^{2+} and Mg^{2+} concentration of the media was determined by an atomic absorption spectrophotometer and expressed in milligram percent. Broth MIC was determined by inoculating 1×10^6 organism from an overnight culture into MHB and recording the lowest concentration of antibiotic which yielded a clear tube at 24 h. All clear tubes were subcultured onto blood agar and the minimal bactericidal concentration (MBC) was that concentration which yielded less than 5 colonies at 24 h.

Standard reference strains *Escherichia coli* 53.153 and *P. aeruginosa* 58.38 obtained from the collection of the Pasteur Institute were kindly provided by Yves Chabbert. Each plate was inoculated with 30 clinical isolates and both reference strains.

Criteria for susceptibility. Isolates were considered susceptible if they were inhibited at or below the peak serum level achieved during administration of drug to patients or normal volunteers. For gentamicin, this was 4 μg/ml at a dose of 1.5 mg/kg; for amikacin and aminodeoxybutirosin, 16 μg/ml at a dose of 7.5 mg/kg; and for carbenicillin and BL-P1654, 128 μg/ml for a dose of 4 g and 1 g, respectively. The inhibitory index refers to the ratio between the mean peak serum levels given above and the MIC for at least ⅔ of each species tested (22).

RESULTS

Each isolate was tested a minimum of three times by the agar dilution method to determine the MIC. The two standard reference strains were tested on each plate as an internal control. Reference strains were thus rechecked more than 20 times each, and showed no greater than a one dilution variation.

Susceptibility of clinical isolates to three aminoglycosides. The in vitro susceptibility of clinical isolates of four different species are represented in Fig. 1-4.

The concentration of gentamicin to inhibit 50% of the isolates of *P. mirabilis* was 1.5 μg/ml; for amikacin, 4 μg/ml; and for aminodeoxybutirosin, 8 μg/ml (Fig. 1). Over 95% of isolates were inhibited by all three drugs within their

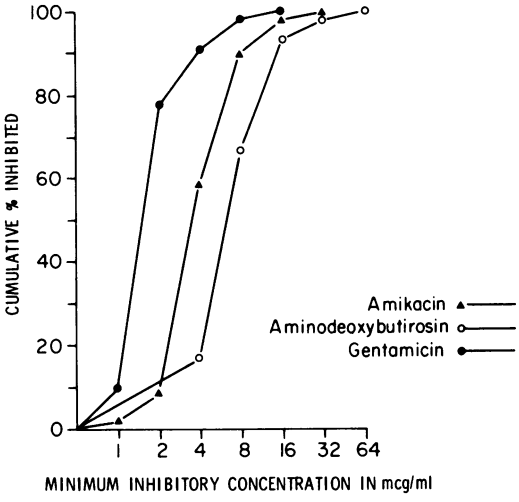

FIG. 1. *The activity of gentamicin, amikacin, and aminodeoxybutirosin against 58 clinical isolates of Proteus mirabilis.*

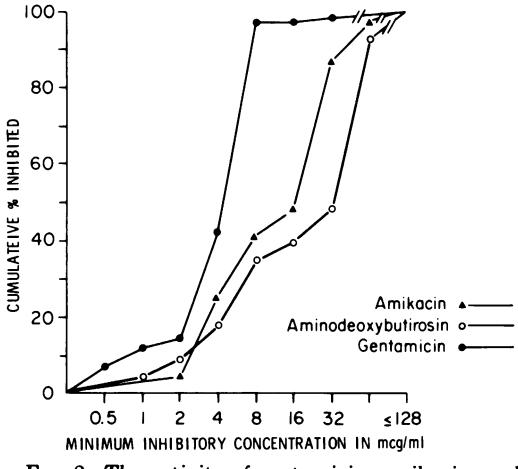

FIG. 2. *The activity of gentamicin, amikacin, and aminodeoxybutirosin against 57 clinical isolates of Pseudomonas aeruginosa.*

therapeutic range. Inhibition of 50% of isolates of *P. aeruginosa* by gentamicin, amikacin, and aminodeoxybutirosin was achieved at 4, 16, and 32 μg/ml, respectively (Fig. 2). With these agar dilution tests, the curve representing cumulative percent inhibition of *P. aeruginosa* by gentamicin and amikacin showed its greatest increment at one dilution above the mean peak serum levels for each drug. Fifty percent of indole-positive *Proteus* sp. was susceptible to gentamicin at 1 μg/ml, amikacin at 2 μg/ml, and aminodeoxybutirosin at 4 μg/ml (Fig. 3). At the mean peak serum levels, 81% of isolates were inhibited by gentamicin, 95% were inhibited by amikacin, and 96% were inhibited by

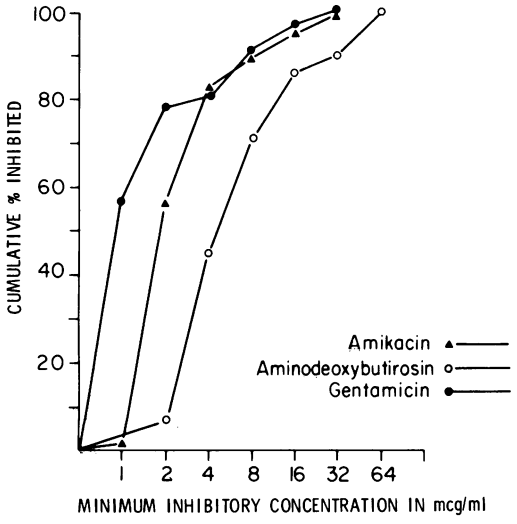

FIG. 3. *The activity of gentamicin, amikacin, and aminodeoxybutirosin against 58 clinical isolates of indole + Proteus sp.*

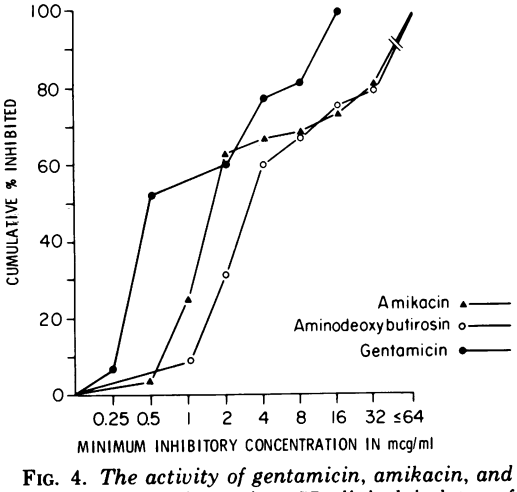

FIG. 4. *The activity of gentamicin, amikacin, and aminodeoxybutirosin against 57 clinical isolates of Serratia marcescens.*

aminodeoxybutirosin. Fifty percent of the *Serratia* strains were susceptible to 0.5, 1.5, and 2.5 μg/ml of gentamicin, amikacin, and aminodeoxybutirosin, respectively. At mean peak serum levels, gentamicin inhibited 77% of these strains, amikacin 74% of strains, and aminodeoxybutirosin 75% of strains.

Susceptibility of clinical isolates to two penicillins. At 8 μg/ml for BL-P1654 and between 16 to 32 μg/ml for carbenicillin, approximately 50% of *P. aeruginosa* strains were inhibited. At 128 μg/ml (peak serum level), 95% of strains were inhibited by both agents (Fig. 5). Fifty percent of *S. marcenscens* was inhibited by 16 μg/ml of BL-P1654 and carbenicillin, and at 128 μg/ml the MIC for 90% of isolates was exceeded by both drugs. Both carbenicillin and BL-P1654 were quite active against *P. mirabilis*, as greater than 90% of isolates were inhibited by either agents at only 4 μg/ml (Fig. 7). In contrast, the activity of the two agents against indole-positive *Proteus* sp. differed markedly (Fig. 7). The concentration of 4 μg of carbenicillin per ml, compared with 128 μg of BL-P1654 per ml, inhibited 50% of indole-positive *Proteus* sp.

Inhibitory index. The relevance of activity by weight of an antimicrobial agent may be less

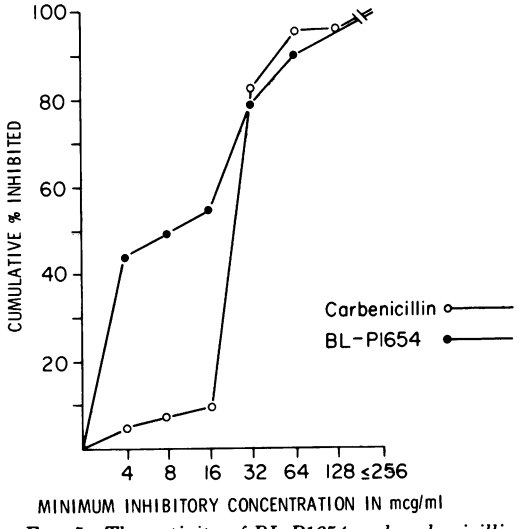

FIG. 5. *The activity of BL-P1654 and carbenicillin against 57 clinical isolates of Pseudomonas aeruginosa.*

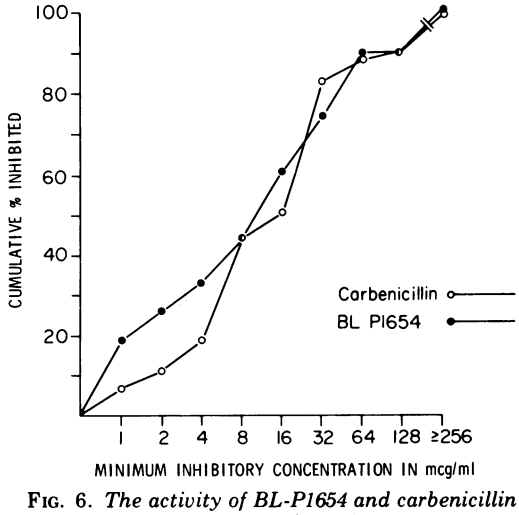

FIG. 6. *The activity of BL-P1654 and carbenicillin against 57 clinical isolates of Serratia marcescens.*

meaningful than the relationship between in vitro activity and the achievable peak serum levels. The inhibitory index is defined as the ratio of the mean peak serum level to the lowest antibiotic concentration required to inhibit two-thirds of the isolates (Table 1). Thus, the greater the ratio the higher the blood levels are in relation to the pattern of MICs. Viewed in this manner, the three aminoglycosides are comparable except that amikacin appears to offer an advantage over gentamicin against *Serratia* and carbenicillin is distinctly superior to BL-P1654 against indole-positive *Proteus* sp.

Comparison of MBC/MIC for BL-P1654 and carbenicillin. The tube dilution method, performed according to the ICS guidelines to determine the MIC and MBC of 12 representative clinical isolates and both reference strains, indicated that for *Pseudomonas* and some *Serratia* the MBC/MIC ratio for BL-P1654 was much greater than for carbenicillin (Table 2). No difference was seen for *Proteus* sp.

Comparison of methods and effect of media. The MIC was determined for 12 clinical isolates and both standard reference organisms by the tube dilution method in Mueller-Hinton broth and compared with those obtained by the agar dilution method (Table 3). Except for *Pseudomonas*, the results were comparable and did not differ by more than a single dilution. For *P. aeruginosa*, the aminoglycosides were least active on 1.5% agar, and most active in MHB probably reflecting the differences in the concentration of Ca^{2+} + Mg^{2+} in the media (Table 4).

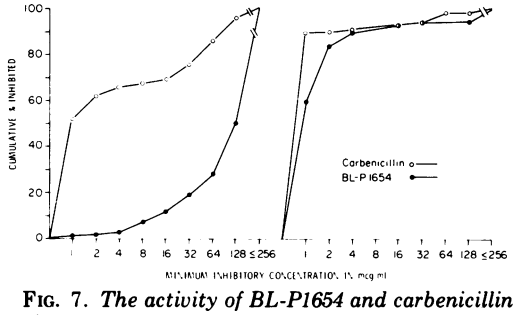

FIG. 7. *The activity of BL-P1654 and carbenicillin against Proteus sp. (Left) Against indole + Proteus sp. carbenicillin showed significantly greater activity. (Right) Against P. mirabilis the activity of the two penicillins was similar.*

DISCUSSION

The spectrum of these aminoglycoside antibiotics was similar but on a weight basis gentamicin was clearly the most active, amikacin intermediate, and aminodeoxybutirosin least active. This order of activity against the four species of bacteria tested was in agreement with previous reports (22, 23). To factor for the differences in pharmacokinetics between amikacin and gentamicin (6), inhibitory indices were calculated to relate potency by weight with achievable serum levels (22). Using this approach, amikacin appears to offer no theoretical advantage over gentamicin, except against isolates of *S. marcescens*. It should be borne in mind, however, that highly gentamicin-resistant strains are often susceptible to amikacin, whereas the few strains studied which are resistant to amikacin are also gentamicin resistant (5, 18). Amikacin is effective against bacterial strains resistant to other aminoglycosides because it is a poor substrate for enzymes that inactivate aminoglycosides by phosphorylation, or adenylation (2, 18). Comparative clinical studies will be required to determine whether one of these agents has any therapeutic advantages over the other.

Aminodeoxybutirosin is a chemical modification of butirosin, the latter being obtained from fermentation filtrates of *Bacillus circulans*. Butirosin and aminodeoxybutirosin resemble amikacin in activity and pharmacokinetics (7, 11). A previous report showed aminodeoxybutirosin to have greater activity against *Pseudomonas* and *Serratia* than butirosin (personal communication, M. W. Fisher, Parke-Davis and Co.),

TABLE 1. *Inhibitory index: Ratio of mean peak serum level per concentration to inhibit ⅔ of isolates (μg/ml)*

Determinants	Gentamicin (4)[a]	Amikacin (16)[a]	Aminodeoxybutirosin (16)[a]	Carbenicillin (128)[a]	BL-P1654 (128)[a]
Pseudomonas aeruginosa (57 isolates)	0.5 (4/8)	0.5 (16/32)	0.25 (16/64)	4 (128/32)	4 (128/32)
Serratia marcescens (57 isolates)	1 (4/4)	4 (16/4)	2 (16/8)	4 (128/32)	4 (128/32)
Indole + *Proteus* sp. (58 isolates)	2 (4/2)	2 (16/8)	2 (16/8)	128 (128/1)	64 (128/2)
Indole − *Proteus* sp. (58 isolates)	2 (4/2)	4 (16/4)	2 (16/8)	32 (128/4)	<1 (128/>128)

[a] Mean peak serum level.

TABLE 2. *Comparison of MBC-MIC ratio between carbenicillin and BL-P1654 in 14 clinical isolates*

Determinants	Carbenicillin		BL-P1654	
	MBC/MIC	Ratio	MBC/MIC	Ratio
Pseudomonas aeruginosa Pasteur Institute 58.38	128/64	2	512/16	32
Escherichia coli Pasteur Institute 54.127	8/4	2	16/2	8
Serratia marcescens 66-1	256/64	4	128/64	2
S. marcescens 829	64/64	1	256/32	8
S. marcescens 688	16/8	2	62/32	2
S. marcescens 670-5	64/64	1	1024/32	32
P. aeruginosa 1887	512/256	2	512/32	16
P. aeruginosa 2120	126/64	2	512/16	32
P. aeruginosa 2395	16/16	1	512/16	32
P. aeruginosa 3559	128/28	1	512/32	16
Proteus rettgeri 120	8/4	2	32/32	1
Proteus morganii 130	1/1	1	4/2	2
Proteus mirabilis 383	2/2	1	4/4	1
P. mirabilis 241	2/2	1	4/2	2

TABLE 3. *Comparison of methods and effect of media on the activity of three aminoglycosides*

Determinants	Gentamicin			Amikacin			Aminodeoxybutirosin		
	Agar dilution (MHB + 4% agar)	Broth dilution		Agar dilution (MHB + 4% agar)	Broth dilution		Agar dilution (MHB + 4% agar)	Broth dilution	
		MHB + 1.5% agar	MHB		MHB + 1.5% agar	MHB		MHB + 1.5% agar	MHB
Escherichia coli Pasteur Institute 54.127	1	0.5	0.5	1	1	1	2	2	1
Pseudomonas aeruginosa Pasteur Institute 58.38	16	4	1	16	8	2	16	8	2
P. aeruginosa 2120		4	1		8	2		8	2
P. aeruginosa 1887		4	1		4	4		8	2
P. aeruginosa 3556		4	2		8	2		64	16
P. aeruginosa 3286		4	1		8	4			
Serratia marcescens 07		1	1		8	16		4	4
S. marcescens 670-5		1	2		2	4		4	4
Proteus morganii 130	1		1	4		8	4		8
Proteus rettgeri	16		16	8		16	64		32

TABLE 4. *Divalent cation concentration in three types of media as determined by atomic absorption spectrophotometry*

Determinants	Ca^{2+} (mg%)	Mg^{2+} (mg%)	$Ca^{2+} + Mg^{2+}$ (mg%)
MHB + 4% agar	3.7	6.0	9.7
MHB + 1.5% agar	2.0	4.0	6.0
MHB	1.9	0.73	2.63
Normal human serum	10	2.4	12.4

but our results showed no advantage of aminodeoxybutirosin over amikacin. Cross-resistance between gentamicin and butirosin (and presumably aminodeoxybutirosin) commonly occurs (18).

The effect of divalent cation concentration in culture media on the activity of gentamicin against *Pseudomonas* has been extensively investigated and is confirmed in this report (9, 10, 15). The interaction of Ca^{2+} and Mg^{2+} with the bacterium probably occurs at the cell wall and this increases the structural stability of the cell wall resulting in increased resistance to aminoglycoside antibiotics (24). Based upon broth dilution susceptibility in this study and average peak serum levels, greater than 95% of the *Pseudomonas* would be susceptible to gentamicin and amikacin. An important question is whether the rather high degree of resistance encountered in this study compared to earlier reports from this and other laboratories can be reconciled on the basis of technical differences and/or the use of media with varying divalent cation content. Since one dilution variation of MICs in relation to peak serum levels dramati-

cally changes the prevalence of resistant strains and since as much as eightfold differences are observed between broth and agar tests, these factors probably explain the variations that have been encountered. The interpretation of this data with regard to the physiologic state of the host is even more important. Since the effect of Mg^{2+} is greater than that of Ca^{2+}, and since the concentration of Mg^{2+} in MHA is closer to that in human serum, the results of tests using MHA may be more clinically relevant than those using MHB.

The two penicillins compared in this study have a comparable spectrum. Bodey and Stewart (4) and Price et al. (16) have shown BL-P1654 to be more active in vitro than carbenicillin against *Pseudomonas* and *Serratia*, but Adler and Finland could demonstrate no significant difference against *Pseudomonas* (1). Our data agree with the latter study. The reason for the difference in results is probably related to the marked effect of culture media on BL-P1654 (17, 21). The weakly basic BL-P1654 has up to a 40-fold reduction in activity on MHA as compared to nutrient broth. Carbenicillin, which is acidic, does not show this variation. MHA is a more reliable indicator of the relative in vivo potency of BL-P1654 and carbenicillin than nutrient agar (17). The action of carbenicillin is markedly greater than BL-P1654 against indole-positive *Proteus* sp. in agreement with previous work (4). Further, in testing of BL-P1654, a wide discrepancy was observed between MIC and MBCs. Overall, BL-P1654 compared to carbenicillin appears no more active against *P. aeruginosa* and *S. marcescens* as determined by the agar dilution method, has markedly less bactericidal activity against these two species and is less active against indole-positive *Proteus* sp.

ACKNOWLEDGMENTS

This study was supported in part by Public Health Service training grant AI-309 from the National Institute of Allergy and Infectious Diseases, and by Bristol Laboratories, Schering Corporation, and Parke-Davis.

The expert technical assistance of Nalinee Linpisal, Shahnaz Raza, and Dorothy Kelly is gratefully acknowledged.

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