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# Comparison and Evaluation of Carbenicillin Disks in Diffusion Susceptibility Testing

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A broad variety of bacterial strains, including 79 *Pseudomonas aeruginosa*, were studied in an in vitro evaluation of carbenicillin disk susceptibility testing. Regression analysis with both 50-μg and 100-μg carbenicillin disks was carried out. Organisms having minimal inhibitory concentration values of 100 to 200 μg/ml demonstrated zones of less than 11 mm with the 50-μg disk, resulting in very little opportunity for appropriate discrimination of results. The line of regression for the 50-μg disk intersected the ordinate at a point just above the minimal inhibitory concentration value considered to be the limit of intermediate susceptibility for *Pseudomonas*. These considerations, together with evidence of greater disk content variation in the 50-μg than in the 100-μg disks assayed, considerable manufacturer-to-manufacturer variability with the 50-μg disk, and the more appropriate performance of the 100-μg disk, lead us to conclude that the 100-μg disk better serves the clinical test requirements for this agent than does the 50-μg disk, which is currently the only disk available for laboratory testing.

Carbenicillin (disodium-alpha-carboxybenzyl penicillin), although a relatively new antimicrobial agent, has become very much a part of the antibiotic armamentarium of the physician treating infections caused by *Pseudomonas aeruginosa*, *Proteus* species, *Providencia*, and, to a lesser extent, strains of *Enterobacter* and *Escherichia coli* resistant to other antibiotics (3, 12, 16).

Susceptibility testing is now carried out in most diagnostic microbiology laboratories on a routine basis with carbenicillin. Currently, only the 50-μg disk is approved for carbenicillin testing by the Food and Drug Administration (6, 9). Previously work was done with disks of varied antibiotic content (10-12, 17, 20, 21). However, subsequent to the advent of the Food and Drug Administration decision, a few in vitro studies were published which contain 50-μg disk data (16, 19, 22, 23).

The study reported here was undertaken due to an ongoing awareness in our laboratory that organisms having minimal inhibitory concentration (MIC) values of 100 to 200 μg of carbenicillin per ml may demonstrate zones of 12 mm or less (interpreted as resistant) with the 50-μg disk. These strains include those which, by MIC values, are considered susceptible or intermediately susceptible when approaching *Pseudomonas* infections therapeutically with carbenicillin. Carbenicillin presents unique laboratory testing problems. It has a wide range of activity, relatively high dosage requirements in the therapy of *Pseudomonas* infections, and requires an appropriately discriminating disk content for the testing of these higher MIC values. Therefore, an evaluation was undertaken to assess the extent of the possible limitations of the 50-μg carbenicillin disk and to ascertain if a 100-μg disk might resolve these limitations.

## MATERIALS AND METHODS

Two hundred sixty-eight strains of bacteria, with representatives of 15 different species, were analyzed for study purposes. Included were 79 strains of *P. aeruginosa*. All organisms were clinical isolates from the University of Minnesota Clinical Microbiology Laboratories and were identified by standard laboratory means (2, 7).

For antibiotic susceptibility testing, the inoculum from a single colony of each strain was incubated overnight at 35 C in Trypticase soy broth (BBL) (soybean-casein digest medium, USP). This culture suspension was then standardized against an 0.5 MacFarland opacity standard (0.5 ml of 1% $BaCl_2$ plus 99.5 ml of 1% $H_2SO_4$ [0.36 N]).

The disk diffusion method employed was a slight modification of the method of Bauer et al. (1). The modification incorporated was the use of a single colony for inoculum in this research setting, rather than the use of 3 to 10 colonies as practiced in the

routine clinical laboratory setting to detect culture heterogeneity. A swab was placed in the standardized suspension described above, rotated against the sides of the tubes to remove excess fluid, and then streaked in three directions over the surface of Mueller-Hinton agar plates (BBL) having an agar depth of 4 to 5 mm. Two strengths of commercially prepared disks (BBL) (50, 100 $\mu$g) were employed for the regression graphs included here. For comparison studies, the 50-$\mu$g disks of three commercial manufacturers were utilized (BBL, Difco, Pfizer). Two agar plates were used for each strain tested. After 18 h of incubation, zone sizes were determined by two different individuals. The four values thus obtained were recorded for each strain for each disk strength of the carbenicillin disks. These four values were then averaged to obtain the value reported.

A $10^{-2}$ dilution of the standardized suspension was inoculated onto Mueller-Hinton agar (BBL) by means of the replicating device of Steers, Foltz, and Graves for agar dilution studies (19). The inoculum delivered was approximately $10^4$ organisms. Agar dilution studies on the swarming strains of *Proteus* were carried out with clear plastic tubular rings. Readings were taken after 18 h of incubation at 35 C. A barely visible haze or a single colony was disregarded in reading the end point, as described in the method employed above (8).

Diffusion zone values for each disk strength and the MIC readings from the agar dilution studies were plotted on scattergrams. Regression calculations were made by the formula of least squares as adapted for computer computation.

A control organism (*E. coli* ATCC #25922) was included each time a replicator plate was used and each time diffusion testing was done. A sample of the commercial disks used was assayed both by a dilution technique, in which the disks were eluted and compared by broth assay with known standards, and by a comparison of inhibition zones obtained with the commercially prepared disks and with disks prepared in our laboratory with a carefully measured amount of antibiotic.

Disks were prepared in our laboratory in 10-$\mu$g increments resulting in 15 concentrations ranging from 10 to 150 $\mu$g. Each disk content was tested 10 times each against a strain of *E. coli* (ATCC #25922), *P. aeruginosa*, and *Staphylococcus aureus* (ATCC #25923), again by the standardized disk susceptibility method described above. The readings from the replicated studies done were averaged for each of the three organisms to give a value that could be used in plotting a standard curve (4, 8, 14).

Organism counts in representative samples of the standardized suspension were carried out by the method of Miles and Misra (5). Log dilutions of the five *E. coli* and five *P. aeruginosa* standardized suspensions thus tested were made to determine the effect of inoculum variation on disk diffusion and agar dilution results.

Medium composition and method of preparing the standard inoculum were examined to determine their effects on the reproducibility of results for any given batch of disks.

Media from four manufacturers (BBL Division of Bioquest, Difco Laboratories, Mogul Diagnostics, Pfizer Diagnostics) were tested using susceptible strains of *E. coli* and *P. aeruginosa* and both 50- and 100-$\mu$g disks. Five plates of each medium were streaked with each organism, and four disks of each content were placed randomly on each plate.

The method of preparing the standard inoculum was varied by using a standardized light source and background black lines for matching the MacFarland standard (the routine procedure) for one inoculum and by using ordinary laboratory lighting and background for the second. Five strains each of *E. coli* and *P. aeruginosa* were standardized by both methods and streaked by the standard method onto Mueller-Hinton agar plates. Four disks of each content were placed randomly on each seeded plate.

Values obtained from the routine susceptibility testing of 669 strains of *P. aeruginosa* with the 50-$\mu$g disk were tabulated to determine the population distribution of zone sizes of consecutive strains tested in the daily routine of the University of Minnesota Hospitals Diagnostic Microbiology Laboratories.

## RESULTS

Table 1 summarizes the agar dilution MIC values for organisms tested with carbenicillin. The results demonstrate the broad range of organism susceptibility among both gram-negative and gram-positive strains. Of particular importance are the *P. aeruginosa* strains which demonstrated a clustering of values in the 12.5 to 100 $\mu$g/ml range. The strains analyzed from among the four *Proteus* species and from *Providencia* and *E. coli* represented, on the whole, much lower MIC values than the *P. aeruginosa*. The *Enterobacter* strains demonstrate a biphasic distribution of organisms, whereas the *Klebsiella* strains were all rather resistant when compared to the other *Enterobacteriaceae* tested. Results with the gram-positive cocci were varied, with group A beta hemolytic streptococci and *Diplococcus pneumoniae* being most susceptible.

Figures 1 and 2 represent the regression graphs (scattergrams) for the 50- and 100-$\mu$g carbenicillin disks, respectively. The MIC values are represented on the ordinate by a log scale, whereas the zone sizes in millimeters are represented on the abscissa. The regression line is shown and the mathematic analysis of these two figures is assembled in Table 2.

Figure 3 represents a comparison of the scattergram plots of selected organisms, for both the 50- and 100-$\mu$g disks, as superimposed to allow visual evaluation of zone size and MIC relationships.

Table 3 outlines the results of bacterial counts made from the bacterial suspensions standardized with the turbidity standards de-

TABLE 1. *Carbenicillin agar dilution studies*

| Organism | No. of strains | MIC (μg/ml) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.2 | 0.4 | 0.8 | 1.6 | 3.1 | 6.3 | 12.5 | 25 | 50 | 100 | 200 | 400 | 800 | >800 |
| *Pseudomonas aeruginosa* | 79 | 1 | 1 | 1 | | | 1 | 19 | 19 | 24 | 6 | 2 | 2 | 2 | 1 |
| *Proteus mirabilis* | 7 | | 2 | 3 | 2 | | | | | | | | | | |
| *Proteus vulgaris* | 7 | | | 2 | 2 | | 2 | | | | 1 | | | | |
| *Proteus rettgeri* | 8 | | 2 | 1 | | 2 | 1 | 1 | | | | | 1 | | |
| *Proteus morganii* | 8 | | | 6 | 2 | | | | | | | | | | |
| *Providencia* | 20 | | | 8 | 6 | 1 | 2 | 1 | 2 | | | | | | |
| *Escherichia coli* | 21 | | | 3 | 6 | 9 | | | | 1 | 1 | | 1 | | |
| *Enterobacter* | 20 | | | | | 11 | 1 | | | 1 | 4 | 1 | | | 2 |
| *Klebsiella* | 34 | | | | | | | 1 | 1 | 2 | 14 | 7 | 3 | 1 | 5 |
| *Staphylococcus aureus* | 15 | 1 | 4 | 3 | 1 | | 5 | | 1 | | | | | | |
| *Staphylococcus epidermidis* | 10 | 1 | 2 | | 4 | 1 | | | 1 | | 1 | | | | |
| *Enterococcus* | 10 | | | | | | 1 | | 8 | 1 | | | | | |
| *Diplococcus pneumoniae* | 10 | 3 | 7 | | | | | | | | | | | | |
| *Group A beta hemolytic streptococci* | 9 | 6 | 3 | | | | | | | | | | | | |
| *Beta hemolytic streptococci, non-group A* | 10 | 2 | | 3 | 2 | 3 | | | | | | | | | |

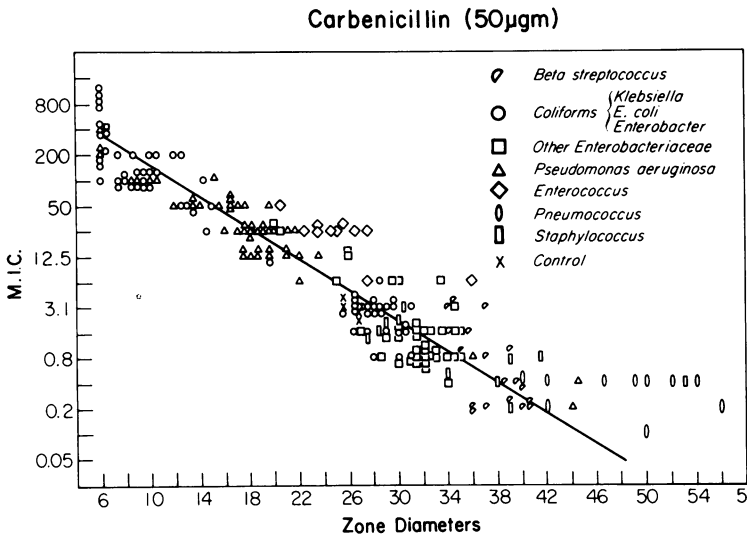


FIG. 1. *Regression graph for carbenicillin obtained by testing with the 50-μg disk.*

scribed earlier. Counts of cultures standardized in this manner routinely reveal numbers between $3 \times 10^7$ and $1 \times 10^8$ colony-forming units (CFU)/ml for most coccal and bacillary organisms. However, as demonstrated in Table 3, *P. aeruginosa* regularly produced counts in the range of $1 \times 10^8$ to $4 \times 10^8$ CFU/ml.

Varying the inoculum by a one-log dilution factor showed the greatest effect to be that of varying the inoculum in the susceptibility tests from $10^8$ to $10^7$ CFU/ml. This particular numerical alteration is of the kind which may be encountered in the preparation of a standard inoculum. The mean zone size difference with this inoculum change was 1.8 mm for both *E. coli* and *P. aeruginosa* with the range being 0.6 to 4.0 mm and 0.1 to 4.1 mm, respectively. This effect was independent of disk content. Other log dilutions (CFU/ml) ($10^9$, $10^6$, $10^5$) did not show changes of the same magnitude. *E. coli* and *P. aeruginosa* were thus equally affected by reducing the inoculum 10-fold.

Changing disk contents at the standard inoculum for disk susceptibility testing demonstrated that the zones produced with *P. aeruginosa* were drastically affected by halving the disk content from 100 to 50 μg, whereas *E. coli* was only moderately affected. The differ-

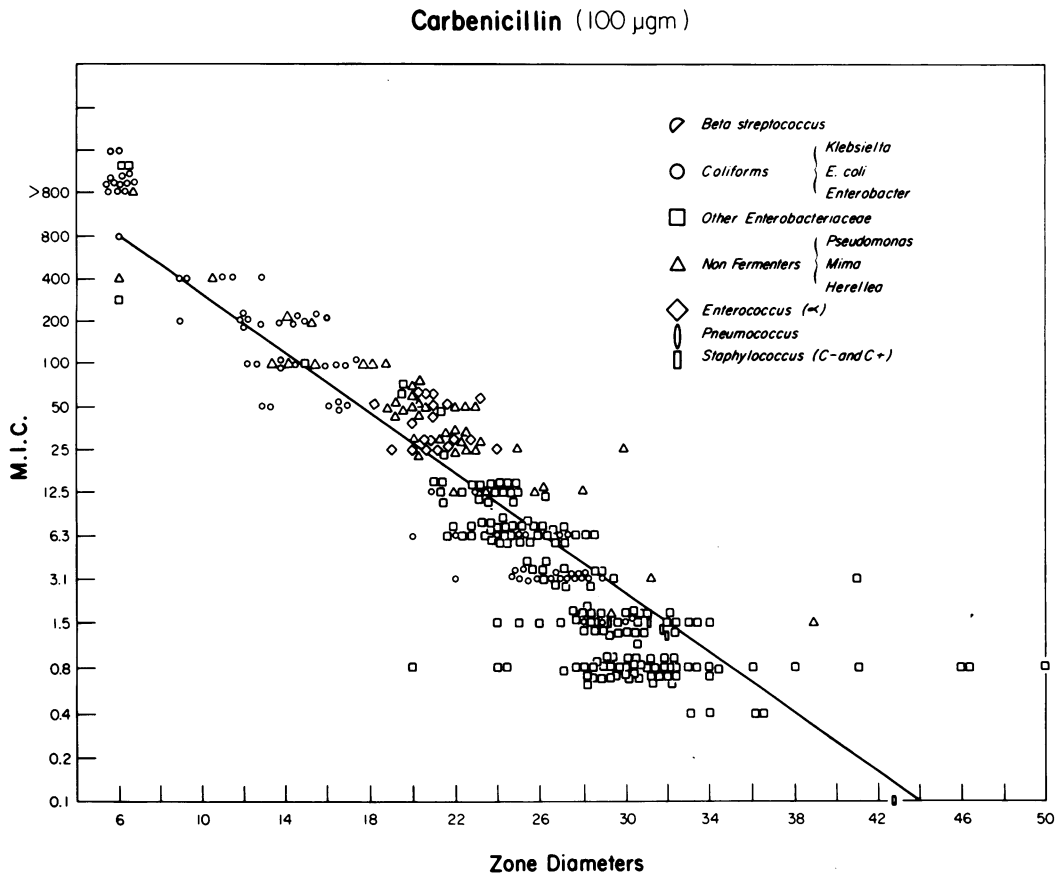


FIG. 2. *Regression graph for carbenicillin obtained by testing with the 100-µg disk.*

TABLE 2. *Carbenicillin susceptibility mathematical analysis*

| Disk content (µg) | No. of strains | Mean of X[a] | Mean of Y[b] | SD[c] of X | SD of Y | SD of Y/X | Correlation coefficient | Y intercept | Slope | SE[d] of slope | SE of Y intercept |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 50 | 253 | 25.11462 | .80187 | 10.01045 | .84816 | .34843 | −0.91209 | 2.74271 | −0.07728 | .00219 | .05926 |
| 100 | 216 | 24.53241 | .96312 | 6.15187 | .77181 | .42903 | −0.83213 | 3.52428 | −0.10440 | .00476 | .12028 |

[a] X = disk zone size in millimeters. Zone size is the independent variable in calculations.
[b] Y = minimal inhibitory concentration in micrograms per milliliter (log scale).
[c] SD, Standard deviation.
[d] SE, Standard error.

ence in zone sizes between the 100- and 50-µg disks ranged from 1.0 to 3.6 mm (mean 2.3 mm) for *E. coli* and from 4.0 to 11.6 mm (mean 7.5 mm) for *P. aeruginosa*.

Zone variations on the same strain from the same standardized suspension were determined for nine *Proteus* strains, eight *P. aeruginosa*, seven *Klebsiella*, and one *E. coli*. Readings were made with the Fisher-Lilly zone reader to the nearest 0.1 mm. Readings were made by two technologists and resulted in a mean variation of 0.5 mm for technician A for both the 50- and 100-µg disks, whereas technician B had mean reading variations of 0.6 mm with the 50-µg disk and 0.4 with the 100-µg disk. The variation from plate to plate was slightly greater with the 50-µg disks as regards both mean and range values. However, there was no statistical significance to this variability.

The results of the bioassay of carbenicillin disks are shown in Table 4. Five lots of 50-µg and four lots of 100-µg disks were assayed. The

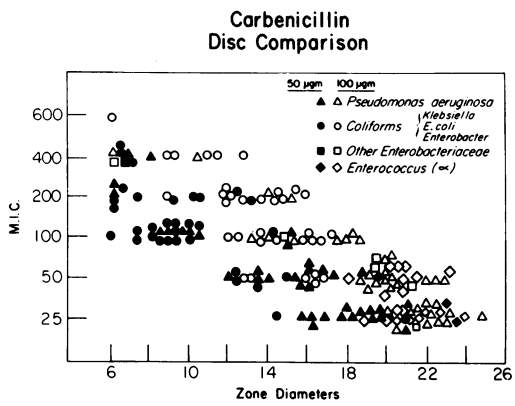


FIG. 3. *Regression graph comparison of values obtained with organisms tested simultaneously with the 50- and 100-μg carbenicillin disks.*

TABLE 3. *Sample bacterial counts from turbidity standardized inoculum*

| Organism | Bacterial count |
|---|---|
| *Escherichia coli* (ATCC #25922) | $7.04 \times 10^7$ |
| *E. coli* (Maah's) | $6.36 \times 10^7$ |
| *E. coli* #1 | $3.62 \times 10^7$ |
| *E. coli* #2 | $3.40 \times 10^7$ |
| *E. coli* #3 | $6.25 \times 10^7$ |
| *E. coli* #4 | $8.80 \times 10^7$ |
| *E. coli* #5 | $3.45 \times 10^7$ |
| *Staphylococcus aureus* (ATCC #25923) | $5.01 \times 10^7$ |
| *S. aureus* ("C") | $5.44 \times 10^7$ |
| *Pseudomonas aeruginosa* #1 | $2.76 \times 10^8$ |
| *P. aeruginosa* #2 | $2.58 \times 10^8$ |
| *P. aeruginosa* #3 | $2.48 \times 10^8$ |
| *P. aeruginosa* #4 | $2.44 \times 10^8$ |
| *P. aeruginosa* #5 | $2.12 \times 10^8$ |
| *P. aeruginosa* #6 | $2.25 \times 10^8$ |
| *P. aeruginosa* #7 | $1.89 \times 10^8$ |
| *P. aeruginosa* #8 | $4.37 \times 10^8$ |
| *P. aeruginosa* #9 | $1.06 \times 10^8$ |
| *P. aeruginosa* #10 | $1.34 \times 10^8$ |
| *P. aeruginosa* #11 | $4.03 \times 10^8$ |
| *P. aeruginosa* #12 | $1.93 \times 10^8$ |
| *P. aeruginosa* #13 | $1.35 \times 10^8$ |
| *Klebsiella pneumoniae* | $9.20 \times 10^7$ |
| *Enterococcus* | $5.20 \times 10^7$ |

mean batch content of the "50-μg" disks ranged from 33 to 70 μg with a mean error of 35%. The mean batch content of the "100-μg" disks ranged from 99 to 135 μg with a mean error of 21%.

When the 50-μg disks of three commercial manufacturers were compared, a considerable discrepancy occurred. Figure 4 outlines these differences. In each case, the disks had been received directly from the manufacturer or the manufacturer's local commercial outlet and had outdates of several months hence. The manufacturer whose disks seemed most out of line had an outdate of 11 months hence. In each case, storage of the disks prior to our receipt was stated to have been at 3 to 6 C. A certain consistency was evident with the disk of each manufacturer, with those from manufacturer #1 giving the largest zones generally and those from manufacturer #3 resulting in the smallest zone sizes.

Figure 5 shows the carbenicillin standard curve. The disks used in this study were the disks prepared in our laboratory in 10-μg increments. Single strains of *S. aureus*, *E. coli*, and *P. aeruginosa* were analyzed. The results are plotted with the zone diameter on the ordinate, and the disk content is recorded on a log scale

TABLE 4. *Carbenicillin disk assay*

| Disk size | Lot | Manufacturer | No. tested | Mean content (μg) | Range | Mean error (%) |
|---|---|---|---|---|---|---|
| 50-μg disk | a | #1 | 20 | 59 | 44–70 | 18 |
| | b | #1 | 10 | 66 | 58–72 | 32 |
| | c | #2 | 10 | 33 | 31–35 | 34 |
| | d | #3 | 10 | 70 | 66–76 | 40 |
| | e | #3 | 10 | 70 | 65–76 | 40 |
| 100-μg disk | a | #1 | 20 | 99 | 90–108 | 1 |
| | b | #1 | 10 | 128 | 104–153 | 28 |
| | c | #1 | 10 | 121 | 114–130 | 21 |
| | d | #1 | 10 | 135 | 123–150 | 35 |

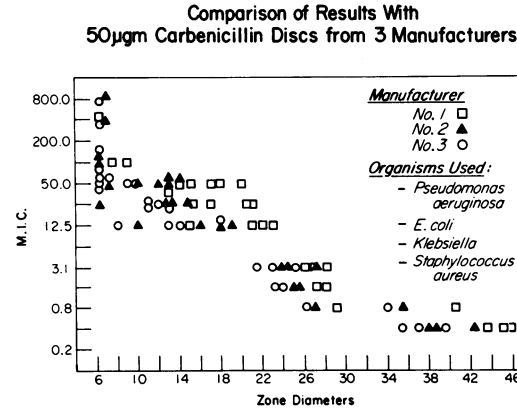


FIG. 4. *Regression graph comparison of values obtained with organisms tested simultaneously with 50-μg carbenicillin disks from three different manufacturers.*

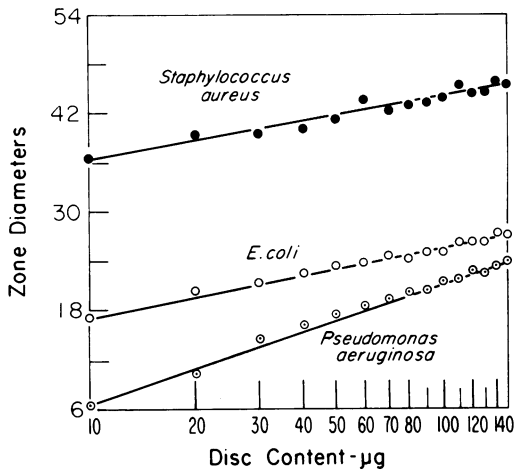


FIG. 5. *Carbenicillin standard curves obtained using three control organisms and disks prepared in 10-µg increments.*

on the abscissa. The standard curve is used to check regression results and is helpful in assessing appropriate disk strength (4, 14). In this instance there was a continual linear relationship for each of the three organisms tested.

The media comparison studies revealed batch-to-batch variation as was expected. However, the interactions between medium content, organism (*Pseudomonas* or *E. coli*), and amount of antibiotic in the disks were so complex they defied simple statistical analysis. For example, with the 100-µg disk: medium #1 gave consistently smaller zones than medium #2 for both *E. coli* and *Pseudomonas*. However, comparison of the same media using the 50-µg disk gave smaller zones with *E. coli* but larger zones with *Pseudomonas*. Statistical analysis of disk variability within a batch revealed no statistical difference between the 50- and 100-µg disks used in this part of the study.

No significant differences were found when the standard inocula were prepared by two different methods, that is, using black lines on a white surface as a background or using no special background at all.

Figure 6 shows the population distribution of 669 strains of *P. aeruginosa* tested against the 50-µg carbenicillin disk in the Diagnostic Microbiology Laboratories at the University of Minnesota Hospitals. Using the currently recommended guidelines (9), 68.0% of these strains would be called susceptible, 11.8% would be called intermediate, and 20.2% would be designated resistant.

Figure 7 represents a population distribution of organisms tested against the 100-µg carbenicillin disk and includes both *P. aeruginosa* and *Enterobacteriaceae* strains, with suggested zone sizes indicated for susceptible, intermediate, and resistant for each group of organisms.

## DISCUSSION

In arriving at a decision as to the appropriate potency of a disk to be used for disk diffusion antibiotic susceptibility testing, one must first carry out regression studies to determine the relationship of the disk diffusion results with appropriate dilution studies. By applying the mathematical formula of least squares, a regression line is developed, based on the assumption that there is a linear relationship between zone size and $\log_2$ scale of MIC. This principle holds true for most antibiotics and indeed holds true for carbenicillin as well.

In determining disk potency, one is particularly concerned about the distribution of organisms about the regression line. Sufficient numbers should be analyzed to adequately test the scatter of zone size at each MIC value. In

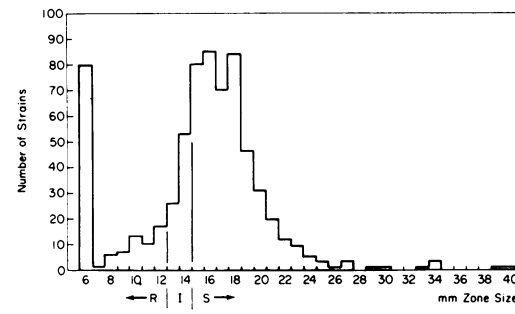


FIG. 6. *The population distribution of 669 strains of Pseudomonas aeruginosa tested with the 50-µg carbenicillin disk.*

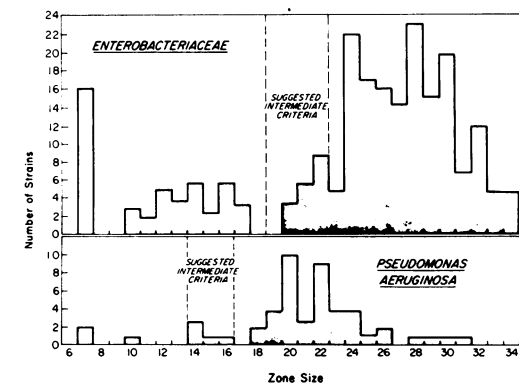


FIG. 7. *The population distribution of strains of Enterobacteriaceae (top) and Pseudomonas aeruginosa (bottom) tested with the 100-µg carbenicillin disk with suggested zone size breakpoints.*

addition, there should be sufficient disk potency to allow for appropriate zone discrimination at the MIC values which correlate with therapeutic usefulness.

Such is not the case with the 50-μg disk regression graph as illustrated in Fig. 1, and yet this is currently the only disk potency approved and available commercially for clinical laboratory testing (6, 9). Organisms having MIC values of 100 to 200 μg/ml often demonstrate zones of less than 11 mm with the 50 μg disk, and yet these values include organisms which are considered susceptible or intermediately susceptible when approaching *Pseudomonas* infections therapeutically with carbenicillin. The line of regression for the 50-μg disk intersects the ordinate at a point just above the range of intermediate susceptibility for *P. aeruginosa*. Because of the scatter or standard deviation of organism plots about this line, as in Fig. 1, organisms having susceptible or intermediately susceptible MIC values will give resistant results by disk testing with this disk. There is no corollary to this type of limitation in any other currently used disk testing situation.

The regression graph of the 100-μg carbenicillin disk (Fig. 2) gives a regression line which is somewhat steeper than that obtained with the 50-μ disk, and the zone sizes demonstrated by *P. aeruginosa* with the higher MIC values which are still within the range of therapeutic reach allow for more appropriate discrimination. The continuum of organism scatter is present, and the pattern of low MIC organisms is in an appropriate zone size distribution involving primarily zone sizes of the higher 20 and lower to mid 30 range. Furthermore, the regression line intersects the ordinate well above the intermediate range, and the standard deviation figures for both the X and Y axes are lower with the 100-μg disk (Table 2).

Figure 3 demonstrates the combined organism plots of the 50-μg disk and the 100-μg disk for the MIC values above 25 μg. As is evident, the area of 100 to 200 μg/ml MIC values, the problem area of discrimination with the 50-μg disk, is resolved with the 100-μg disk and allows for greater discrimination in this range of MIC values.

The pronounced change in zone sizes between the 50- and 100-μg disks seen with *P. aeruginosa* (Results) emphasizes the weakness of the 50-μg disk. The *Pseudomonas* strain showing the greatest difference in zone sizes had an MIC of 50 μg/ml for carbenicillin. It was found to be susceptible according to the 100-μg disk (zone = 25.9 mm) but of intermediate susceptibility according to the 50-μg disk (zone = 14.3). Two *Pseudomonas* with MICs of 100 μg/ml were found to be susceptible with the 100-μg disk but resistant with the 50-μg disk.

The rather pronounced change of zone size seen with *Pseudomonas* when switching disk content tends to indicate that, with this organism, the 50-μg disk does not perform as a "high" content disk. When the marked effect on *P. aeruginosa* of varying disk content is considered in tandem with the failure of the 50-μg disk to discriminate critical MIC values, one is left with the valid concern about the effects of disk content variability. This concern is further justified by the greater disk assay error evident in the 50-μg disks assayed as compared to the 100-μg disks. The greater variability seen with the 50-μg disk is reflected in the larger standard deviations seen with the 50 μg regression line (Table 2). In addition, and in keeping with the greater variability in assay values, there were somewhat greater variations when results of duplicate plate testing with the 50-μg disk were compared with the 100-μg disk values.

Perhaps one of the most disconcerting findings in this study was the pronounced differences demonstrated with the 50-μg disks obtained from three major manufacturers of antibiotic susceptibility testing materials. As shown in Fig. 4, the variety of results was rather pronounced with most strains. It should be stressed that the disks from the three manufacturers were tested on the same plate in each instance, in order to obviate test condition differences. Discrimination would obviously be markedly compromised should a laboratory receive a batch of disks similar to that which we received from "manufacturer #3." The shift with these disks is rather pronounced, and would cause susceptible or intermediate organisms to be called resistant in many instances (Fig. 4). Studies of a similar nature could not be carried out with the 100-μg disk as disks from only one manufacturer were available to us.

Standard curve studies (Fig. 5) demonstrate a linear relationship of zone diameters versus disk content over the range tested. A break in this linear relationship would, for most antibiotics, suggest that the disk potency for susceptibility testing should not exceed the potency indicated by the disk at the point where the linear relationship failed. The linear relationship obtained suggests that there is no theoretical reason for limiting the disk to 50 μg.

The distribution of organisms on the regression line for carbenicillin is similar to that seen with other semisynthetic penicillins and the

cephalosporins (13, 15). The presence of this wide spectrum of activity of carbenicillin is even more pronounced than with related compounds such as ampicillin because of the large doses employed therapeutically with carbenicillin against *P. aeruginosa*. It is pertinent, then, to consider that the testing situation reflects this range of activity. Not only is a more discriminating disk needed, but, as has been advocated previously for the 50-μg disk, separate criteria should be established for *P. aeruginosa* as differentiated from species of the nonfermentative gram-negative bacteria and from the *Enterobacteriaceae*. The suggestions we would put forth (Fig. 7) are based on analysis of the 100-μg disk regression graph. The zone size of 17 mm will include organisms with MIC values of 100 μg/ml, but exclude those with 200 μg/ml values. This 100-μg level seems to represent an achievable level when high dose therapy is undertaken with carbenicillin with *P. aeruginosa*. Zone sizes of 13 to 16 mm will include organisms in the range of 100 to 200 μg/ml but will exclude organisms with values of 400 μg ml. Similarly, a zone size of 23 mm or greater represents 12.5 μg/ml or less, considered to be a realistic level in approaching infections due to *Enterobacteriaceae*, although it is obvious that higher levels can be achieved just as with *Pseudomonas*. In both instances we would suggest the use of intermediate values as outlined in Fig. 7 (13 to 16 mm and 19 to 22 mm). These recommendations correlate closely with previous recommendations for the 100-μg carbenicillin disk.

Documentation for these recommendations is further evident in the bar graphs of organism distribution (Fig. 6 and 7). The separation of bacterial populations tends to fit the zone size breakpoints outlined above. There is not a completely differentiated diphasic population, but the majority of strains are either susceptible or resistant by the suggested criteria.

It is our recommendation that the 100-μg disk be approved for laboratory use, and that it replace the 50-μg disk currently in use. Our recommendations for zone size breakpoints are outlined above and would serve the two groups of organisms most appropriately treated clinically with carbenicillin.

## ACKNOWLEDGMENTS

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