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# In Vitro Susceptibility of *Pseudomonas aeruginosa* to Carbenicillin and the Combination of Carbenicillin and Gentamicin

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One hundred and eleven strains of *Pseudomonas aeruginosa* isolated from clinical material were studied for susceptibility to carbenicillin. Of the strains, 80% were inhibited by 125 μg/ml or more. The combination of carbenicillin and gentamicin was shown to have inhibitory and bactericidal synergistic effect on 15 of 16 strains of *P. aeruginosa* tested. There was poor correlation between the single-disc sensitivity method and the tube dilution method.

Carbenicillin (disodium α-carboxybenzyl penicillin) is a semisynthetic penicillin active against a wide range of gram-negative bacteria (5). The antibiotic is minimally to moderately active against *Pseudomonas aeruginosa* as well (2, 5). Of strains of *P. aeruginosa* studied by British authors (5), 67% were inhibited by less than 100 μg/ml of carbenicillin. However, recent studies from the United States revealed a higher proportion of resistant strains (2, 8).

It is the purpose of this communication to report the in vitro susceptibility of 111 clinical strains of *P. aeruginosa* and its mucoid variants (3), the lack of correlation between the single-disc antibiotic susceptibility testing method and the serial twofold tube dilution method, and the demonstration of synergism of the combination of carbenicillin and gentamicin against selected strains of *P. aeruginosa*.

## MATERIALS AND METHODS

**P. aeruginosa.** All strains of *P. aeruginosa* used in this study were obtained from patients, including some with cystic fibrosis, in the wards of the Cincinnati General Hospital, Cincinnati Childrens Hospital, and the Drake Memorial Hospital. They were identified in the bacteriology laboratories of the three hospitals.

**Carbenicillin and gentamicin.** Carbenicillin as a disodium salt was supplied by Beecham Pharmaceuticals, Clifton, N.J. It was dissolved in phosphate buffer (*p*H 6) and stored at 4 C. Gentamicin as gentamicin sulfate, laboratory standard, was kindly supplied by Schering Corporation, Bloomfield, N.J. It was dissolved in sterile 0.9% NaCl solution and stored at 4 C. The BBL carbenicillin antibiotic disc with a potency of 100 μg was supplied by Beecham Pharmaceuticals.

**Susceptibility testing methods.** Minimum inhibitory concentration (MIC) was determined by a standard twofold serial tube dilution method by using a total volume of 1 ml. A 0.5-ml amount of Antibiotic Medium no. 3 (AB no. 3; Difco) was pipetted into each of a series of test tubes (1.2 by 10 cm), and a 0.5-ml amount of carbenicillin solution was introduced into the first tube. The antibiotic was then serially diluted in a twofold manner. The last tube contained no carbenicillin and served as a positive control. A 0.4-ml amount of AB no. 3 was then added to each tube. Finally 0.1 ml of a $10^{-2}$ dilution of an 18-hr culture of *P. aeruginosa* grown in Trypticase Soy Broth (BBL) was inoculated into each tube to make the final concentration of the inoculum $10^{-3}$ (approximately $10^5$ colony-forming units). The MIC was read as that concentration of the antibiotic in the clear tube next to the first tube turbid with growth, after 18 hr of incubation at 37 C. From each clear tube, 0.01 ml was then subcultured onto Trypticase Soy Agar (BBL) and incubated at 37 C. The original series of tubes was incubated for an additional 18 hr at 37 C. The minimum bactericidal concentration (MBC) was determined from the 36-hour reading of the tubes. The MBC, as determined by the tube cultures, corresponded with the results of subcultures on Trypticase Soy Agar, i.e., the tubes which had no visible growth at 36 hr either were sterile or grew fewer than 25 colonies on the agar subculture.

Disc susceptibility tests were performed by the single disc method of Bauer and associates (1).

**Tests for synergism.** The test for synergism with the combination of carbenicillin and gentamicin was carried out by the broth dilution method described by Sabath et al. (7). A "checkerboard" arrangement of test tubes (1.2 by 10 cm) was used to test the effect of the paired antibiotics. Usually 60 tubes arranged in six rows of 10 each were used. A given horizontal row contained the same concentration of carbenicillin, and a given vertical row contained the same concentration

of gentamicin. Horizontal rows were prepared so that individual tubes contained twofold decreases in gentamicin concentrations. The vertical rows were prepared so that individual tubes contained twofold increases in carbenicillin concentrations. The first horizontal row contained twofold decreases in gentamicin concentrations alone to determine the MIC of this antibiotic for the organism. The 10th vertical row contained no antibiotic and served as a positive control. The inoculum added was approximately $10^5$ colony-forming units. The medium was AB no. 3. Tubes were incubated for 18 hr at 37 C and observed for visible growth. Results were plotted on an arithmetic scale as in Fig. 1. The ordinate represents increasing concentrations of carbenicillin, and the abscissa increasing concentrations of gentamicin. Each point plotted represents the inhibition of growth achieved by a given combination of antibiotics as read at 18 hr. The resultant isobols (lines joining the points for each combination) were drawn and interpreted in the usual fashion (7).

## RESULTS

The in vitro susceptibility of *P. aeruginosa* to carbenicillin is graphically depicted in Fig. 2. Of the organisms tested in this study, 20% were inhibited by 62.5 μg/ml or less. Another 25% were inhibited by 125 μg/ml.

The amount of antibiotic required to produce a bactericidal effect was higher. Of the organisms, 7% showed no growth at 36 hr in the presence of 62.5 μg/ml of carbenicillin. On the other hand, 20% grew in 500 μg/ml of the antibiotic. Those strains which were identified as the mucoid type had the same pattern of susceptibility to carbenicillin.

The MIC of ampicillin against 12 strains susceptible to 125 μg/ml of carbenicillin or less was determined. All 12 strains required 500 μg/ml or more of ampicillin to inhibit growth.

The effect of inoculum size upon the MIC was investigated in 15 strains (Table 1). The MIC of carbenicillin for these organisms ranged from 31.2 to 250 μg/ml when the inoculum was $10^5$

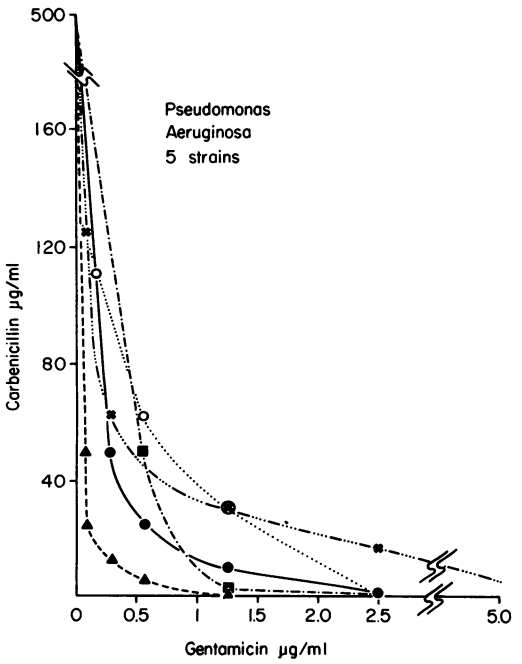


FIG. 1. *Isobolograms showing synergism between carbenicillin and gentamicin on five strains of Pseudomonas aeruginosa.*

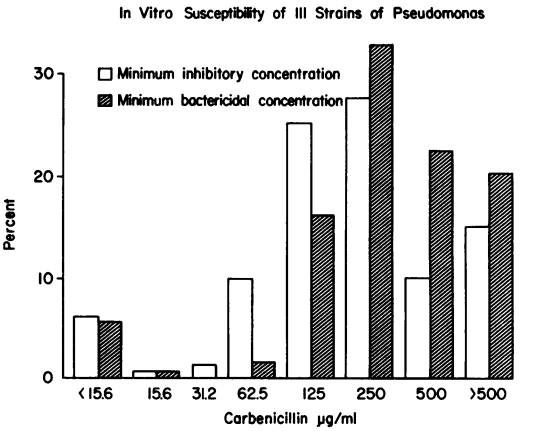


FIG. 2. *In vitro susceptibility of 111 strains of Pseudomonas aeruginosa to carbenicillin as determined by the tube dilution method.*

TABLE 1. *Minimum inhibitory concentration of carbenicillin for P. aeruginosa with varying inoculum size*

| Strain | Inoculum | | |
|---|---|---|---|
| | $10^{5a}$ | $10^4$ | $10^3$ |
| Mo | 31.2 | 31.2 | 31.2 |
| De | 125.0 | 125.0 | 125.0 |
| Pe | 62.5 | 31.2 | 31.2 |
| Br | 62.5 | 62.5 | 62.5 |
| Fi | 62.5 | 125.0 | 62.5 |
| Sa | 125.0 | 62.5 | 62.5 |
| Ro | 125.0 | 31.2 | 31.2 |
| Gu | 250.0 | 125.0 | 125.0 |
| Gr | 62.5 | 62.5 | 31.2 |
| Ki | 125.0 | 62.5 | 62.5 |
| He | 125.0 | 125.0 | 31.2 |
| Mc | 125.0 | 250.0 | 125.0 |
| V. H. | <0.45 | <0.45 | <0.45 |
| Sy | 125.0 | 62.5 | 62.5 |
| Gi | >500.0 | >500.0 | >500.0 |

[a] Colony-forming units.

TABLE 2. *Synergism between carbenicillin (25 μg/ml) and gentamicin*[a]

| Strain | CBN MIC | GMC MIC | Inhibitory concn CBN + GMC (μg/ml) | CBN MBC | GMC MBC | Bactericidal concn CBN + GMC (μg/ml) |
|---|---|---|---|---|---|---|
| 1 | >500 | 2.5 | 0.625 | >500 | 2.5 | 1.25 |
| 2 | >500 | 2.5 | 1.25 | >500 | 5.0 | 2.5 |
| 3 | 500 | 1.25 | 0.156 | 500 | 2.5 | 0.156 |
| 4 | 250 | 1.25 | 0.156 | 500 | 2.5 | 0.625 |
| 5 | 125 | 0.625 | 0.078 | 125 | 2.5 | 0.312 |
| 6 | 125 | 10 | 2.5 | 250 | 20 | 10 |
| 7 | 125 | 2.5 | 0.625 | 250 | 5 | 1.25 |
| 8 | 62.5 | 1.25 | 0.625 | 125 | 5 | 1.25 |
| 9 | 62.5 | 1.25 | 0.156 | 125 | 2.5 | 0.625 |
| 10 | 62.5 | 5 | 1.25 | 125 | >5 | 1.25 |
| 11 | 31.2 | 0.625 | <0.039 | 62.5 | 1.25 | 0.156 |

[a] Abbreviations: CBN = carbenicillin, GMC = gentamicin, MIC = minimum inhibitory concentration, MBC = minimum bactericidal concentration.

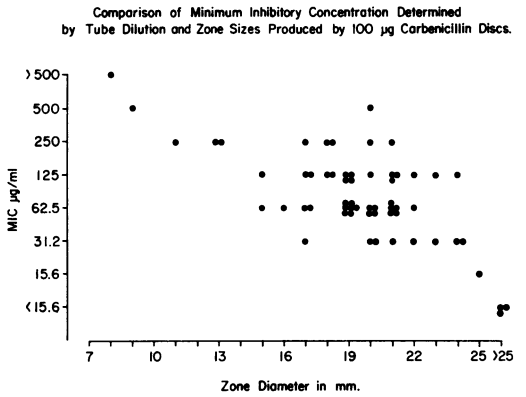


FIG. 3. *Minimum inhibitory concentration of carbenicillin for 66 strains of Pseudomonas aeruginosa determined by the tube dilution method compared to zone sizes produced by 100 μg carbenicillin disc.*

colony-forming units. The variation in growth inhibition at 18 hr and bactericidal effect at 36 hr was insignificant with smaller inocula, $10^4$ or $10^3$ colony-forming units.

Correlation of zone diameters produced by 100-μg discs with the MIC as determined by tube dilution is shown in Fig. 3. Among strains with the same MIC, the distribution of zone diameters was wide. For example, the zone diameters of strains with an MIC of 250 μg/ml varied from 11 to 21 mm, overlapping the diameters of strains with an MIC of 31.2 μg/ml, which ranged from 17 to 24 mm.

The combination of carbenicillin and gentamicin resulted in synergistic activity against 15 of 16 strains tested (Tables 2 and 3). The MIC of carbenicillin ranged from 31.2 to 500 μg/ml. The MIC of gentamicin ranged from 0.78 to 10 μg/ml.

TABLE 3. *Synergism between carbenicillin (50 μg/ml) and gentamicin*[a]

| Strain | CBN MIC | GMC MIC | Inhibitory concn. CBN + GMC (μg/ml) | CBN MBC | GMC MBC | Bactericidal concn CBN + GMC (μg/ml) |
|---|---|---|---|---|---|---|
| 12 | 250 | 1.56 | 0.4 | 500 | 3.12 | 0.78 |
| 13 | 125 | 0.78 | 0.17 | 250 | 0.78 | 0.17 |
| 14 | 125 | 5 | 1.25 | 250 | 5 | 2.5 |
| 15 | 62.5 | 2.5 | 0.625 | 500 | 2.5 | 5.0 |

[a] Abbreviations as in Table 2.

In eight strains, the combination of 25 μg/ml or less of carbenicillin (Table 2) and 0.625 μg/ml or less of gentamicin achieved inhibition of growth at 18 hr. The MBC of carbenicillin ranged from 62.5 μg/ml to more than 500 μg/ml, and the MBC of gentamicin ranged from 0.78 to 20 μg/ml. The combination of 25 μg/ml or less of carbenicillin and 0.625 μg/ml or less of gentamicin was bactericidal in five strains (Table 2). Four strains demonstrated both inhibitory and bactericidal synergism with the use of the combination of gentamicin and 50 μg/ml of carbenicillin (Table 3). In the 16th strain, no consistent synergistic effect was observed. The MIC values of gentamicin and carbenicillin for this strain were 1.25 μg/ml and 500 μg/ml, respectively. In combination with 50 μg/ml of carbenicillin, 0.625 μg/ml of gentamicin was required to achieve inhibition of growth at 18 hr, but no enhancement of bactericidal effect was noted. The graphic representation of the effect of the combination of these two antibiotics upon five strains is shown in Fig. 1.

## DISCUSSION

The susceptibility of *P. aeruginosa* to carbenicillin in this study is in a range similar to that reported by Bodey and Terrell (2), and Smith and Finland (8). The level of carbenicillin required to achieve inhibition of growth of 80% of these strains is 125 μg/ml or higher, a level which is difficult to achieve in the blood even with therapeutic schedules recommended by previous investigators (5). This concentration of drug, however, is easily reached in the urine (5). There was little difference between the MIC and MBC when susceptible organisms were tested. However, more resistant organisms, as defined by MIC values, required even more antibiotic to achieve bactericidal effect. Strains sensitive to 125 μg/ml of carbenicillin were very resistant to ampicillin, a semisynthetic penicillin structurally similar to carbenicillin.

Determination of the susceptibility of *P. aeruginosa* to carbenicillin by the use of the single disc method is difficult. The reasons for this are twofold. First, the distribution of the MIC values determined by tube dilution (Fig. 2) closely resembles a normal distribution curve rather than the bimodal pattern demonstrated with other antibiotics (6). Second, the wide scatter of zone diameters (Fig. 3) obscures any obvious distinction between sensitive and resistant organisms. The lack of consistent suppression of growth of *P. aeruginosa* by the 100-μg carbenicillin disc has been noted previously (8), and there is no apparent explanation for this phenomenon.

The in vitro synergism of the combination of gentamicin and carbenicillin against *P. aeruginosa* was demonstrated. Concentrations of both antibiotics required to achieve inhibition of growth or bacterial killing are easily achieved in the blood (1.25 μg/ml of gentamicin and 25 μg/ml of carbenicillin). The results of a preliminary investigation in our laboratory could possibly explain the mechanism of synergism. We have successfully induced spheroplasts of *P. aeruginosa* by carbenicillin (10). One clinical strain of *P. aeruginosa*, with an MIC of 10 μg/ml of gentamicin, was converted to an unstable spheroplast by carbenicillin. The growth of the spheroplasts was inhibited by 0.625 μg/ml of gentamicin (*unpublished data*). The organism was obviously more susceptible to gentamicin after its cell wall synthesis was inhibited by carbenicillin. Spheroplasts of *Staphylococcus aureus* have also been shown to be more sensitive than their parent forms to antibiotics which inhibit protein synthesis (9). Other possible explanations for the mechanism of synergism between antibiotics were reviewed by Jawetz (4).

The combination of carbenicillin and gentamicin may prove to be useful in the treatment of infections due to *P. aeruginosa*. Testing of individual organisms would be necessary, since an antibiotic combination which is generally synergistic against all strains of the same organism has not been found (4). This is illustrated by the failure to demonstrate synergism between gentamicin and carbenicillin at the concentrations studied in one of the 16 strains of *P. aeruginosa* tested. This organism was very resistant to carbenicillin (MIC > 500 μg/ml), which may account for the failure to demonstrate synergism when a relatively low concentration (50 μg/ml) of carbenicillin was used in combination with gentamicin.

When combination of carbenicillin and gentamicin is demonstrated in vitro, it would be possible to use lower doses of gentamicin, a drug with ototoxic and nephrotoxic potential. This would be of especial value in neonates, the elderly, and patients with renal insufficiency. It would also enable the use of lower doses of carbenicillin, since the serum level of 25 μg/ml is achievable with intramuscular injection of 1 g of carbenicillin (5).

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