Susceptibility of *Pseudomonas aeruginosa* to Carbenicillin

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Ninety clinical isolates of *Pseudomonas aeruginosa* were examined for susceptibility to carbenicillin by the broth dilution and disc diffusion methods. Inhibition zone diameters varied at given minimal inhibitory concentration levels of the antibiotic. Nevertheless, the results obtained allowed the proposal of the following tentative criteria for the interpretation of inhibition zones. *Pseudomonadaceae* yielding zones of inhibition measuring at least 10 and 16 mm in diameter around 25- and 100-µg discs, respectively, are sensitive to this antibiotic when examined by the standardized Bauer-Kirby method of disc susceptibility testing. Isolates characterized by zones of less than 100 mm in diameter around 25-µg discs should be tested with 100-µg discs before they are reported as resistant or resistant to carbenicillin.

Carbenicillin, a semisynthetic, penicillinase-susceptible penicillin (carboxybenzyl penicillin), has been shown to be active against a variety of *Enterobacteriaceae* and *Pseudomonadaceae* commonly resistant to other penicillins (1, 3–7, 11, 12). Most investigators employ bacterial inocula in the stationary phase of growth for disc diffusion, broth dilution, or agar dilution, or all three tests, although penicillins are more active against organisms in the logarithmic phase of growth. It is also known that various sizes of bacterial inoculum drastically affect the activity of carbenicillin (13).

The present study was undertaken in an effort to determine tentative criteria for the interpretation of the sizes of inhibition zones obtained with 25- and 100-µg carbenicillin discs against clinical isolates of *Pseudomonas aeruginosa*, employing a standardized disc diffusion method and bacterial inocula in the logarithmic phase of growth.

**MATERIALS AND METHODS**

**Bacteria.** Ninety isolates of *P. aeruginosa* from various clinical sources were characterized through the oxidase reaction; the production of pigments or a metallic sheen, or both; gelatin liquefaction; and the oxidative metabolism of carbohydrates.

**Media.** Mueller-Hinton broth (MHB) and agar (MHA) were purchased from Difco. The organisms under study were maintained on Brain Heart Infusion agar slants (Difco).

**Carbenicillin.** Carbenicillin powder (batch A0026MA) was a gift from Beecham Pharmaceuticals, Clifton, N.J., as were BBL 25- and 100-µg carbenicillin discs (batches 801059 and 810045, respectively). The antibiotic was dissolved in sterile distilled water to an activity of 10,000 µg/ml, passed through membrane filters (Millipore Corp., 0.22 µm), and dispensed in 2-ml portions into sterile screw-capped vials which were frozen and kept stored at −65°C. The vials were never reused after thawing.

**Sensitivity tests.** The isolates, including a laboratory control strain of *Escherichia coli* of known antibiotic susceptibility, were grown in Trypticase Soy Broth (BBL) for 5 hr (logarithmic growth phase), after which the turbidity was adjusted to that of McFarland BaSO₄ standard no. 0.5 (corresponding to 1.5 × 10⁶ organisms/ml). Disc susceptibility tests were performed precisely by the technique of Bauer et al. (2).

The isolates were tested by the broth dilution sensitivity method simultaneously, utilizing the microtiter technique (Cooke Engineering Co., Alexandria, Va.). For this purpose, the standardized suspensions of organisms were diluted 50-fold further by transferring 0.2 ml of organisms to 9.8 ml of MHB (3 × 10⁶ organisms/ml). To 0.05 ml of serial twofold dilutions of carbenicillin (range, 1,000 to 1 µg/ml), 0.05 ml of bacterial inoculum was added into U-shaped wells of sterile, disposable microtiter plates; control wells received 0.05 ml of MHB and 0.05 ml of bacterial inoculum. Thus, the assay wells contained 1.5 × 10⁶ organisms/ml at zero time, and the final concentrations of carbenicillin in a log₂ dilution series ranged from 500 to 0.5 µg/ml. The microtiter and disc sensitivity plates were incubated at 35°C for 18 hr. The minimal inhibitory concentration (MIC) of carbenicillin was defined as the lowest concentration of antibiotic completely inhibiting bacterial growth as judged by visual inspection; a mirror facilitated gross readings.
RESULTS

The MIC values of carbenicillin against 90 clinical isolates of *P. aeruginosa* are listed in Table 1; 72 (80%) of the isolates were inhibited by 62.5 μg of carbenicillin per ml or less. Three strains required 250 μg/ml for inhibition, whereas 15 strains were inhibited by 125 μg of carbenicillin per ml. These data agree with those in the literature. The results obtained with the disc diffusion technique are demonstrated and plotted against the corresponding MIC values in Fig. 1; a total of 22 isolates (24.4%) yielded zones of inhibition measuring less than 10 mm in diameter around 25-μg discs. Of these, seven strains were inhibited by 62.5 μg of carbenicillin per ml, whereas 12 isolates required 125 μg of antibiotic per ml for inhibition. None of the isolates that was inhibited by 31.2 μg of carbenicillin per ml gave zones of inhibition of less than 10 mm in diameter around 25-μg discs. The three isolates, which required 250 μg of carbenicillin per ml for inhibition, yielded no zone of inhibition around 25-μg discs, whereas one isolate was characterized by a zone of 10 mm around 100-μg discs. None of the isolates that was inhibited by 125 μg of carbenicillin per ml or less yielded zones of less than 16 mm in diameter around the 100-μg carbenicillin discs.

Of 179 additional clinical isolates of *P. aeruginosa* that had been exposed to 25-μg carbenicillin discs, a total of 35 (23.4%) yielded zones of less than 10 mm in diameter, whereas 144 (75.7%) gave zones of greater than 10 mm.

**DISCUSSION**

Isolates of *P. aeruginosa* were tested for susceptibility to carbenicillin while in the logarithmic phase of growth. Routinely, clinical isolates are incubated for several hours in a suitable broth, after which they are tested by the disc susceptibility method. Furthermore, it is well known that penicillins are less active against organisms in the stationary phase of growth. It should also be stressed that the same medium without and with agar should be utilized for correlative broth dilution and disc susceptibility tests. This is why MHB and MHA were employed in this study.

Phair and co-workers (9) noted that a significant number of their *P. aeruginosa* isolates gave a wide scatter of zone diameters at given MIC levels of carbenicillin. This is also apparent from our data, with both the 25- and 100-μg discs. Fairly often, gross inspection of primary culture plates after overnight incubation fails to discriminate between non-lactose-fermenting *Enterobacteriaceae* and atypically appearing colonies of *P. aeruginosa* and other *Pseudomonadaceae*. If one were to follow the recommendation of Beecham Pharmaceuticals (P. J. Wise, personal communication), namely to employ 25-μg carbenicillin discs for *Enterobacteriaceae* and 100-μg discs for *Pseudomonadaceae*, the clinical laboratory and, therefore, the physician in charge would lose an entire day before carbenicillin susceptibility results would be available. Therefore, the following tentative criteria are proposed for the interpretation of the disc susceptibility of *Pseudomonadaceae* to carbenicillin. Typical colonies of *P. aeruginosa* are tested by the disc sensitivity method with 100-μg carbenicillin discs; those isolates that yield inhibition zones measuring less than 16 mm in diameter are reported as resistant to the antibiotic. Atypically appearing colonies of *Pseudomonadaceae* are exposed to 25-μg discs. Those isolates that are characterized by zones of inhibition of less than 10 mm in diameter are subsequently tested with 100-μg discs; again, a zone of less than 16 mm in diameter is interpreted as indicative of resistance.

Several studies with regard to the pharma-

**Table 1. Susceptibility of Pseudomonas aeruginosa to carbenicillin (broth dilution technique)**

| MIC* (μg/ml) | No. of strains inhibited | Per cent | Cumulative no. of strains inhibited | Cumulative per cent |
|--------------|-------------------------|----------|-----------------------------------|---------------------|
| 15.6         | 0                       | 0        | 0                                 | 0                   |
| 31.2         | 16                      | 17.8     | 16                                | 17.8                |
| 62.5         | 56                      | 62.2     | 72                                | 80.0                |
| 125          | 15                      | 16.7     | 87                                | 96.7                |
| 250          | 3                       | 3.3      | 90                                | 100                 |
| 500          | 0                       | 0        |                                    |                     |

*Minimal inhibitory concentration.*
Cology of carbenicillin in humans have established that intravenous administration of the antibiotic leads to high peak blood and, especially, urine levels, thus rendering isolates of *P. aeruginosa* that require as much as 125 μg of carbenicillin per ml for inhibition amenable to appropriate chemotherapy (8, 10, 13).

**ACKNOWLEDGMENT**

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**ADDENDUM IN PROOF**

Meanwhile, 50-μg carbenicillin discs have become available; those *P. aeruginosa* isolates that required 250 μg of carbenicillin per ml for inhibition yielded zones measuring 6 and 7 mm in diameter, whereas isolates inhibited by 125 μg of carbenicillin per ml or less gave inhibition zones of 14 mm or more in diameter.

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ERRATUM

Rapid Semiquantitative Method for Screening Large Numbers of Virus Samples by Negative Staining Electron Microscopy

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Volume 20, no. 2, page 261, column 1, line 1: Change “staining” to “starting.”

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Volume 20, no. 4, page 630, abstract: The last sentence should read as follows: “Isolates characterized by zones of less than 10 mm in diameter around 25-µg discs should be tested with 100-µg discs before they are reported as sensitive or resistant to carbenicillin.”