

BL-P1654, Ticarcillin, and Carbenicillin: In Vitro Comparison Alone and in Combination with Gentamicin Against *Pseudomonas aeruginosa*

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Minimum inhibitory concentrations of carbenicillin, ticarcillin, and BL-P1654 were determined for 89 clinical isolates of *Pseudomonas aeruginosa*. Ticarcillin was generally twice as active and BL-P1654 eight to 16 times as active as carbenicillin. Usually carbenicillin and ticarcillin killed at the same concentration or twice the concentration needed to inhibit, whereas 400 μg of BL-P1654 per ml was not bactericidal for the majority of isolates tested. The inhibitory effect of all three drugs varied markedly with the size of bacterial inoculum. When therapeutically achievable concentrations were used, adding gentamicin enhanced the inhibitory and bactericidal activity of all three penicillin derivatives for the majority of isolates. However, inhibition of isolates highly resistant to gentamicin was not improved by combining the semisynthetic penicillins with gentamicin.

Pseudomonads are responsible for 10 to 15% of gram-negative infections (1, 6, 7). Carbenicillin and gentamicin have replaced the polymyxins as the antibiotics of choice for systemic infections caused by these organisms. The marginal activity of carbenicillin against pseudomonads has necessitated the use of very large doses of this antibiotic (4). As a consequence, sodium overload (8), hypokalemia (9), and problems with coagulation (5) have been reported. Gentamicin also is known to have a narrow therapeutic-to-toxic ratio. These problems as well as the emergence of resistant *Pseudomonas* strains against both these antibiotics (2, 12) have necessitated a search for additional drugs with activity against these organisms.

Two new semisynthetic penicillins with activity against pseudomonads have been released for clinical investigation. Ticarcillin (BRL 2288) is similar to carbenicillin in structure and spectrum (13). BL-P1654 resembles ampicillin in structure and spectrum with additional antipseudomonal activity (11). This in vitro study compares these two antibiotics with carbenicillin, alone and in combination with gentamicin against *Pseudomonas aeruginosa*.

MATERIALS AND METHODS

The University of Maryland Hospital, Baltimore VA Hospital, and the Baltimore Cancer Research

Center supplied 89 clinical isolates of *P. aeruginosa*. Some of these isolates were selected because they were resistant to carbenicillin or gentamicin or both by routine disk susceptibility testing.

Cultures were maintained on Trypticase soy agar (BBL) at -70°C until studied. Each isolate was plated onto blood agar and passed twice in Trypticase soy broth (BBL) prior to testing to insure purity and luxuriant growth.

Stock antibiotic solutions were prepared freshly each day in concentrations of 1,000 $\mu\text{g}/\text{ml}$ for ticarcillin and BL-P1654 and 25,000 $\mu\text{g}/\text{ml}$ for carbenicillin. All comparative studies were performed in parallel in Trypticase soy broth.

The minimal inhibitory concentration (MIC) for each antibiotic was determined by the tube dilution technique. Twofold dilutions of the antibiotic were formed in a volume of 0.5 ml to which the inoculum of 0.5 ml of a 10^{-8} dilution of an overnight culture was added. The MIC was read as the lowest concentration which prevented grossly detectable growth after overnight incubation at 37°C . The minimal bactericidal concentration (MBC) was determined by subculturing the broth of clear tubes with a 0.01-ml calibrated loop onto blood agar plates and noting the lowest antibiotic concentration which resulted in the growth of less than 10 colonies.

The influence of the inoculum size on the inhibitory concentrations of each antibiotic was determined for nine strains by using inocula of 10^{-2} , 10^{-4} , and 10^{-6} dilutions of an overnight culture as well as the standard 10^{-8} dilution as described above. The results are expressed in terms of the approximate inoculum (bacteria per milliliter) resulting from these dilutions.

The rate of killing of pseudomonads by the antibiotics was determined by adding 1 ml of a 10^{-2} dilution of an overnight culture to 9 ml of broth to which the appropriate concentrations of the antibiotic or antibiotics had been added. Colony counts were performed at 4, 8, 24, and in some cases 48 h by removing 0.5 ml, diluting appropriately in 0.85% saline, subculturing into melted Trypticase soy agar, and counting the colonies after 24 h of incubation.

After 48 h of contact with 100 μg of BL-P1654 per ml, the development of resistance was measured for five isolates. The MIC of BL-P1654 was determined as previously described using 0.5 ml of a 10^{-3} dilution of the surviving bacteria as the inoculum. Simultaneously, the MIC of BL-P1654 against the respective strains which had not been previously subjected to the antibiotic were performed using the same dilution of an overnight culture as the inoculum. After 24 h of incubation, the broth was filtered to remove the surviving bacteria and assayed for BL-P1654 by a modified agar well diffusion method described by Bennett et al. (3), using *P. aeruginosa* ATCC 10701 as the assay organism.

The effect of gentamicin on the inhibitory activity of each of the penicillin derivatives was observed using a microtiter variation of the checkerboard technique as previously described (10). Briefly, twofold dilutions of the penicillin derivatives in 0.05 ml were made in one direction using the automated microtiter dilutor. Twofold dilutions of gentamicin in the same volume were added manually in the perpendicular direction using a disposable pipette delivering 0.05 ml per drop. Where each of the antibiotics was used alone, 0.05 ml of broth was added to achieve a volume of 0.1 ml. The inoculum of 0.1 ml of a 10^{-3} dilution of an overnight culture was added to each well by delivering 2 drops from a 0.05-ml drop disposable pipette. The plate was sealed with plastic tape and gently agitated. A small hole was punched in the tape over each well with a 26-gauge needle to allow aerobic conditions. Inhibitory end points were determined after overnight incubation at 37 C by noting the lowest concentration of antibiotics alone and in combination which allowed no visible growth.

The bactericidal effect of the antibiotic combinations was observed by using twofold dilutions of each penicillin alone and in combination with 1.2 μg of gentamicin per ml in 1 ml of broth. One tube contained 1.2 μg of gentamicin per ml alone. The same volume of a 10^{-3} dilution of an overnight culture served as the inoculum. After overnight incubation, the clear tubes were subcultured onto blood agar plates using a 0.01-ml calibrated loop; the MBC was defined as the lowest concentration of each penicillin alone and with gentamicin which resulted in the growth of less than 10 colonies.

RESULTS

The in vitro activities of carbenicillin, ticarcillin, and BL-P1654 are shown in Table 1. In general, ticarcillin was twice as active and BL-P1654 was eight to 16 times as active as carbenicillin. No isolate was more susceptible to

TABLE 1. Comparative MICs of the three semisynthetic penicillin derivatives against 89 clinical isolates of *P. aeruginosa*

Antibiotic	Number of isolates with an MIC ($\mu\text{g}/\text{ml}$) of:							
	≤ 3.1	6.2	12.5	25	50	100	200	≥ 400
Carbenicillin	1	0	0	0	22	42	17	7
Ticarcillin	1	0	1	21	43	16	4	3
BL-P1654	3	26	37	7	8	2	4	2

carbenicillin than to ticarcillin, and only one isolate was more susceptible to ticarcillin than to BL-P1654. Carbenicillin (100 $\mu\text{g}/\text{ml}$) inhibited 73% of the isolates whereas ticarcillin at this concentration inhibited 92%. BL-P1654 in the same concentrations inhibited 93% of strains but 82% were inhibited by 25 $\mu\text{g}/\text{ml}$.

The cross resistance between ticarcillin, BL-P1654, and carbenicillin is shown in Table 2. Those isolates susceptible to (MIC ≤ 100) or moderately resistant (MIC = 200) to carbenicillin were, with one exception, susceptible to 100 μg or less of either ticarcillin or BL-P1654 per ml. However, the more highly carbenicillin-resistant strains (MIC ≥ 400 $\mu\text{g}/\text{ml}$) were generally resistant to 100 μg of both ticarcillin and BL-P1654 per ml.

Varying the number of bacteria in the inoculum markedly altered the MIC for all three penicillin derivatives and produced the greatest effect upon the MIC of BL-P1654 (Fig. 1). Strains were susceptible to 6.2 μg or less of this antibiotic per ml with an inoculum of approximately 10^8 bacteria per ml, whereas they were resistant to 100 $\mu\text{g}/\text{ml}$ when 10^7 bacteria per ml was used as the inoculum. Similar results were obtained when ticarcillin and carbenicillin were tested with the high inoculum.

The MBC of each antibiotic for nine isolates is shown in Fig. 2. In most cases, the MBC was the same or one tube higher than the MIC when carbenicillin and ticarcillin were tested. However, for BL-P1654, bactericidal concentrations were 16- to 64-fold higher than the inhibitory concentrations for all strains. The great discrepancy between inhibitory and bactericidal ability of BL-P1654 prompted an examination of the comparative rates of killing by the antibiotics; the killing of one strain by 100 and 200 μg of each penicillin per ml is seen in Fig. 3. Both concentrations of ticarcillin and 200 μg of carbenicillin per ml killed completely, but even 200 μg of BL-P1654 was not sufficient to prevent regrowth of organisms. The rate of killing of five additional strains of *Pseudomonas* by 100 μg of BL-P1654 per ml was similar, i.e., initial killing of one to two logs of bacteria within the first 8 h

TABLE 2. Cross resistance between ticarcillin, BL-P1654, and carbenicillin for 89 isolates of *P. aeruginosa*

Carbenicillin MIC	No. of isolates	Ticarcillin MIC ($\mu\text{g/ml}$)					BL-P1654 MIC ($\mu\text{g/ml}$)				
		≤ 25	50	100	200	≥ 400	≤ 25	50	100	200	≥ 400
≤ 100	65	21	40	4	0	0	61	4	0	0	0
200	17	2	3	11	1	0	12	2	2	1	0
≥ 400	7	0	0	1	3	3	0	2	0	3	2

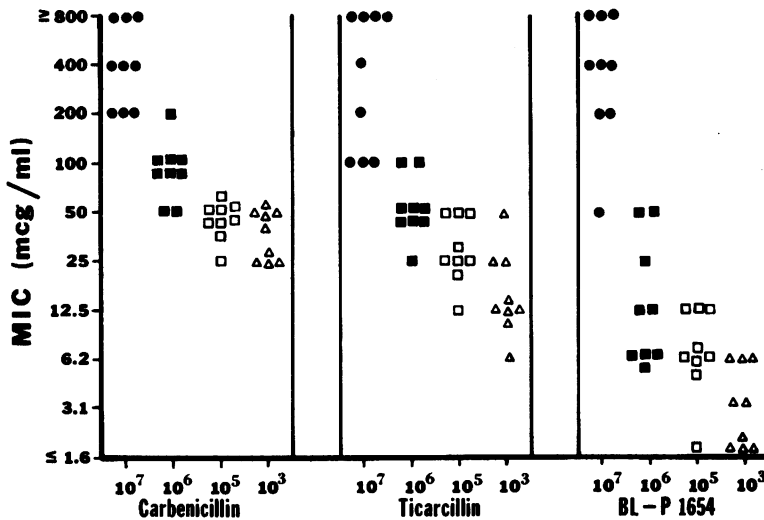


FIG. 1. Effect of varying the inoculum size (indicated on the horizontal axis as the logs of bacteria per milliliter) on MIC for the three semisynthetic penicillins against *P. aeruginosa*. The standard inoculum contained approximately 10^6 bacteria/ml.

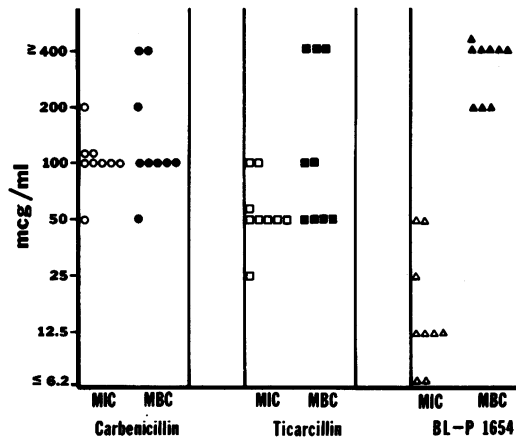


FIG. 2. Comparative MICs and MBCs for the three semisynthetic penicillin derivatives against nine isolates of *P. aeruginosa*.

and, thereafter, regrowth of organisms. By 48 h, gross cloudiness of the broth was seen in most cases. Less than 25% of the BL-P1654 was lost after 24 h of incubation with the bacteria; therefore, antibiotic deterioration did not account for the regrowth of organisms.

When the inhibitory concentrations were determined on those organisms exposed to BL-P1654 for 48 h, invariably more resistance was seen as compared with organisms incubated in the absence of antibiotic; for 4 of 5 isolates there was at least a fourfold increase in the MIC.

The combination of each of the semisynthetic penicillins with gentamicin markedly enhanced inhibition for the majority of strains. Figure 4 compares the MICs of each of the penicillin derivatives alone to those obtained by adding a subinhibitory concentration of 0.6 μg of gentamicin per ml. Carbenicillin alone or with gentamicin was the least active of the penicillins; BL-P1654 alone and in combination with gentamicin was the most active. Thus, 75% of the isolates were inhibited by 3.1 μg of BL-P1654 per ml, 12.5 μg of ticarcillin per ml and 25 μg of carbenicillin per ml when in combination with 0.6 μg of gentamicin per ml.

The increased inhibitory effect obtained by the semisynthetic penicillin-gentamicin combination in concentrations usually achieved clinically was demonstrated for almost identical numbers of isolates regardless of which penicil-

lin was in the combination. Using concentrations of 100 $\mu\text{g/ml}$ or less for carbenicillin and 5 $\mu\text{g/ml}$ or less of gentamicin, 74 of the 89 isolates were inhibited by lower concentrations of the antibiotics in combination than when the most effective of the drugs was used singly. An additional isolate met this criterion when the ticarcillin-gentamicin and BL-P-gentamicin combinations were evaluated at these concentrations. The remaining isolates were inhibited no better by the antibiotic combination than by the most effective antibiotic alone. Improvement of inhibition by the combination was correlated with susceptibility to gentamicin as

seen in Table 3. All of the isolates which were inhibited by lower concentrations of the antibiotics in combination were susceptible to 40 μg or less of gentamicin per ml. Conversely, those whose inhibition was not improved by combining the drugs were with few exceptions highly resistant to gentamicin ($\geq 80 \mu\text{g/ml}$).

The bactericidal as well as the inhibitory capacity for the penicillins was enhanced by the addition of gentamicin. This is illustrated for 21 isolates which were relatively susceptible to gentamicin in Fig. 5. The addition of 1.2 μg of gentamicin per ml lowered the concentration of BL-P1654 necessary for bactericidal activity to

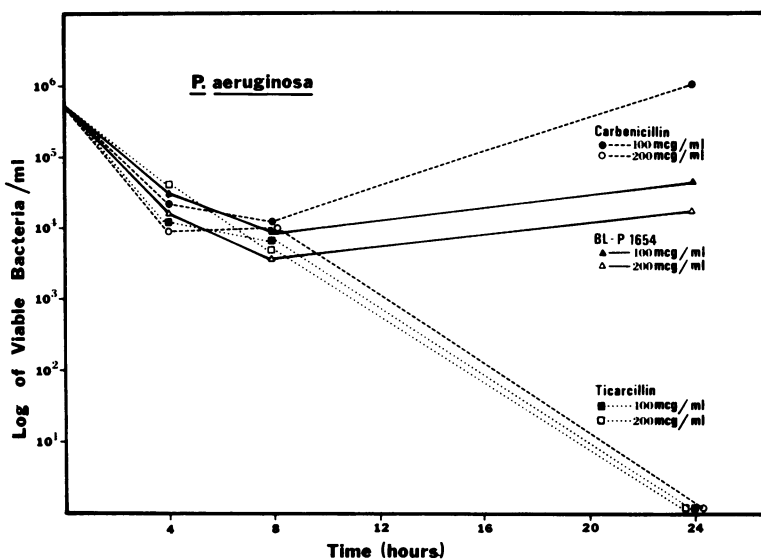


FIG. 3. The comparative rate of killing of one strain of *P. aeruginosa* by 100- $\mu\text{g/ml}$ and 200- $\mu\text{g/ml}$ concentrations of the three semisynthetic penicillins.

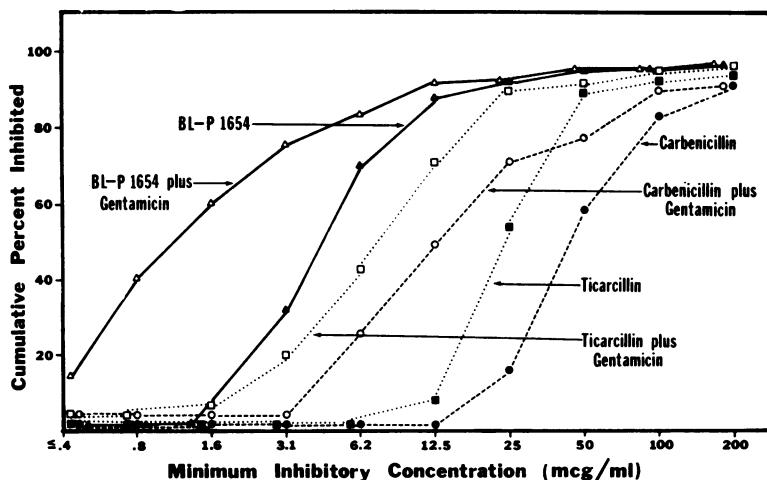


FIG. 4. Comparison of MICs for the three penicillin derivatives alone and in combination with a subinhibitory concentration of 0.6 μg of gentamicin per ml against 89 isolates of *P. aeruginosa*.

TABLE 3. The relationship of the isolates gentamicin susceptibility to the effect produced by therapeutic concentrations of the three penicillin-gentamicin combinations

Semisynthetic penicillin in combination	Increased inhibition by combination			No effect by combination		
	≤5 ^a	10 to 40	≥80	≤5	10 to 40	≥80
Carbenicillin . . .	68	6	0	0	2	13
Ticarillin	67	8	0	0	1	13
BL-P1654	68	7	0	0	1	13

^a Gentamicin MIC in micrograms per milliliter.

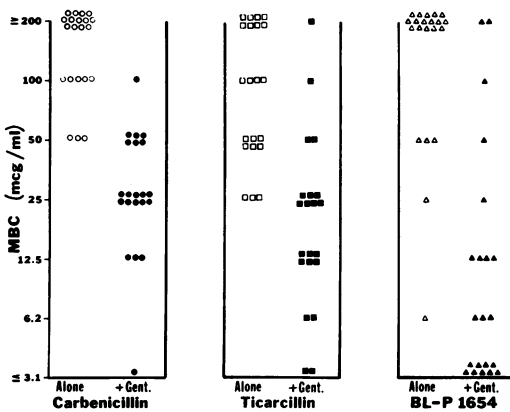


FIG. 5. A comparison of MBCs for each of the three semisynthetic penicillins alone and in combination with 1.2 µg of gentamicin per ml for 21 *P. aeruginosa* isolates.

12.5 µg/ml or less for all but five of the 21 isolates tested. Ticarcillin was the next most active and carbenicillin the least active of the three antibiotics. The MBC for gentamicin alone was at least 5 µg/ml for all but one of these isolates.

DISCUSSION

Serum levels obtained with ticarcillin are comparable to those obtained with carbenicillin (R. D. Libke, J. T. Clarke, E. D. Ralph, R. P. Luthy, and W. M. M. Kirby, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 14th, San Francisco, Calif., Abstr. 405, 1974). This study demonstrates that, in vitro, ticarcillin has twice the activity of carbenicillin against *P. aeruginosa*. Continued clinical evaluation of this antibiotic alone and in combination with gentamicin is clearly indicated.

The effect of BL-P1654 on *P. aeruginosa* is markedly altered by the inoculum size and only slightly bactericidal. Resistance to the antibiotic rapidly develops in vitro. These properties

suggest that this antibiotic, when used alone, may not be as effective clinically as the inhibitory concentrations would indicate. The addition of gentamicin increased the bactericidal as well as the inhibitory capacity of all three of the semisynthetic penicillins against those strains which were not highly resistant to gentamicin. The BL-P1654-gentamicin combination was the most active against the majority of *Pseudomonas* isolates and, therefore, may offer a potential advantage which must be assessed by careful clinical trials.

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