LABORATORY DETERMINATION OF ANTIBIOTIC SUSCEPTIBILITY TO CARBENICILLIN

by

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A THESIS
Presented to the Department of Clinical Pathology
and the Graduate Division of the
University of Oregon Medical School
in partial fulfillment of
the requirements for the degree of
Master of Science

June 1971

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ACKNOWLEDGEMENTS

The author wishes to express her sincere appreciation to Dr. T. Hutchens, Dr. M. Berroth and the other members of the Department of Clinical Pathology for their assistance during the course of this study. I also would like to thank Miss Diana Harry for typing this thesis.

Especially I would like to acknowledge the guidance, encouragement and friendship of Dr. A.L. Rashad, Professor in charge of this thesis.

Financial support which made this research possible was provided by a training grant from the United States Public Health Service.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

A. Historical Note on Antimicrobial Agents

In the period soon after microorganisms were implicated as disease producers, Pasteur made many important discoveries in immunology. Scientists of that era were thus mainly interested in vaccines as means of controlling infectious diseases. In 1909, Ehrlich synthesized organic compounds of arsenic, but it was not until his 606th attempt that he finally found one which would kill Trepanema pallidum, the spirochete of syphilis, without harming the host. In this way he introduced the concept of selective toxicity of chemotherapy. Following Ehrlich's discovery, several investigators started the search for synthetic chemicals with greater affinity for parasitic cells than for host cells.

Much hope was placed on dyes because of their selective staining of infectious agents, but it was not until 1935 that Domagk found that the dye prontosil had the capability to cure mice of bacterial infections. Later he found that the antibacterial activity was due to the sulfanilamide moiety of prontosil. The activity of sulfanilamide against bacteria is due to its ability to block the conversion of PABA (para-aminobenzoic acid) to folic acid, an important co-enzyme in bacterial cell metabolism.

Because of the discovery of sulfanilamide, Fildes suggested that chemotherapeutic agents might be found among structural analogues of metabolites. Shortly thereafter, analogues of amino acids, vitamins, purines and pyrimidines were synthesized, but none proved to be a use-

ful antibacterial agent.

The history of antibiotics actually begins with the discovery of penicillin by Fleming in 1929. The discovery happened quite by accident. A Petri dish in which Fleming was culturing Staphylococci became contaminated with the fungus Penicillium. By noticing that the growth of the bacteria was inhibited in the area around the fungus, he concluded that this inhibition was due to a chemical substance which was liberated by the mold. He isolated the Penicillium, grew it in pure culture and demonstrated that filtrates derived from the fungus were bactericidal as well as non-toxic to animals. However, Fleming abandoned his work on penicillin at this point due to technical difficulties in the purification process.

Florey and Chain in 1939 succeeded in isolating and purifying penicillin in a quantity that enabled them to test its therapeutic value in clinical situations. After striking success in achieving cures of a variety of bacterial infections a full-scale effort was begun in the United States to produce penicillin in great quantities.

A systematic search for other useful antibiotics was launched. Schatz and Waksman at Rutgers University were successful in isolating the second effective antibiotic, streptomycin, from Actinomycetes. Their discovery stimulated many other laboratories to do similar routine testing and soon other antibiotics such as chloramphenicol and tetracycline, both produced by Actinomycetes, were discovered. Waksman in 1952 defined an antibiotic as any substance produced by one microorganism which has an inhibitory action on another.

The successful practice of chemotherapy revolutionized medicine and soon was extended to cover nearly every bacterial disease of man. Presently most of the bacterial diseases can be effectively treated with antibiotics. It must be remembered, however, that it is not always easy to find a useful antibiotic. Hundreds of agents have been discovered, but could not be used because they were either too toxic or too unstable.

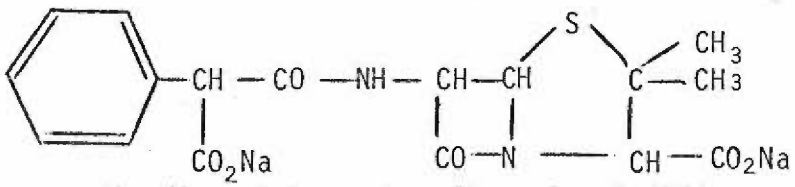
The subject of this thesis is carbenicillin, one of the most recently introduced penicillins. The penicillins may be classified into two main groups. The first group includes penicillins that are susceptible to penicillinase such as penicillin G, ampicillin and carbenicillin. The second group includes penicillins that are resistant to penicillinase such as methicillin, nafcillin and cloxacillin. The penicillins are one of the least toxic groups of antimicrobial agents available at the present time. Their chief toxicity is hypersensitivity reactions. Penicillin G has a limited spectrum of activity. It is mainly active against gram-positive bacteria, Neisseria and the spirochetes. Ampicillin and carbenicillin, on the other hand, have a much broader spectrum of activity. They are active against both gram-positive and gram-negative bacteria. The penicillinase-resistant penicillins are less active than penicillin G and they are chiefly used in the treatment of infections caused by penicillinase producing Staphylococci.

The mechanism of the antibacterial action of penicillins have been under survey for nearly three decades. The earliest microscopic studies

revealed that morphological changes occurred in penicillin treated cells and that only growing bacteria were affected. The bizarre forms observed with sublethal doses of penicillin suggested that the drug acted on the cell wall of the bacteria. Subsequently many investigators have provided evidence that penicillin interferes with the synthesis of the cell wall of actively growing bacteria.

B. Carbenicillin

Carbenicillin (disodium alpha-carboxylbenzylpenicillin) is a new, broad-spectrum, semi-synthetic penicillin first described by Acred and associates in 1967 (2). All penicillins are monocarboxylic acids with a beta-lactam/thiazolidine ring (nucleus) attached to an acyl side-chain by an amide linkage. Differences in their antibacterial activity, absorption and acid stability are determined mainly by the chemical structure of the side-chain while their solubility in water depends chiefly upon the salt used. The use of ionized substituents at the alpha carbon of the side-chain seems to increase its activity against gram-negative bacilli (25). The chemical structure of carbenicillin is very much like ampicillin except that it has a carboxyl group instead of an amino group in the alpha position of the 6-amino penicillanic acid nucleus. Its action is virtually like that of all the penicillins; it interferes with the cross-linking of muramic acid with the rest of the cell wall.



disodium alpha-carboxylbenzylpenicillin

One of the remarkable features of carbenicillin is that exceptionally high blood levels can be achieved after intravenous administration. Levels as high as 125 µg/ml can be readily achieved in the blood. Another unique feature of carbenicillin is that it is the only penicillin known to be active against the Pseudomonas and indol positive Proteus organisms. These bacteria tend to be highly resistant to many of the available antimicrobial agents including ampicillin. The drug of choice for the treatment of Pseudomonas infections is polymyxin B, an antibiotic that is highly nephrotoxic. The drug of choice for indol positive Proteus organisms is either kanamycin or gentamicin. These drugs are also highly toxic agents (nephrotoxic). Patients having impaired renal function and suffering from Pseudomonas (or indol positive Proteus) infections usually present a dilema for the treating physician. The introduction of carbenicillin, a drug which is almost free from side-effects, offers much hope for the treatment of such infections.

The recommended routes of administration of carbenicillin are either intramuscularly or intravenously. It cannot be given orally because it is very poorly absorbed and is unstable in acid solution (its half-life at a pH of 2 at 37°C is onlyabout 30 minutes).

C. General Remarks About in vitro Susceptibility Tests

One type of susceptibility test that has been used early in the era of antibiotics was based on a comparison of results obtained by testing the in vitro activity of a given drug against the infecting organism with results obtained from a known bacterial strain. The reference strain was one which was known from previous clinical trials

to respond to the drug in question. If the in vitro activity of a given drug against the causative organism was found to be similar to that of the reference strain, the drug was selected for therapy and the prognosis was believed to be good. On the other hand, if the organism causing the disease was found to be more resistant than the reference strain, another antimicrobial agent was sought for treatment. (11)

A second type of in vitro susceptibility determination that has not gained much popularity, is based on testing tissue fluid obtained from the infected site from patients receiving chemotherapy against the infecting organism. This is not always easy to perform or even possible in most cases.

The most practical and most widely used approach is to test the activity of the antibiotic in vitro directly against the infecting bacterium and utilize established criteria for determining whether the organism is susceptible or not. These criteria are based on correlating minimum inhibitory concentrations (MICs) to blood levels attained with ordinary dosage schedules. The MIC is defined as the least amount of antibiotic that inhibits bacterial growth. An organism is considered as sensitive to a given antibiotic if the MIC is lower than the attainable blood level. On the other hand, if the MIC is greater than the blood level the organism is considered resistant.

It is important to note that the results of an in vitro susceptibility test must not be interpreted in absolute values. The results are influenced by the size of inoculum, pH of medium, type of medium,

and length of incubation (33). In order to be able to obtain reproducible results it is important that the test system be as close to in vivo conditions as possible in respect to pH, temperature, etc. Furthermore, the test conditions must be adequately standardized and the endpoints should be chosen consistently.

MICs for various antimicrobial agents can be determined by means of the broth dilution or agar dilution tests. In the broth dilution method a measured volume of a standardized inoculum of the organism is added to serial twofold dilutions of the antibiotic. After overnight incubation at 37°C, the MIC is usually read as the least concentration of antibiotic that inhibits grossly visible growth. The minimum bactericidal concentrations (MBCs) may be obtained by subculturing each tube onto solid media. The bactericidal concentration is the least amount of antibiotic that kills the organism, i.e., shows no growth on subculture. One of the disadvantages of the broth dilution test is that only the maximum resistance of the bacterial population is measured, or, in other words, the results are determined solely by the most resistant variants. Thus, differences in the sensitivity of the bacterial population cannot be recognized. Another disadvantage is that many tubes are required for each organism; this makes it difficult to test a large number of bacterial strains simultaneously. Furthermore, fastidious organisms which require the presence of blood for growth cannot be tested by this method. The blood makes the medium appear cloudy and thus an accurate endpoint cannot be read.

In the agar dilution test, serial twofold dilutions of the anti-

biotic are added to premeasured amounts of melted agar cooled to about 40°C. The antibiotic-agar mixture is poured into sterile Petri dishes and allowed to harden. A standardized inoculum of each organism is then placed onto the agar and the MICs are read after overnight incubation at 37°C. Inoculation of the plates is usually performed by means of the Steer's replicator which allows a total of 32 bacterial strains to be tested simultaneously.

The agar dilution method has several advantages: a large number of organisms can be tested simultaneously; contaminants, if present, can be detected readily; and blood may be added to the medium when fastidious organisms such as Streptococcus and gonococcus are tested. Furthermore, the proportion of resistant variants in the population can be easily determined.

Although the agar and broth dilution methods are valuable in determining the susceptibility of an organism they are not generally used for the routine testing in a clinical laboratory. They are relatively expensive, time consuming, and require technical skill. It is for all these reasons that the agar diffusion method was developed. The agar diffusion test is simple to perform and less costly than the dilution tests. The diffusion test has gained much popularity and has become the routine test used in the hospital laboratory for determining drug susceptibility of organisms. The test is carried out by streaking a standardized inoculum of the organism onto an agar plate and then applying antibiotic discs onto the surface of the agar by means of flamed forceps or an automatic disc dispenser. After overnight

incubation, zones of inhibition are measured to the nearest millimeter by means of a caliper.

The agar diffusion test is influenced by a variety of factors including ionic concentration of medium, depth of agar, concentration of antibiotic in the disc, speed of diffusion of drug, growth rate of organism and the size of inoculum (33). In order to be able to obtain reproducible results, Bauer, Kirby, Sherris and Turck (3) at Seattle developed an agar diffusion method in which they standardized all of the factors known to influence the results of this test. It is important to note that a zone of inhibition around a 50 µg disc does not mean that the MIC of the organism is 50 µg/ml. Furthermore, the largest zone of inhibition is not always indicative of the drug of choice. There is a tendency for the zones to be large for antibiotics with a low molecular weight. Conversely, antibiotics with a high molecular weight usually give rise to small zones of inhibition.

Before the results of the agar diffusion test can be interpreted, a correlation of the diameter of zones of inhibition with MICs must first be carried out. For any given antibiotic there is an approximately linear relationship between log MICs and zone sizes for organisms with reasonably comparable growth rates. Such a relationship is usually represented in the form of a regression line. Once a regression line in plotted for an antibiotic one can easily determine what zones of inhibition obtained in the diffusion test represent susceptibility or resistance. This is usually done by establishing "break points" on the regression lines. An MIC value equivalent to

the level of antibiotic that can be readily attained in the blood is usually taken as the break point. The diameter of the zone of inhibition corresponding to this MIC value is derived from the regression line. If this diameter is, for example, 16 mm, organisms with zones measuring greater than 16 mm are classified as susceptible. During the last 15 years, Kirby, Bauer, Sherris and their colleagues at the University of Washington in Seattle have plotted regression lines for all of the commonly used antimicrobial agents. From such regression lines they were able to derive criteria that can be used readily in the clinical laboratory for determining the in vitro susceptibility of microorganisms from measurement of the zones of inhibition.

In spite of the fact that the diffusion test is the most popular in vitro susceptibility test used at the present time the dilution tests still remain very valuable methods particularly when an MIC determination is needed. The following is a list of the indications for performing a dilution test: (a) to determine MICs or MBCs of more resistant organisms when an unusually high dosage of a given antibiotic is being contemplated in therapy; (b) to test slow growing organisms for which routinely used diffusion tests have not been adequately standardized; and (c) to obtain quantitative data on new antibiotics for use in establishing a correlation between diffusion test results and MICs.

D. Purpose of the Present Investigation

The present investigation was performed in order to: (a) establish criteria for determining the susceptibility of organisms to carbenicil-

lin by means of the agar diffusion test; (b) determine the pattern of susceptibility to carbenicillin of the pathogenic microorganisms isolated at the University of Oregon Medical Center; (c) study the influence of alteration of the test conditions (e.g., varying inoculum size, enriching medium with blood or prolonging incubation time) on the results of the dilution and diffusion tests; (d) determine the stability of carbenicillin discs that are used in the diffusion tests; and finally (e) explore some of the underlying factors associated with resistance to carbenicillin.

MATERIALS AND METHODS

A. Bacterial Strains

All bacterial strains used in this study were fresh clinical isolates from our laboratory. The 327 strains tested are listed in Table 1. These strains were stored at -60°C in trypticase soy broth enriched with 50% calf serum.

B. Antibiotic

Disodium carbenicillin was provided in 5 gram sterile vials by Chas. Pfizer & Co., New York. Antibiotic stock solutions were prepared with sterile distilled water (10,000 µg/ml) and stored at -20°C in 2 dram screw-cap vials. Lower drug concentrations were made up shortly before use. Carbenicillin discs of 100 µg, 50 µg, and 30 µg potencies were provided by Chas. Pfizer & Co. and Beecham Laboratories. They were stored at -20°C and warmed to room temperature immediately before use.

C. Media

The medium used for all tests was Mueller-Hinton (MH) broth or MH agar. The pH of the medium was 7.2. For fastidious organisms such as enterococcus, Corynebacterium diphtheriae, beta-hemolytic streptococcus group A, Pneumococcus, and HB-1, 5% sterile defibrinated sheep blood was added to the agar.

D. The Agar Dilution Method

The agar dilution tests were performed using Steers' inocula replicator. Carbenicillin dilutions were incorporated in MH agar at 50-55°C to give final concentrations in a Log_2 dilution series. Plates

TABLE 1

LIST OF BACTERIAL STRAINS TESTED

ORGANISM	NUMBER OF STRAINS
Escherichia coli	23
Enterobacter	13
Klebsiella	19
Serratia	20
Providencia	20
Citrobacter	1
Salmonella	6
Shigella	5
Arizona	2
Proteus mirabilis	20
Proteus vulgaris	11
Proteus rettgeri	14
Proteus morganii	16
Pseudomonas aeruginosa	33
Pseudomonas species	17
EO-1	7
Staphylococcus aureus (penicillin-resistant)	23
Staphylococcus aureus (penicillin-sensitive)	23
Enterococcus	18
Beta-hemolytic streptococcus group A	10
Pneumococcus	4
Achromobacter	1
Aeromonas	1
Alkaligenes	5
Herrellea	1
Pasteurella multocida	1
Pasteurella pseudotuberculosis	1
Bordetella bronchiseptica	1
Corynebacterium diphtheriae	5
Group I-B	1
Group IV-C	1
Group IV-F	3
HB-1	1
TOTAL	327

were poured in 90 mm Petri dishes to a depth of 2.5 to 3 mm, stored at 4°C and used within 24 hours. An inoculating suspension was prepared equivalent in density to approximately 10^8 organisms/ml (0.5 McFarland standard). An inoculum of each organism was transferred to the surface of the agar plates on one of the 32 prongs of the replicator head; each prong delivered about 0.003 ml. Thirty-two tests could thus be made simultaneously. As a control, a standard strain of Escherichia coli with a known carbenicillin MIC value (31.2 µg/ml) was tested simultaneously with every agar dilution test. Control plates containing no antibiotic were also included in each test.

E. The Agar Diffusion Method

Tubes of trypticase soy broth were inoculated from the frozen stock vials with the organisms to be tested. These tubes were then incubated for approximately five hours to produce a bacterial suspension of moderate cloudiness. The suspension was then diluted, if necessary, with saline solution to a density equivalent to that of a standard (approximately 10^8 organisms/ml). This standard was prepared by adding 0.5 ml of 1% $BaCl_2$ to 99.5 ml of 1% H_2SO_4 (0.36N). For the sensitivity plates, large (15 cm) Petri dishes were used with MH agar (5 to 6 mm in depth). Plates were dried for about 30 minutes before inoculation and were used within four days of preparation.

The bacterial broth suspension was streaked evenly in three planes onto the surface of the medium with a cotton swab (not a wire loop or glass rod). Surplus suspension was removed from the swab by being rotated against the side of the tube before the plates were seeded.

After the inoculum had dried (3 to 5 minutes) the discs were placed on the agar with flamed forceps or a single disc applicator and gently pressed down to ensure contact. Plates were incubated within 30 minutes.

After overnight incubation, the zone diameters (including the 6 mm disc) were measured with a caliper. A reading of 6 mm indicates no zone. The endpoint is taken as complete inhibition of growth as determined by the naked eye. Swarming of Proteus strains was not inhibited and a veil of swarming into an inhibition zone was ignored. As a control, a sensitive standard strain of Escherichia coli was tested simultaneously with every agar diffusion test.

F. Broth Dilution Test

A five hour trypticase soy broth culture of each organism was standardized to an opacity equivalent to a 0.5 McFarland standard (approximately 10^8 organisms/ml), diluted 1:200 and added to serial twofold dilutions of carbenicillin. Final drug concentrations ranged from 0.025 µg/ml to 1000 µg/ml. The total volume in each tube was 2 ml (1 ml of carbenicillin solution + 1 ml of organism suspension). After mixing the drug and inoculum the tubes were incubated at 37°C. The MICs were read and recorded as the first tube with no visible growth as judged by the naked eye at the end of 18 hours. A second reading was made at the end of 42 hours. For each organism tested, a control tube with no drug was run simultaneously and in each run a sensitive standard Escherichia coli strain of known susceptibility (carbenicillin MIC = 31.2 µg/ml) was included.

The MBCs were determined by taking one loopful from the last tube

with visible growth and from all of the tubes with no visible growth after the 18 hour incubation period and streaking them onto blood agar plates. The plates were incubated overnight at 37°C. The MBC was taken as the least concentration of carbenicillin showing no growth.

G. Test of the Ability of Bacteria to Inactivate Carbenicillin

Trypticase soy broth was inoculated with organisms (a total of 77 strains were tested) and incubated for about five hours at 37°C. Each organism suspension was standardized to an opacity equivalent to a 0.5 McFarland standard. Equal volumes (1 ml each) of the standardized inoculum and of carbenicillin solution containing 200 µg/ml were mixed. The final concentration of carbenicillin in the mixture was 100 µg/ml. The organism-carbenicillin mixture was incubated at 37°C for 2 hours.

A sensitive Staphylococcus aureus strain (carbenicillin MIC = 1.9 µg/ml) adjusted to a 0.5 McFarland standard was streaked with a sterile cotton swab onto a large MH agar plate. Six wells about 17 mm (5/8 inch) in diameter were punched into the agar of each plate with a sterile glass test tube. One milliliter of the organism-carbenicillin mixture was pipetted into the wells. Each organism was tested in triplicate (i.e., 3 wells for each organism). The plates were incubated overnight (18 hours) at 37°C. The zones of inhibition around the wells were measured to the nearest millimeter with calipers. A control well with no organism and containing 100 µg/ml of carbenicillin was run simultaneously. The control wells were also done in triplicate. The difference in measurement between the diameters of the zone of inhibition surrounding the wells containing the organism-carbenicillin mixture and the zone surrounding the control well containing only

carbenicillin was taken as a measure of the degree of inactivation of the antibiotic.

H. Stability of the Antibiotic

Carbenicillin discs of 100 µg and 50 µg potencies were kept with and without dessicant at -60°C, -20°C, room temperature, and 37°C for four months and agar diffusion tests were performed once a week using the standard Escherichia coli strain.

RESULTS

A. Agar Dilution MICs

MICs of carbenicillin for 248 bacterial strains as determined by the agar dilution technique are shown in Figures 1-4. Figure 1 illustrates the MICs of 23 Escherichia coli, 13 Enterobacter, 20 Serratia and 19 Klebsiella. Twenty-two of the Escherichia coli strains tested were inhibited by 15.6 µg/ml or less; only 1 strain was highly resistant with an MIC of greater than 1000 µg/ml. Similarly, all Enterobacter strains were highly susceptible, their MICs were 7.8 µg/ml or less. Nineteen of the Serratia strains were inhibited by 31.2 µg/ml or less; 1 strain required 500 µg/ml. The majority of the Klebsiella, on the other hand, were highly resistant. Sixteen strains had MICs of 250 µg/ml or greater and the remaining 3 strains had MICs of 62.5-125 µg/ml.

The MICs of the Proteus and Providencia are illustrated in Figure 2. It can be seen that these organisms tended to exhibit a bimodal distribution with two distinct populations, a susceptible one and a highly resistant one. Seventy-five percent of the Proteus mirabilis strains were susceptible to 15.6 µg/ml or less, the remaining 25% were inhibited by 500 µg/ml or more. Similarly, 69% of the Proteus morganii strains were susceptible to 15.6 µg/ml or less and the remaining 31% had MICs of 125 µg/ml or greater. Likewise, 71% of the Proteus rettgeri strains had MICs of 15.6 µg/ml or less and the other 29% required 1000 µg/ml or more for inhibition. In the case of Proteus vulgaris, however, only 18% of the strains were susceptible to 62.5

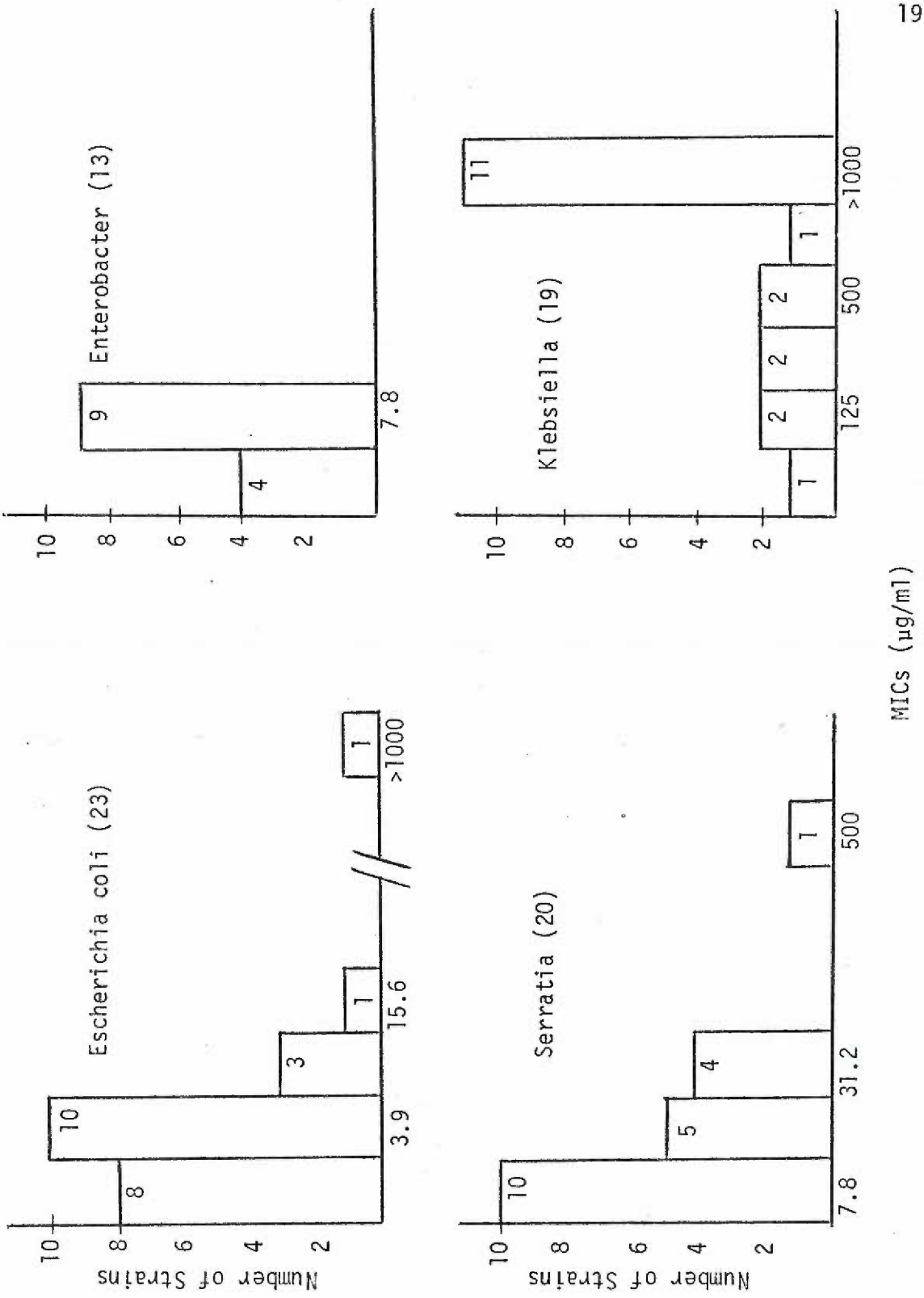

Enterobacter (13)
9
4
7.8
Klebsiella (19)
11
1
2
2
2
1
125
500
>1000
Escherichia coli (23)
8
10
3
1
1
3.9
15.6
>1000
Serratia (20)
10
5
4
1
7.8
31.2
500
Number of Strains
MICs (µg/ml)

FIGURE 1

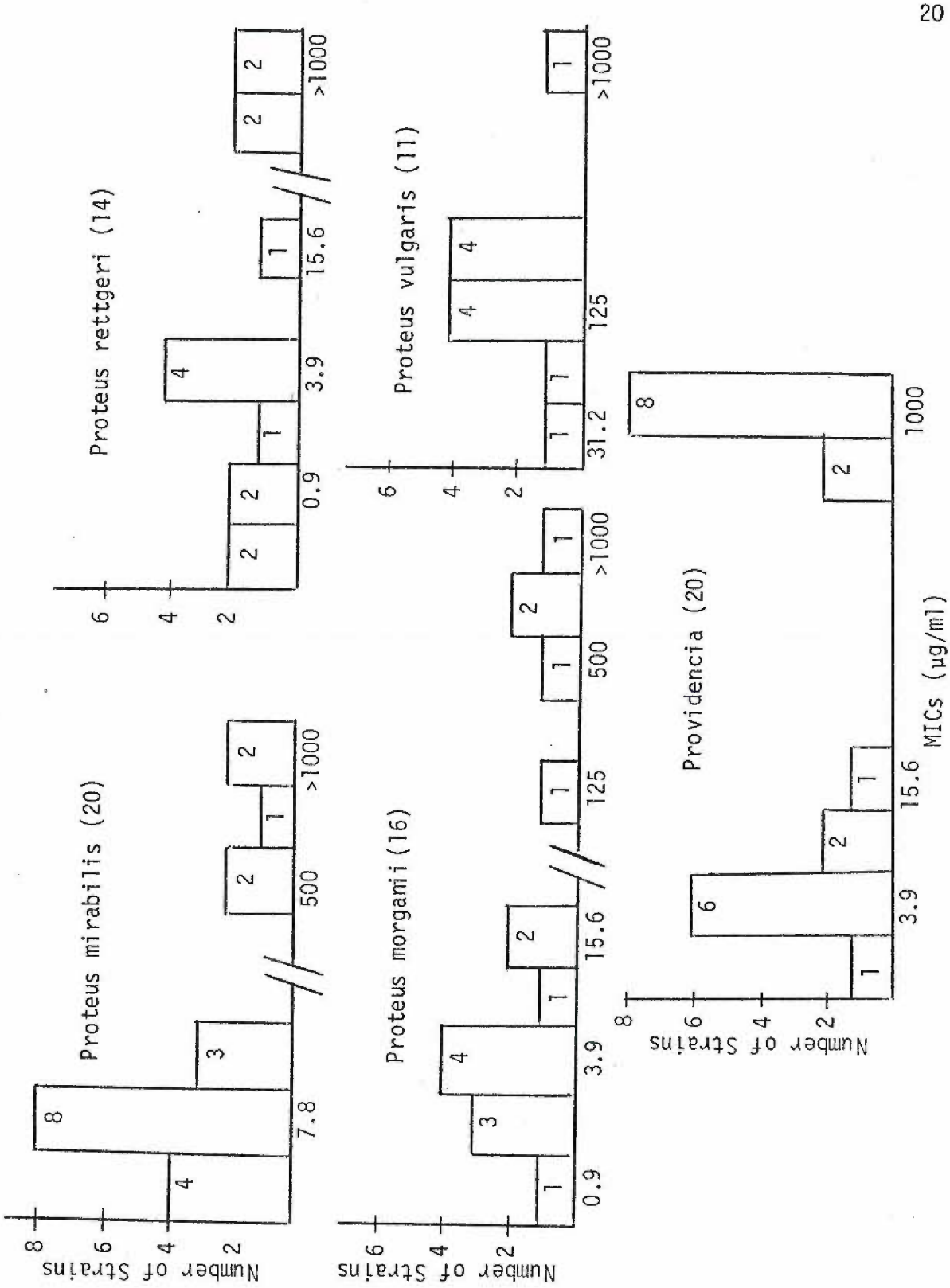

Proteus rettgeri (14)
Proteus vulgaris (11)
Proteus mirabilis (20)
Proteus morgani (16)
Providencia (20)
Number of Strains
MICs (µg/ml)
0.9
3.9
15.6
31.2
125
500
1000
>1000
7.8

FIGURE 2

µg/ml or less. The MICs of the remaining 82% of the strains were 125 µg/ml or greater. Half of the 20 Providencia strains had MICs of 15.6 µg/ml or less and the other half had MICs of 500 µg/ml or greater.

Figure 3 illustrates the MICs of the Pseudomonas. Twenty-four of the 33 Pseudomonas aeruginosa strains tested had MICs of 62.5-125 µg/ml. Three strains were inhibited by 1.9 µg/ml or less, 1 strain by 31.2 µg/ml and 5 strains by 250 µg/ml or more. Eleven of the 17 Pseudomonas species strains had MICs of 62.5 µg/ml or less and the remaining 6 strains had MICs of 125 µg/ml or greater.

Figure 4 illustrates the MICs of 46 strains of Staphylococcus aureus and all strains of enterococcus. All of the non-penicillinase producing Staphylococcus aureus were highly susceptible to carbenicillin with MICs of 7.8 µg/ml or less. The penicillinase producing Staphylococcus aureus tended to have slightly higher MICs ranging from 7.8 to 31.2 µg/ml. For enterococcus the MICs were 125 µg/ml for 13 strains, 62.5 µg/ml for 3 strains and 250 µg/ml for the remaining 2.

Agar dilution MICs were also determined for 58 miscellaneous organisms and the results are shown in Table 2. Forty-six of the miscellaneous bacterial strains tested were inhibited by 31.2 µg/ml or less. The remaining 12 strains had MICs of 62.5 µg/ml or greater.

B. Comparison of Broth Dilution and Agar Dilution MICs

MICs for 223 bacterial strains were determined both by the agar dilution and by the broth dilution methods. The results in Table 3 show good correlation between the MICs obtained by the two methods. Eighty-one strains had identical MICs by the two methods, 88 strains

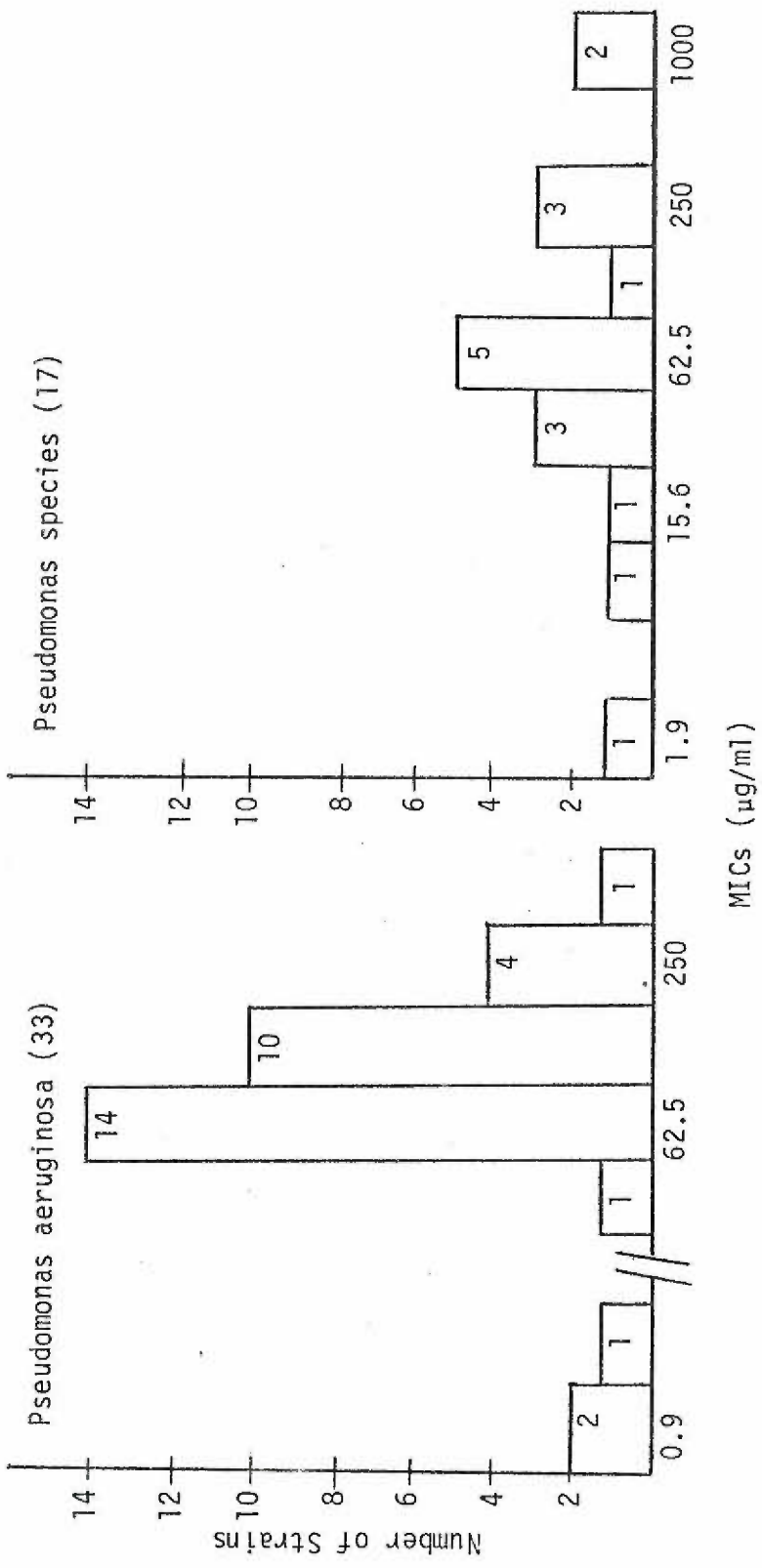

Pseudomonas aeruginosa (33)
Pseudomonas species (17)
Number of Strains
MICs (µg/ml)
0.9
1.9
15.6
62.5
250
1000
2
4
6
8
10
12
14
2
1
14
10
4
1
1
1
3
5
1
3
2

FIGURE 3

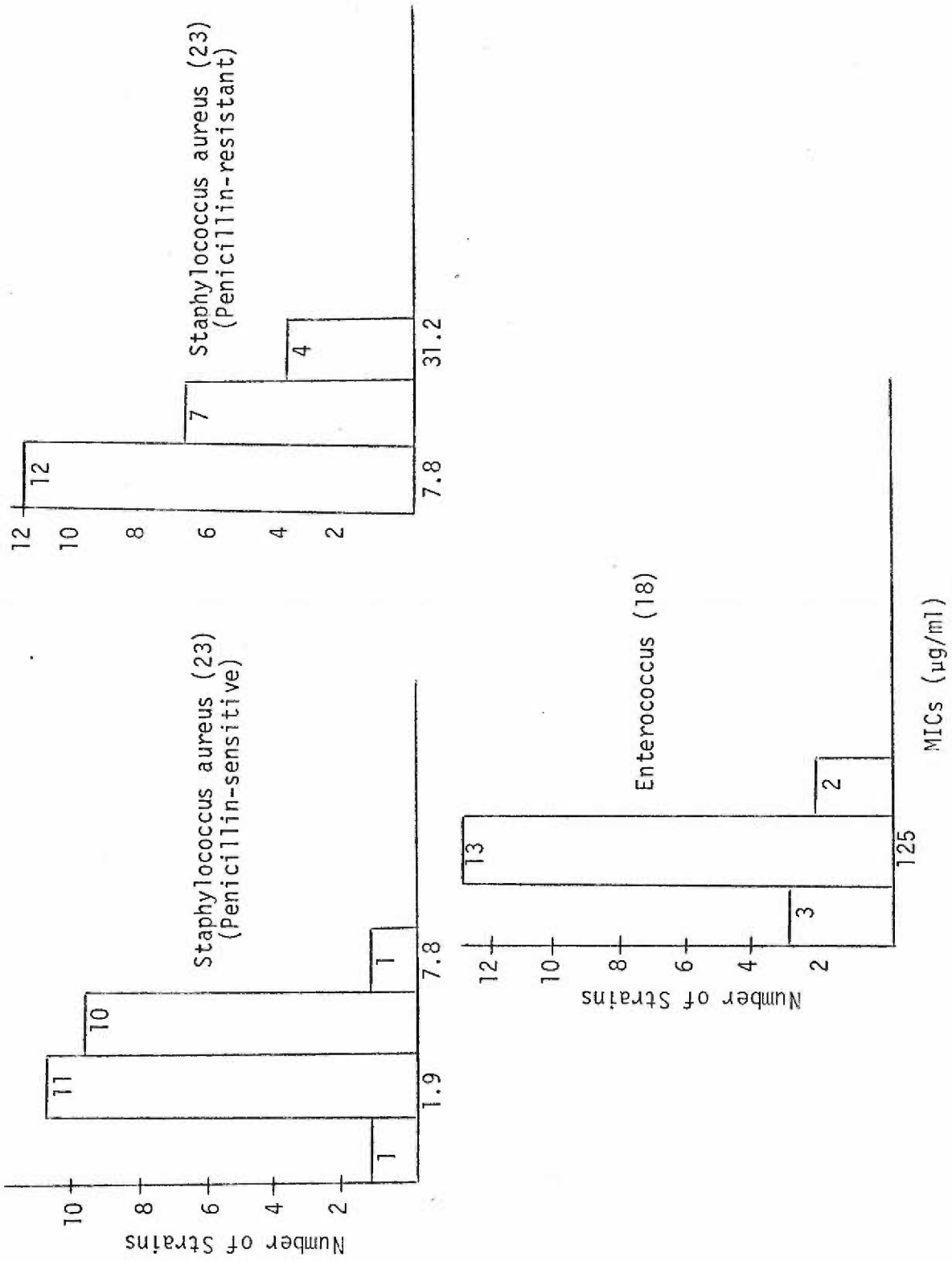

Staphylococcus aureus (23)
(Penicillin-resistant)
12
7
4
1
7.8
31.2
Staphylococcus aureus (23)
(Penicillin-sensitive)
1
11
10
1
1.9
7.8
Enterococcus (18)
2
13
3
2
125
Number of Strains
MICs (µg/ml)

FIGURE 4

TABLE 2

AGAR DILUTION MICs
OF
58 MISCELLANEOUS ORGANISMS

	0.025	0.4	0.9	1.9	3.9	7.8	15.6	31.2	62.5	125	250	500	1000[a]
Achromobacter												1[b]	
Aeromonas								1					
Alkaligenes				3			1		1				
Arizona			1	1									
Beta-Hemolytic Streptococcus Group A		8		1	1								
Bordetella bronchiseptica			1										
Citrobacter				1									
Corynebacterium diphtheriae						2	3						
EO-1								1			2	3	1
Erysipelothrix Insidiosa		1											
Group I-B									1				
Group IV-C								1					
Group IV-F										2			1
Herrellea								1					
HB-1	1												
Pasteurella		2											
Pneumococcus				3	1								
Salmonella					4	1	1						
Shigella			3	1		1							

a- MICs; b- Number of strains

TABLE 3

'FOLD RELATIONSHIP OF BROTH MIC TO AGAR MIC

Organism	-4 or <	-2	0	+2	+4 or >
Escherichia coli	1[c]		5	10	4
Enterobacter		2	4	8	4
Klebsiella			13	2	3
Serratia			2	8	10
Indol pos. Proteus[a]	1	2	8	6	10
Providencia		2	11	6	
Pseudom. aeruginosa		1	5	11	13
Pseudom. species			3	5	5
Staph. aureus (Pen-R)[b]	2	7	8	3	
Staph. aureus (Pen-S)		2	8	9	1
Enterococcus		2	14	2	
Total	4	18	81	70	50

+2 = Broth dilution MIC is twice that of agar dilution MIC.

a - Indol positive Proteus

b - Penicillin-resistant Staphylococcus aureus

c - Number of strains

exhibited twofold differences and 54 strains showed fourfold or greater differences. There was a tendency for the broth dilution MICs to be slightly higher than the agar dilution MICs.

C. Effect of Duration of Incubation on Broth Dilution MICs

The effect of prolonging the duration of incubation (from 18 hours to 42 hours) on the broth dilution MICs of 260 bacterial strains is illustrated in Table 4. There was no change of MIC values in 174 strains. Seventy-seven strains showed only a twofold increase in MIC with the longer incubation period and 9 strains had a fourfold or greater increase. These findings show that MICs determined by the broth dilution method may increase with prolongation of the incubation time.

D. 'Fold Relationship of Broth MIC to MBC

Table 5 shows the 'fold relationship of the broth MIC to the MBC. In 217 out of 249 bacterial strains tested the inhibitory concentrations were equal to the bactericidal concentrations. The MBC was greater than the MIC by twofold in 22 strains and by fourfold or more in 7 strains. In only 3 strains was the MBC twofold lower than the MIC. These findings support the fact that carbenicillin is a bactericidal agent. In bacteriostatic drugs the MBCs are much higher than the MICs.

E. Effect of Enriching MH Medium with Blood or Heated Blood on Results of Agar Dilution and Agar Diffusion Tests

Certain fastidious organisms such as Streptococcus and Hemophilus require media containing blood or heated blood for optimum growth. We

TABLE 4

INCREASE IN BROTH DILUTION MICs
BETWEEN 18 HR AND 42 HR INCUBATION

Organism	'Fold Increase in MIC		
	0	+2	+4 or >
Escherichia coli	15[c]	3	1
Enterobacter	15	4	1
Klebsiella	19		
Serratia	15	5	
Proteus mirabilis	13	7	
Indol pos. Proteus[a]	27	11	
Providencia	15	5	
Pseudom. aeruginosa	17	11	2
Pseudom. species	4	7	2
Staph. aureus (Pen-R)[b]	11	7	2
Staph. aureus (Pen-S)	16	4	
Enterococcus	7	12	1
Total	174	77	9

+2 = MIC at 42 hr is twice that at 18 hr.

a - Indol positive Proteus

b - Penicillin-resistant Staphylococcus aureus

c - Number of strains

TABLE 5

'FOLD RELATIONSHIP OF BROTH MIC TO BROTH MBC

Organism	-4 or <	-2	0	+2	+4 or >
Escherichia coli			18[c]	1	1
Enterobacter			13		
Klebsiella			18		
Serratia			17	3	
Prot. mirabilis			17	2	1
Indol pos. Proteus[a]		1	31	4	1
Providencia			18	2	
Pseudom. aeruginosa			29		1
Pseudom. species			11	1	1
Staph. aureus (Pen-R)[b]			15	3	2
Staph. aureus (Pen-S)			15	5	
Enterococcus		2	15	1	
Total		3	217	22	7

+2 = Broth MBC is twice the broth MIC.

a - Indol positive Proteus

b - Penicillin-resistant Staphylococcus aureus

c - Number of strains

investigated the influence of enriching the MH medium with blood on the MICs obtained with the agar dilution test. MICs were determined for 62 bacterial strains on three different media: plain MH agar, MH agar enriched with 5% blood, and MH agar enriched with 5% heated or "chocolatized" blood. The bacterial strains tested included 8 Pseudomonas aeruginosa, 6 Pseudomonas species, 7 Klebsiella, 5 Enterobacter, 2 Escherichia coli, 5 Serratia, 4 Proteus morganii, 4 Proteus rettgeri, 5 Providencia, 5 penicillin-resistant and 5 penicillin-sensitive Staphylococcus aureus. The results illustrated in Table 6 show that for most strains (68%) the MICs obtained on all three media were the same. The differences observed in the remaining 32% of the strains tended to fall within ± twofold variations. These results support the view that enriching the medium with blood produces very little or no variation in the MIC values.

The above 62 bacterial strains were also tested by the agar diffusion method using plain MH agar and MH agar enriched with blood or heated blood. The addition of blood to the MH medium produced no significant variations in the diameters of zones of inhibition produced around carbenicillin discs. The variations when present were in the range of ± 2 mm. These results suggest that there is no significant binding or inactivation of carbenicillin by blood.

F. Effect of Altering Inoculum Size or Agar Dilution MICs

MICs were determined for 30 bacterial strains utilizing three different inocula. The strains tested included 2 Enterobacter, 2 enterococci, 4 Klebsiella, 2 Proteus morganii, 2 Proteus rettgeri,

TABLE 6

EFFECT OF ENRICHING MH MEDIUM WITH BLOOD OR HEATED BLOOD ON AGAR DILUTION MICs

	'Fold Change in MIC				
	-4	-2	0	+2	+4
Blood M.H.		13[a]	43	6	
Chocolate M.H.	1	18	39	4	

Total: 62 bacterial strains

+2 = MH agar dilution MIC is twice that of enriched agar MIC.

a - Number of strains

3 Providencia, 3 Pseudomonas aeruginosa, 3 Pseudomonas species, 3 Serratia, 3 penicillin-sensitive and 3 penicillin-resistant Staphylococcus aureus.

The first inoculum was the one routinely used in the agar dilution test (0.5×10^7 organisms/ml). This represented a 1:20 dilution of a bacterial suspension adjusted to an opacity equivalent to a 0.5 McFarland standard. The second inoculum was lighter (0.5×10^6 organisms/ml) and it represented a 1:200 dilution of the 0.5 McFarland standard. The third inoculum was heavier (10^8 organisms/ml) and it was prepared by standardizing the bacterial suspension to an opacity equivalent to a 0.5 McFarland standard.

Table 7 illustrates the 'fold relationship of MICs obtained with the lighter and heavier inocula with MICs determined by the routinely used inoculum. It can be seen that when a lighter inoculum was used minimum variations were observed in the MIC values. The MICs were identical with those obtained with the routine inoculum in 25 strains. A twofold difference was found in the remaining 5 strains. The use of a heavier inoculum, on the other hand, tended to produce MIC values that were higher than those obtained with the routine inoculum. A twofold increase in the MICs was observed in 11 strains and a fourfold or greater increase was found in 7 other strains. In the remaining 12 strains the MICs were equal to the values obtained with the routine inoculum. These observations illustrate how endpoints may be influenced by changes in experimental conditions.

TABLE 7

EFFECT OF ALTERING INOCULUM SIZE (0.5×10^7 org./ml) ON AGAR DILUTION MICs

	'Fold Change in MIC						
	-2	0	+2	+4	+8	+16	+32 or >
0.5×10^6 org./ml	4[a]	25	1				
10^8 org./ml		12	11	2	2	1	2

+2 = MIC is twice that obtained using an inoculum of 0.5×10^7 org./ml.

Total: 30 bacterial strains

a - Number of strains

G. Inactivation of Carbenicillin

Sixty-eight bacterial strains were tested for their ability to inactivate carbenicillin. The organisms selected for this experiment belonged to two main groups: (a) fifty-five bacterial strains that were resistant to carbenicillin with MICs of 250 µg/ml or greater and (b) seven bacterial strains that exhibited a fourfold or greater rise in MICs with an increase in the inoculum size. In addition, 6 Staphylococcus aureus strains were tested, 3 strains were sensitive to penicillin and 3 were resistant. All of these strains had carbenicillin MICs of 31.2 µg/ml or less and they exhibited a twofold rise in MIC when the heavier inoculum was used in the agar dilution test.

The reduction in the diameter of the zones of inhibition around the wells containing the organism-carbenicillin mixture from that around the control well containing carbenicillin alone was taken as a measure of the degree of inactivation of carbenicillin (see Materials and Methods). With the organisms tested we encountered the following three patterns: (a) the zone of inhibition was totally abolished, (b) the zone of inhibition was reduced by 15 mm or less, and (c) the zone of inhibition remained unchanged.

We arbitrarily divided the bacterial strains into four categories and utilized the following criteria for measuring the degree of inactivation of carbenicillin: (a) Maximum inactivation: inactivation was considered maximum when the zone of inhibition was totally abolished as a result of incubating the organism with the carbenicillin solution; (b) Minimum inactivation: inactivation was considered

minimum when the zone of inhibition around the well containing the organism-carbenicillin mixture was reduced by 5-15 mm; (c) Questionable inactivation: inactivation was considered questionable when there was a reduction by less than 5 mm of the zone of inhibition around the well containing the organism-carbenicillin mixture; and (d) No inactivation: inactivation was not present when the zone of inhibition around the well containing the organism-carbenicillin mixture was the same as that produced around the wells with the carbenicillin solution alone.

The results in Table 8 show that 26 out of the 67 bacterial strains tested did not inactivate carbenicillin. Inactivation was maximum for 16 strains, minimum for 12 and questionable for 13. It is of interest to note that the 3 penicillinase producing Staphylococcus aureus strains tested showed a maximum degree of inactivation of carbenicillin. On the other hand, the non-penicillinase producing Staphylococcus aureus did not inactivate carbenicillin at all as determined by this test.

H. Stability of Carbenicillin Discs

The 100 µg and 50 µg carbenicillin discs stored at -60°C and -20°C with and without dessicant showed minimum deterioration over a period of 16 weeks. Diameters of the zones of inhibition were reduced on the average by 2-2.5 mm during this period (Figures 5 and 6). The discs stored at room temperature with dessicant exhibited a reduction in the diameters of zones of inhibition averaging 2.5-3.5 mm (Figure 7). On the other hand, those discs kept at room temperature without dessicant

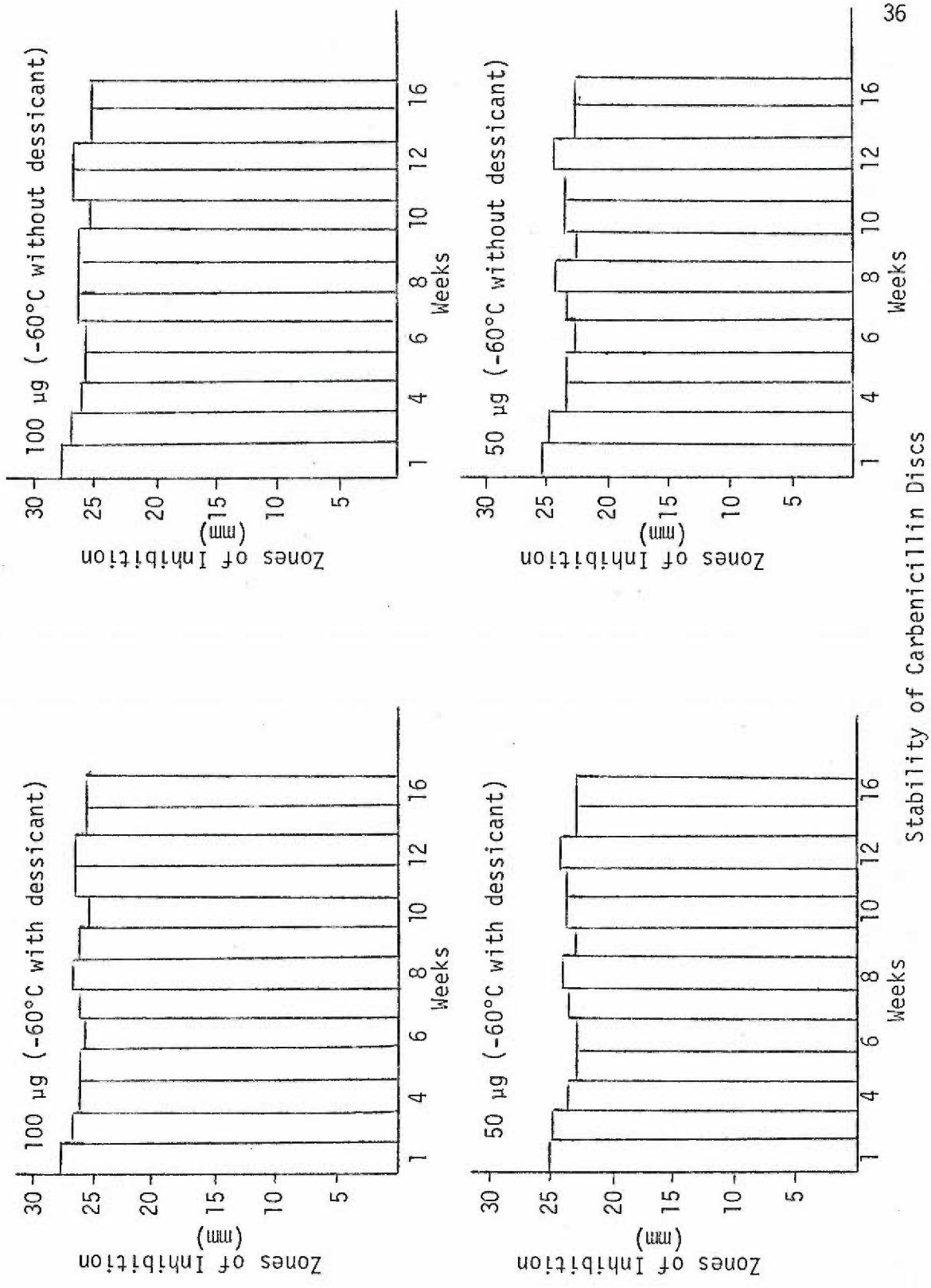
TABLE 8

INACTIVATION OF CARBENICILLIN BY 68 BACTERIAL STRAINS

Organisms	Number of Strains	MIC (µg/ml)	Degree of Inactivation			
			Maximum	Minimum	Questionable	None
Escherichia coli	1	250 or >	1[a]			
Klebsiella	16	" "	7	3	4	2
Proteus mirabilis	5	" "	2	3		
Proteus rettgeri	2	" "		1	1	
Proteus morganii	3	" "			1	2
Providencia	11	" "	3	4	3	1
Pseudom. aeruginosa	4	" "				4
Pseudom. species	5	" "			1	4
EO-1	6	" "			2	4
Enterococcus	1	" "			1	
Group IV-F	1	"		1		
Staphylococcus aureus (penicillin-resistant)	3	31.2 or <	3			
Staphylococcus aureus (penicillin-sensitive)	3	3.9 or <				3
Other organisms[b]	7	31.2 or <				7
Total	68		16	12	13	27

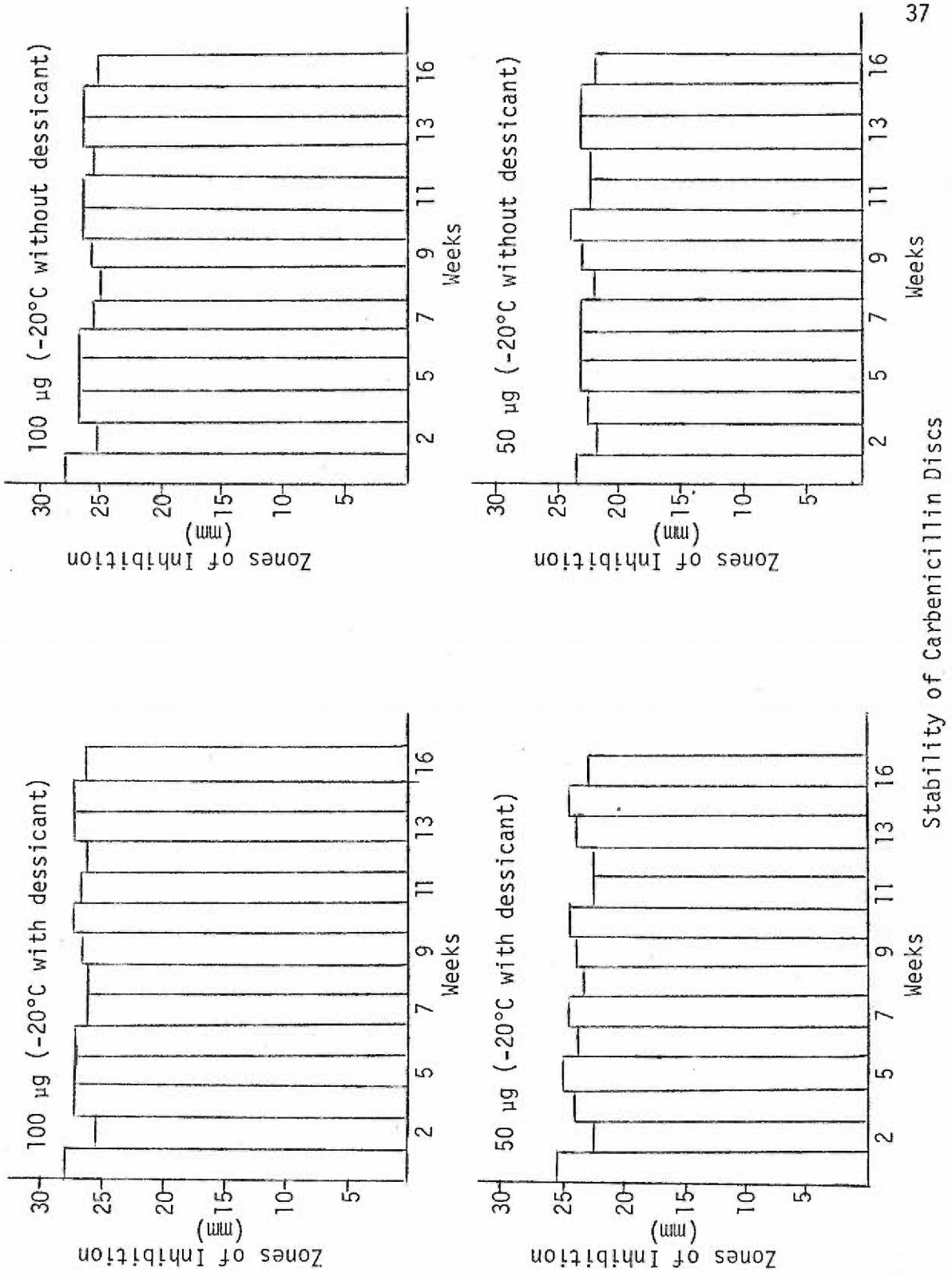
a - Number of strains.

b - These 7 strains are the ones that exhibited fourfold or greater rise in MICs when a heavy inoculum was used. They included 2 Enterobacter, 2 Serratia, 1 Proteus rettgeri, 1 Pseudomonas species, and 1 Providencia.



Stability of Carbenicillin Discs

FIGURE 5



Stability of Carbenicillin Discs

FIGURE 6

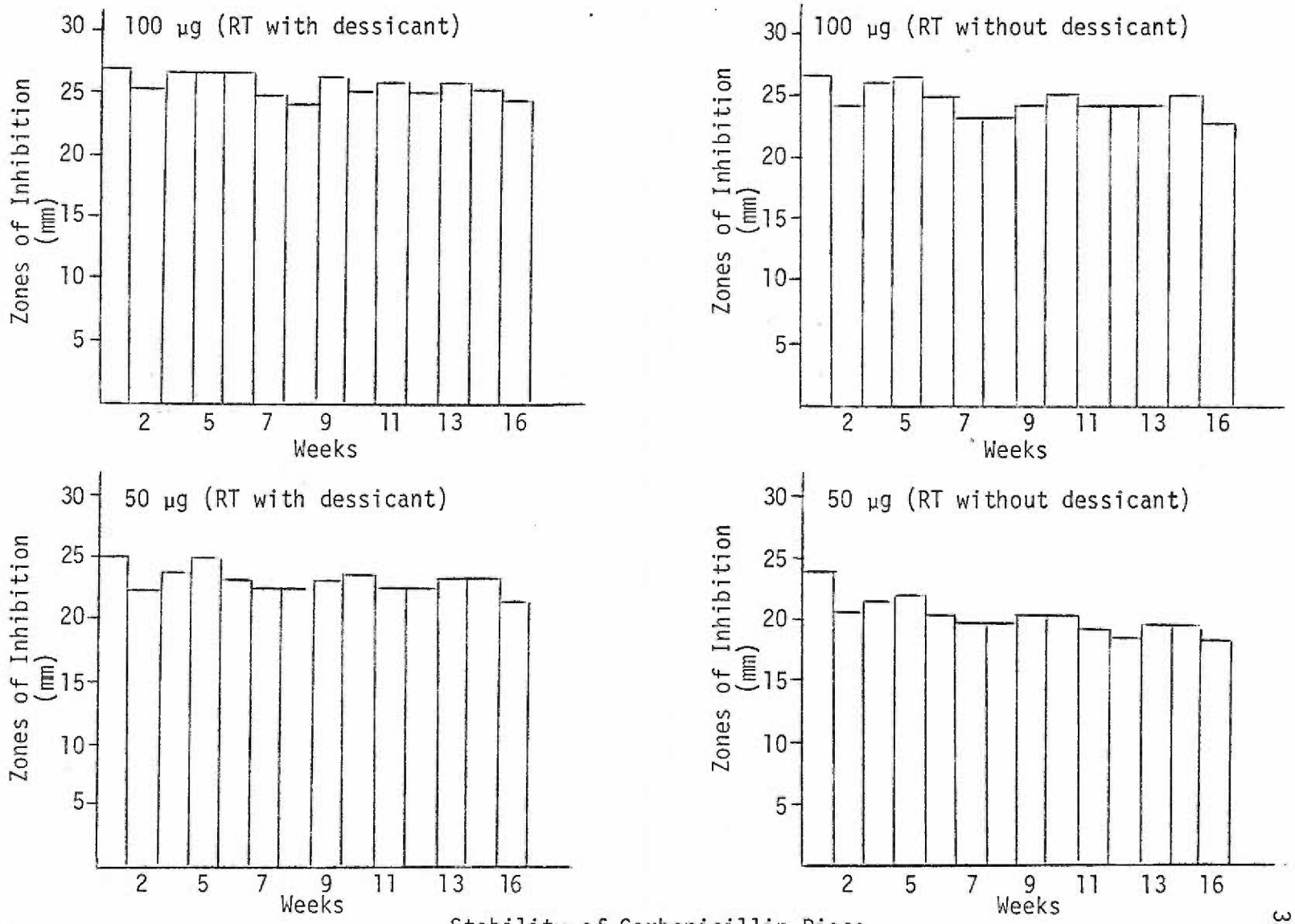


FIGURE 7

Stability of Carbenicillin Discs

showed evidence of deterioration manifested by a reduction of 4-5 mm at the end of the 16 week period. Discs stored at 37°C showed the greatest loss in potency (Figure 8). Those discs kept with dessicant at 37°C showed a reduction in the zone sizes of 4.5-7 mm while those discs kept without dessicant exhibited a 9-16 mm decrease in the zones of inhibition. These results clearly show that the carbenicillin discs are quite stable when stored at -20°C or -60°C for at least a period of 16 weeks.

I. Relationship Between Results of Agar Dilution and Agar Diffusion Tests

The results of diffusion tests cannot be interpreted in quantitative terms from knowledge only of the chemotherapeutic content of the discs because of marked variation in diffusibility of different antibiotics. It is necessary to compare zone sizes obtained by the agar diffusion tests with MICs determined by dilution tests. A total of 254 organisms were tested by the agar dilution and agar diffusion methods. A comparison of the zone sizes obtained with 100 µg, 50 µg, and 30 µg carbenicillin discs with MICs derived from agar dilution tests is illustrated in Figures 9-12. It can be seen that an inverse linear relationship exists between log MICs and zone sizes over a wide range of concentrations. From such regression lines one can easily determine what zone sizes represent susceptibility and which ones represent resistance.

Carbenicillin is used in high doses (30-40 grams per day) for treatment of Pseudomonas infections and in a much lower dose (6-8 grams per

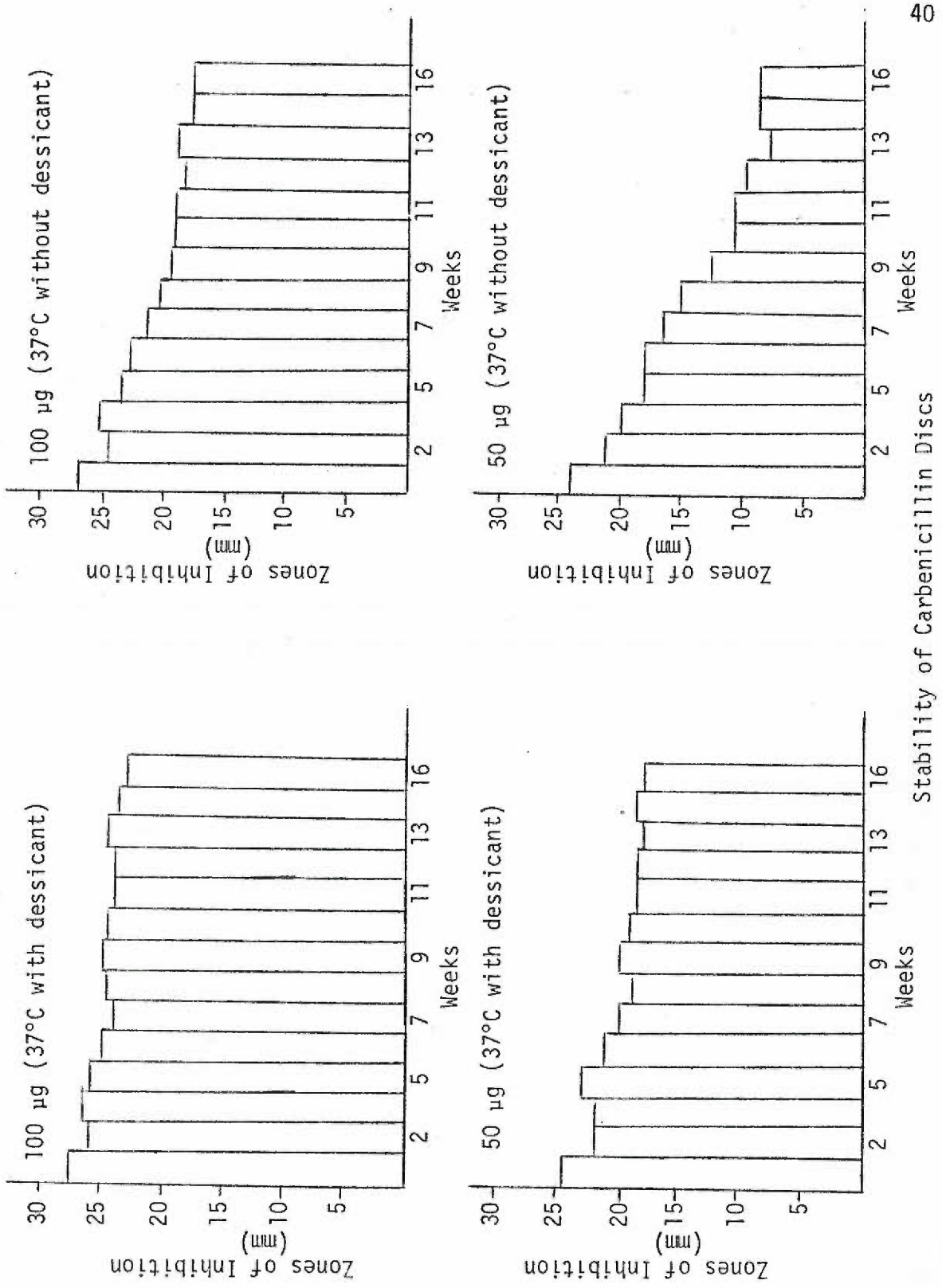

Stability of Carbenicillin Discs
100 µg (37°C without dessicant)
50 µg (37°C without dessicant)
100 µg (37°C with dessicant)
50 µg (37°C with dessicant)
Zones of Inhibition (mm)
Weeks
30
25
20
15
10
5
2
5
7
9
11
13
16

FIGURE 8

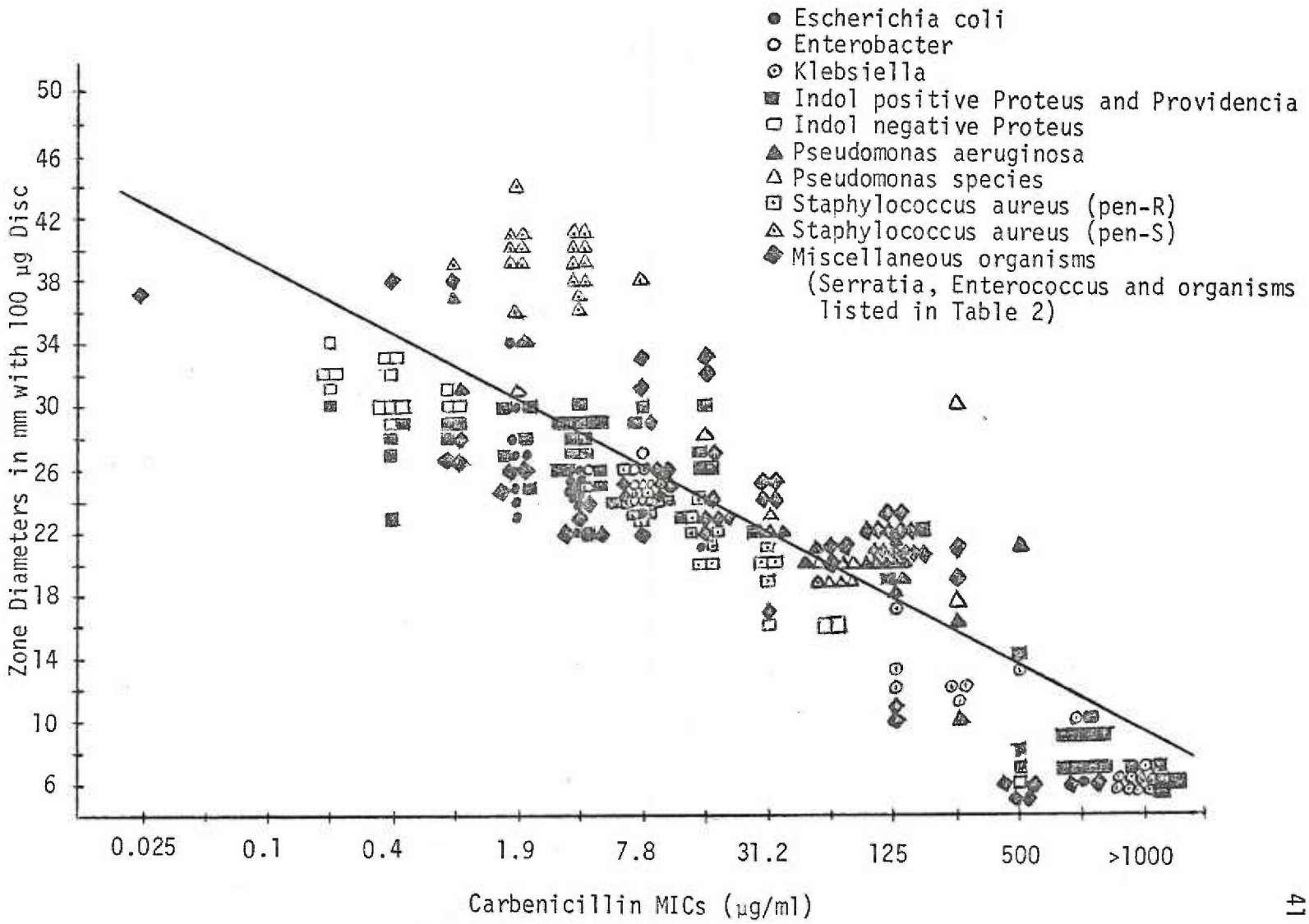

Escherichia coli
Enterobacter
Klebsiella
Indol positive Proteus and Providencia
Indol negative Proteus
Pseudomonas aeruginosa
Pseudomonas species
Staphylococcus aureus (pen-R)
Staphylococcus aureus (pen-S)
Miscellaneous organisms
(Serratia, Enterococcus and organisms listed in Table 2)
Zone Diameters in mm with 100 µg Disc
50
46
42
38
34
30
26
22
18
14
10
6
0.025
0.1
0.4
1.9
7.8
31.2
125
500
>1000
Carbenicillin MICs (µg/ml)

FIGURE 9

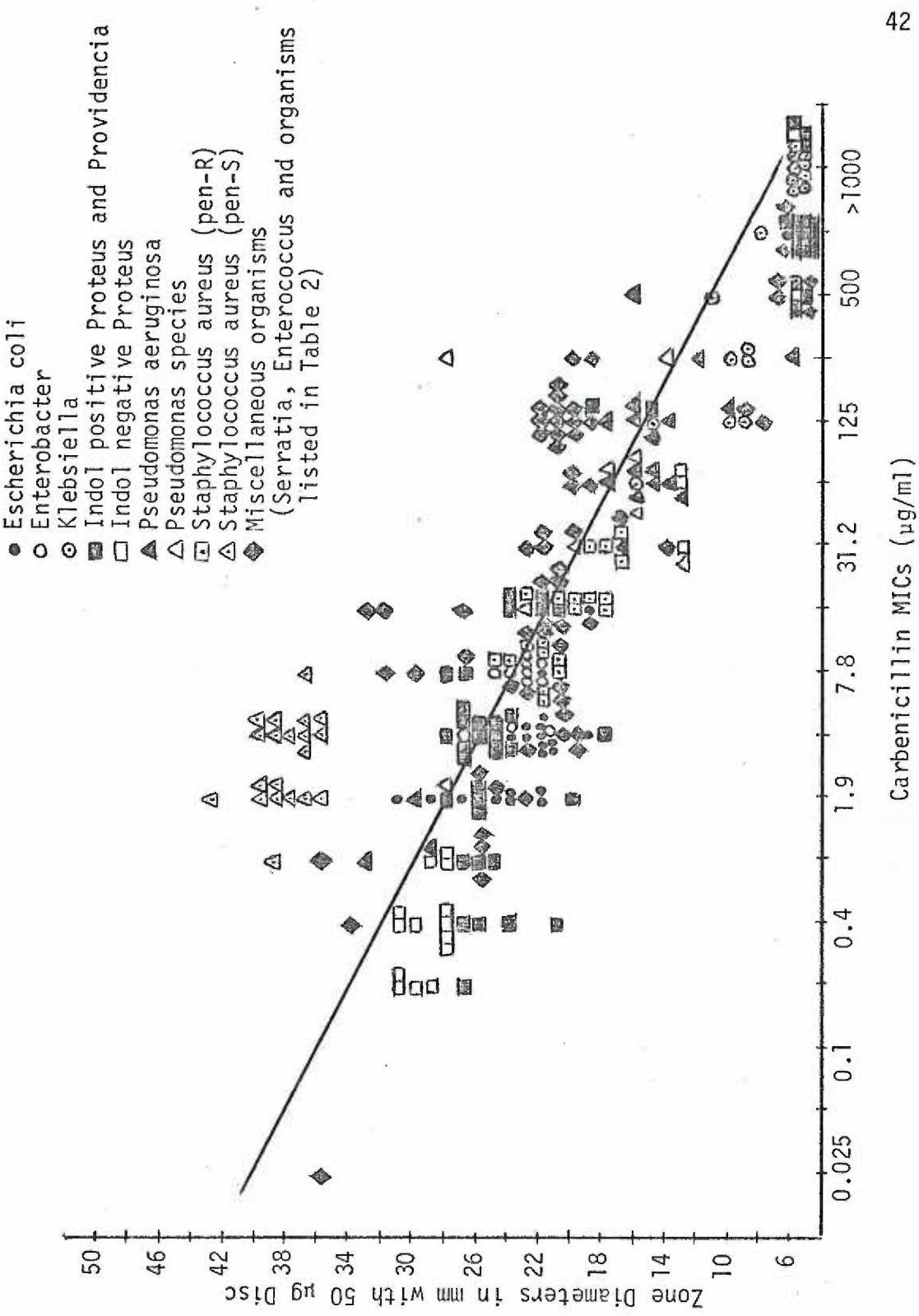

Escherichia coli
Enterobacter
Klebsiella
Indol positive Proteus and Providencia
Indol negative Proteus
Pseudomonas aeruginosa
Pseudomonas species
Staphylococcus aureus (pen-R)
Staphylococcus aureus (pen-S)
Miscellaneous organisms
(Serratia, Enterococcus and organisms listed in Table 2)
Zone Diameters in mm with 50 µg Disc
50
46
42
38
34
30
26
22
18
14
10
6
0.025
0.1
0.4
1.9
7.8
31.2
125
500
>1000
Carbenicillin MICs (µg/ml)

FIGURE 10

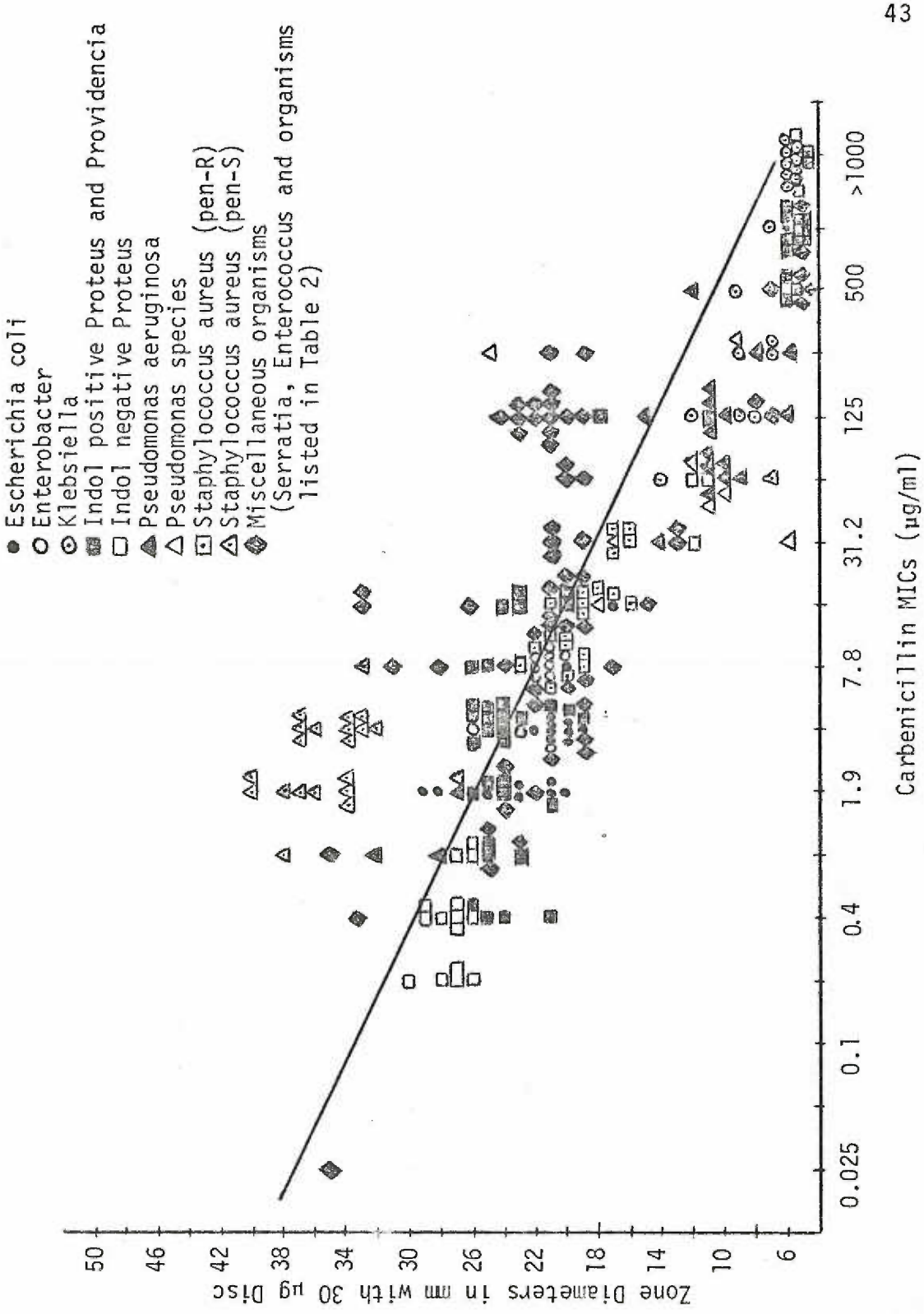

● Escherichia coli
○ Enterobacter
⊙ Klebsiella
■ Indol positive Proteus and Providencia
□ Indol negative Proteus
▲ Pseudomonas aeruginosa
△ Pseudomonas species
⊡ Staphylococcus aureus (pen-R)
◬ Staphylococcus aureus (pen-S)
◆ Miscellaneous organisms
(Serratia, Enterococcus and organisms
listed in Table 2)
Zone Diameters in mm with 30 µg Disc
50
46
42
38
34
30
26
22
18
14
10
6
0.025
0.1
0.4
1.9
7.8
31.2
125
500
>1000
Carbenicillin MICs (µg/ml)

FIGURE 11

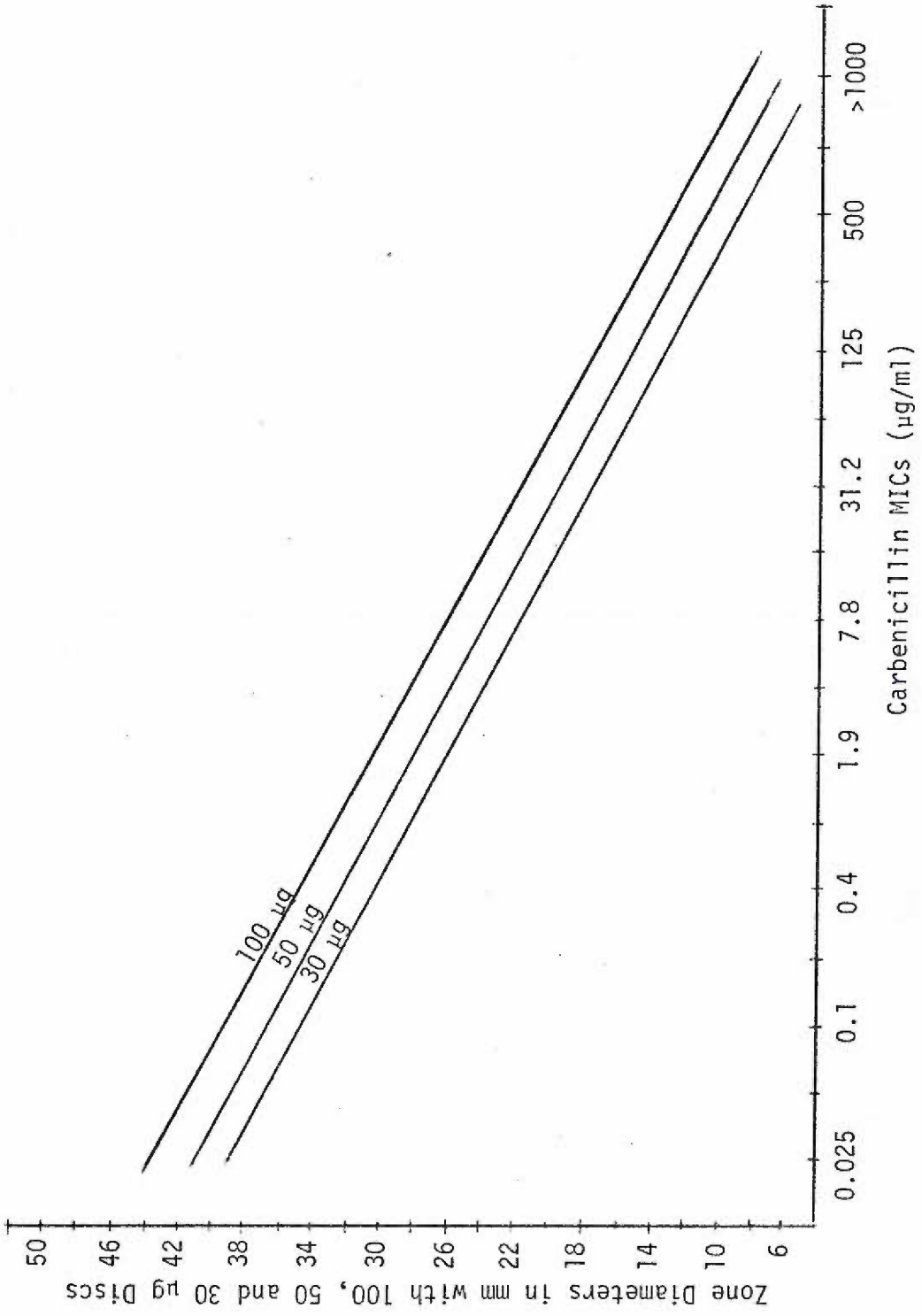

Zone Diameters in mm with 100, 50 and 30 µg Discs
50
46
42
38
34
30
26
22
18
14
10
6
100 µg
50 µg
30 µg
0.025
0.1
0.4
1.9
7.8
31.2
125
500
>1000
Carbenicillin MICs (µg/ml)

FIGURE 12

day) for treatment of infections with other bacteria such as Escherichia coli. The blood level attained with the high dose schedule is about 125 µg/ml and that with the low dose is usually in the range of 16 µg/ml. Thus a Pseudomonas strain with a carbenicillin MIC value less than 125 µg/ml is considered as susceptible. It is evident from the regression lines illustrated in Figure 9 that such a Pseudomonas strain will exhibit a zone of inhibition measuring 18 mm or greater around the 100 µg carbenicillin disc.

J. Proposed Criteria for Determining Susceptibility to Carbenicillin

Table 9 shows our proposed criteria for determining susceptibility to carbenicillin by the agar diffusion technique using the 100, 50, and 30 µg discs. We utilized two different criteria, one for Pseudomonas and a second one for the other organisms. This is because, as mentioned earlier, carbenicillin is used in a relatively high dosage for the treatment of Pseudomonas infections and subsequently a high blood level is usually attained. It can be seen that with the 100 µg carbenicillin disc a zone of inhibition measuring 18 mm or more (corresponding to an MIC value of less than 125 µg/ml) is a criterion of susceptibility for Pseudomonas. For the other organisms a zone of 25 mm or greater (corresponding to an MIC value of 15.6 µg/ml or less) around the 100 µg disc is indicative of susceptibility.

Results of the agar diffusion tests may be interpreted as susceptible, intermediate, or resistant. The "intermediate" class serves as a buffer zone. An organism classified as intermediate may be treatable with carbenicillin. It is advisable, however, to carry

TABLE 9

PROPOSED CRITERIA FOR CARBENICILLIN SUSCEPTIBILITY
BY THE AGAR DIFFUSION METHOD

	Disc Potency	Resistant >250 µg/ml	Intermediate 125-250 µg/ml	Sensitive <125 µg/ml
Pseudomonas	100	14mm or <	15-17mm	18mm or >
	50	12mm or <	13-15mm	16mm or >
	30	10mm or <	11-13mm	14mm or >

	Disc Potency	Resistant >45 µg/ml	Intermediate 15.6-45 µg/ml	Sensitive <15.6 µg/ml
All Other Organisms	100	19mm or <	20-24mm	25mm or >
	50	17mm or <	18-22mm	23mm or >
	30	16mm or <	17-20mm	21mm or >

out MIC and blood level determinations in such cases. If the MIC is below the level attained in the blood, carbenicillin can be used for therapy.

K. Agar Diffusion Susceptibility of 1562 Bacterial Strains Isolated in the Service Laboratory

Tables 10 and 11 illustrate the susceptibility pattern of 952 gram-negative and 610 gram-positive bacterial strains isolated in our service laboratory as determined by the agar diffusion test utilizing the 100 µg carbenicillin disc. It can be seen in Table 10 that 80% of the Pseudomonas aeruginosa and 66% of the Escherichia coli strains were susceptible. With the exception of Proteus vulgaris, the majority of the other Proteus strains were susceptible to carbenicillin. The large percentage of the Mima polymorpha and Herrellea strains were found to be intermediate.

As shown in Table 11 the majority of the beta-hemolytic streptococcus group A (99%), beta-hemolytic streptococcus not group A or D (96%) and Pneumococcus (93%) were susceptible to carbenicillin. All of the penicillin-sensitive Staphylococcus aureus and Streptococcus viridans were susceptible. On the other hand, the majority of the penicillin-resistant Staphylococcus aureus (80%) and enterococcus (66%) were intermediate to carbenicillin.

TABLE 10

SUSCEPTIBILITY OF 952 GRAM-NEGATIVE BACTERIAL STRAINS ISOLATED IN THE SERVICE LABORATORY OF THE UNIVERSITY OF OREGON MEDICAL CENTER AS DETERMINED BY THE AGAR DIFFUSION TEST USING 100 µg CARBENICILLIN DISCS

Organisms	No. of Strains	Susceptible (%)	Intermediate (%)	Resistant (%)
Pseudom. aeruginosa	147	80	11	9
Pseudom. species	19	68	11	21
Escherichia coli	393	66	22	12
Klebsiella	190	1	7	92
Citrobacter	15	20	53	27
Proteus mirabilis	118	81	5	14
Proteus morganii	24	79	13	8
Proteus rettgeri	4	100		
Proteus vulgaris	6	17	33	50
Providencia	2	50		50
Herrellea	24	8	79	13
Mima polymorpha	3	33	67	
Bacteroides	4	75		25
EO-1	3	33	33	33

TABLE 11

SUSCEPTIBILITY OF 610 GRAM-POSITIVE BACTERIAL STRAINS ISOLATED IN THE SERVICE LABORATORY OF THE UNIVERSITY OF OREGON MEDICAL CENTER AS DETERMINED BY THE AGAR DIFFUSION TEST USING 100 µg CARBENICILLIN DISCS

Organisms	No. of Strains	Susceptible (%)	Intermediate (%)	Resistant (%)
Staph. aureus (Pen-R)[a]	251	6	80	14
Staph. aureus (Pen-S)[b]	134	100		
Beta Strep. Group A[c]	78	99	1	
Beta Strep. not Group A or D	48	96	4	
Enterococcus	73	29	66	5
Streptococcus viridans	12	100		
Pneumococcus	14	93	7	

a - Penicillin-resistant Staphylococcus aureus

b - Penicillin-sensitive Staphylococcus aureus

c - Beta-hemolytic streptococcus Group A

DISCUSSION

The results reported in this thesis show that carbenicillin was active against the majority of the Escherichia coli, Enterobacter, Serratia, Proteus (with the exception of Proteus vulgaris) and Pseudomonas strains isolated in our laboratory. Ninety-six percent of the Escherichia coli were susceptible to carbenicillin concentrations of 15.6 µg/ml or less. These results are consistent with the findings of other investigators. Eighty percent of the Escherichia coli studied by Williams and Glasgow (50) were susceptible to concentrations of 12.5 µg/ml or less. Likewise, Stratford (43) and Standiford, Kind and Kirby (42) found that approximately 90% of the Escherichia coli strains they tested had MICs of 12.5 µg/ml or less. English (10) also reported that 74% of 46 strains studied in his laboratory were inhibited by carbenicillin concentrations of 12.5 µg/ml or less. Of 181 strains tested by Rolinson and Sutherland (38) 82% had MICs of 12.5 µg/ml or less. Labowitz and Holloway (20), on the other hand, found that carbenicillin concentrations of 12.5 µg/ml inhibited only 54% of the Escherichia coli strains they examined; the remaining 46% were inhibited by 25 µg/ml to greater than 100 µg/ml. The inoculum size used by the latter investigators was not standardized, it consisted of 1:1000 dilution of an overnight broth culture.

All of the Enterobacter we tested by the agar dilution method had MICs of 7.8 µg/ml or less. Marks and Eickhoff (27), on the other hand, reported that only 20% of 42 Enterobacter strains were inhibited by 12.5 µg/ml or less. These workers, however, did not standardize the inoculum; they used an undiluted overnight broth culture. Other investigators have determined MICs for the Enterobacter-Klebsiella group and

found that the majority of the strains were resistant in these studies (28, 40). However, no attempt was made to distinguish the Enterobacter from Klebsiella. The majority of the strains tested may well have been Klebsiella, an organism known to be highly resistant to carbenicillin.

We found that only 1 of 19 Serratia was resistant (MIC = 500 µg/ml); the remaining strains were inhibited by concentrations of 31.2 µg/ml or less. Standiford et al. (42) reported that 75% of 12 Serratia strains were susceptible to 12.5 µg/ml or less. Williams and Glasgow (50), however, found that 80% of 47 Serratia strains were inhibited by 25 to 500 µg/ml. Likewise, all but 1 of 35 strains tested by Marks and Eickhoff (27) had MICs of 12.5 µg/ml or greater. Similarly, the studies of Meyers, Sabbaj and Weinstein (28) have also shown that carbenicillin was not active against Serratia. It is of interest to note, however, that the inoculum used by Marks and Eickhoff and by Meyers et al. was not standardized. The high MICs reported for Serratia by these workers may be a result of the heavy inoculum employed in their studies. It is also possible that the majority of the Serratia found in their hospitals may be resistant to carbenicillin.

Our Proteus and Providencia showed two distinct populations, a susceptible one and a highly resistant one. The majority of Proteus mirabilis (75%), Proteus morganii (69%) and Proteus rettgeri (72%) strains were susceptible to carbenicillin concentrations of 15.6 µg/ml or less. The Proteus vulgaris, on the other hand, was relatively resistant with MICs of 31.2 µg/ml or greater. Williams and Glasgow (50) have similarly observed a bimodal distribution for 26 Proteus mira-

bilis strains; 21 strains had MICs of 12.5 µg/ml or less and 5 strains were inhibited by 25 to 500 µg/ml. Similar findings have also been reported by Brumfitt, Percival and Leigh (6) for 18 Proteus mirabilis strains. English (10), on the other hand, found that all of the 23 Proteus mirabilis he examined were susceptible to concentrations of 12.5 µg/ml or less. Smith and Finland (41), however, reported that the MICs were 400 µg/ml or greater for 36 Proteus mirabilis strains. It is of interest to note that the inoculum size used by Smith and Finland was higher than that employed in most other studies. The inoculum consisted of an undiluted overnight broth culture.

English (10) determined MICs for 9 Proteus morganii, 20 Proteus vulgaris and 3 Proteus rettgeri. A bimodal distribution was observed in the Proteus vulgaris and Proteus morganii. The MICs tended to be higher in the Proteus vulgaris; approximately 50% of them were inhibited by carbenicillin concentrations of 50-100 µg/ml. English also found that 66% of Proteus morganii and 100% of Proteus rettgeri were susceptible to 6.25 µg/ml or less. Brumfitt et al. (6) reported MICs for 27 indol positive Proteus including 8 Proteus vulgaris strains; all were inhibited by 6 µg/ml or less. Six of 8 indol positive Proteus species studied by Labowitz and Holloway (20) were found to be susceptible to 12.5 µg/ml or less. These investigators, however, did not speciate the Proteus strains they tested.

One half of the 20 Providencia we tested were inhibited by 15.6 µg/ml or less and the other half by 500 µg/ml or greater. Brumfitt et al. (6) determined carbenicillin MICs for 5 Providencia; all were

inhibited by 3 µg/ml or less. Standiford et al. (42) tested 3 strains of Providencia and found that their MICs were 2.5 µg/ml or less.

Our studies show that 55% of the Pseudomonas aeruginosa (33 strains) and 65% of the Pseudomonas species (17 strains) were inhibited by carbenicillin concentrations of 62.5 µg/ml or less. Traub and Raymond (46) tested 90 Pseudomonas aeruginosa and found that 80% were inhibited by 62.5 µg/ml or less. Similarly, Neu and Swarz (31) found that 84% of 77 Pseudomonas aeruginosa strains had MICs of 100 µg/ml or less. Rolinson (37) also reported that 88% of 111 Pseudomonas aeruginosa were inhibited by 12.5 to 50 µg/ml. Likewise, Meyers et al. (28) found that 93% of 40 strains had MIC values of 100 µg/ml or less. Standiford et al. (42) tested 40 Pseudomonas strains and found that 90% were inhibited by 25 to 100 µg/ml. Similar findings have also been reported by Acred et al. (2), Brumfitt et al. (6), Isenberg and Siegel (15), Jones and Lowbury (18), Knudsen et al. (19), Phair et al. (34), and Williams and Glasgow (50). Marks and Eickhoff (27), however, reported that 40% of 92 Pseudomonas aeruginosa strains were inhibited by 6.3 to 100 µg/ml. Similarly, only 28% of 126 strains tested by English (10) were susceptible to 62.5 µg/ml or less. Smith and Finland (41) have also found that only 5% of 71 Pseudomonas aeruginosa strains were inhibited by 100 µg/ml or less. The increased frequency of resistant Pseudomonas strains reported by the latter three groups of investigators may reflect a true resistance of their nosocomial strains. It may well also be a result of the relatively high inoculum they used in their susceptibility tests. A small number of resistant mutants

present in the population can result in high MIC values when a heavy inoculum is used in the broth dilution test.

Our Klebsiella strains exhibited a high degree of resistance to carbenicillin. Ninety-five percent were inhibited by concentrations equivalent to 125 µg/ml or greater. Brumfitt et al. (6) reported MIC values of 200 µg/ml or greater for all 5 Klebsiella strains they tested. Similarly, Labowitz and Holloway (20) found that 73% of their Klebsiella were inhibited by 100 µg/ml or more; the remaining 27% had MICs less than 100 µg/ml. Marks and Eickhoff (27) also reported that 90% of their strains were resistant with MICs of greater than 200 µg/ml. Nineteen of the 24 Klebsiella strains studied by Standiford et al. (42) were inhibited by carbenicillin concentrations of greater than 200 µg/ml. Similar results have been reported by other investigators (6, 15, 28, 40, 50).

All of the 23 non-penicillinase producing Staphylococcus aureus strains we tested were susceptible to carbenicillin concentrations of 7.8 µg/ml or less. The penicillinase producing strains had higher MICs ranging from 7.8 to 31.2 µg/ml. Rolinson and Sutherland (38) studied 7 penicillin-sensitive and 12 penicillin-resistant Staphylococcus aureus and found that the non-penicillinase producing strains were inhibited by 2.5 µg/ml or less. The MICs of the penicillinase producers were much higher, ranging from 12.5 to 125 µg/ml.

Eighty-nine percent of the 18 enterococcus strains we examined had MICs of 125 g/ml or less. Smith and Finland (41) found that the 35 enterococci they tested were susceptible to concentrations ranging

from 50 to 100 µg/ml. Approximately 90% of the strains studied by Marks and Eickhoff (27) were inhibited by 100 µg/ml or less. Silverblatt and Turck (40) have also reported that 75% of their enterococcus strains had MICs of 100 µg/ml or less.

Our data show that 6 Salmonella strains had MICs of 15.6 µg/ml or less and that 5 Shigella were inhibited by less than 15.6 µg/ml. Foz (12) and Rolinson (37) reported that their Salmonella and Shigella strains were inhibited by 5 to 12.5 µg/ml. Two of 8 Salmonella tested by Meyers et al. (28) had MICs of 50 µg/ml; the remaining 6 strains were inhibited by 6 µg/ml or less. These investigators also reported that 3 Shigella were susceptible to 25 µg/ml or less.

We found that 5 Corynebacterium diphtheriae and 4 Alkaligenes were inhibited by carbenicillin concentrations of 15.6 µg/ml or less. One Corynebacterium diphtheriae strain tested by Rolinson (37) had an MIC of 1.25 µg/ml. Meyers et al. (28) examined 3 Alkaligenes and found that 2 strains were susceptible to 6 µg/ml or less while 1 strain required 200 µg/ml for inhibition.

We found that 10 beta-hemolytic streptococcus group A and 4 Pneumococcus strains were susceptible to carbenicillin concentrations of 3.9 µg/ml or less. Smith and Finland (41) also have reported that 28 Pneumococcus strains had MICs of 0.8 µg/ml or less and that 96% of 51 beta-hemolytic streptococcus group A were inhibited by 3.1 µg/ml or less. Similar results have been reported by other investigators (10, 12, 28, 37).

Our results show that MICs obtained by the agar dilution test

correlate well with MICs determined by the broth dilution method. Seventy-five percent of the 223 bacterial strains tested by the two methods showed either identical MICs or twofold variations. The remaining 25% exhibited fourfold or greater differences. Our data also show that broth dilution MICs tended to be slightly higher than agar dilution MICs. Similar findings have been reported by Sherris, Rashad and Lighthart (39). These investigators tested 44 bacterial strains against ampicillin and cephalothin by the broth and agar dilution techniques. They found that the two methods gave MICs that varied by 1 dilution step or less in all but nine cases. They have also shown that MICs tended to be slightly higher in the broth dilution test. With cephalothin, for example, 40% of the strains exhibited slightly higher MICs when tested by the broth method.

The data reported in this thesis clearly illustrate that variations in the experimental conditions can influence the results of the dilution susceptibility tests. When a heavier inoculum (c 10^8 organisms/ml) was used in the agar dilution test, a rise of twofold or greater was observed in MIC values of 60% of the strains. Sherris et al. (39) pointed out the importance of standardizing the technique of _in vitro_ susceptibility tests. They found that a hundredfold decrease in the inoculum size used in the agar dilution test produced a twofold or greater reduction in ampicillin MICs for 45% of the bacterial strains tested. Similar results were also shown with cephalothin. Standiford et al. (42) reported that the size of the inoculum used in the testing of _Pseudomonas_ had a marked effect on the MICs of carbenicillin.

A rise in the inoculum size from 10^3 to 10^7 organisms/ml produced a twofold increase in the MIC values. Similarly, Meyers et al. (28) determined the carbenicillin MICs for 5 Pseudomonas strains utilizing two inocula, a 10^{-6} dilution of an overnight broth culture and a 10^{-2} dilution. A greater than fourfold rise in MIC values was found with the heavier inoculum in all 5 strains. Stratford (43) stressed the importance of standardizing the inoculum size in sensitivity testing with carbenicillin.

The tendency for the MIC values to rise with increase in inoculum size reflects the heterogeneity of the bacterial population we are dealing with. With heavier inocula there is a greater chance of encountering spontaneously occurring resistant mutants that have high MICs. The influence of an increase in the inoculum size on the MICs is most marked in those bacterial species that are capable of producing enzymes which inactivate the drug. For example, in the case of penicillinase producing Staphylococcus aureus, a larger amount of the enzyme is produced when a heavier inoculum is used and this subsequently leads to a greater degree of inactivation of penicillin.

It is of interest to note that when the inoculum we utilized in the agar dilution test was reduced from c 0.5×10^7 organisms/ml to c 0.5×10^6 organisms/ml very little influence on MIC values was observed. Only 4 of 30 bacterial strains showed a twofold decrease in MIC with the lighter inoculum. Similar findings have been reported by Williams and Glasgow (50). These investigators found that a reduction of the inoculum size from c 10^6 organisms to c 10^4 organisms

produced very little effect on the MICs of carbenicillin except with 3 Pseudomonas strains for which a two- to fourfold reduction in MIC was noted with the lighter inoculum.

Our data also show that when endpoints for the broth dilution test were recorded at the end of 42 hours instead of the routinely used 18 hour period, an increase in carbenicillin MICs of twofold or greater was observed in approximately 30% of the bacterial strains tested. Similar results have been reported for other antibiotics. For example, Sherris et al. (39) determined broth dilution MICs for 18 bacterial strains and recorded the endpoints after 12 and 24 hours of incubation. A rise of two- or fourfold in MIC values was observed in approximately two-thirds of the strains tested. Similar findings have also been reported for cephalothin. Wick (49) found that cephalothin MICs determined by the broth dilution technique tended to increase with prolonged incubation. He showed that this was due to antibiotic deterioration which permitted surviving organisms to grow out.

Our results show that MBC values of carbenicillin were identical to the MICs in 217 of 249 bacterial strains tested. Twenty-five other strains exhibited twofold variations and the remaining 7 showed fourfold or greater differences. Meyers et al. (28) have also shown very little difference between MIC and MBC values of carbenicillin. These findings are consistent with the fact that carbenicillin is a bactericidal drug. Similar findings have also been observed with ampicillin and cephalothin.[1] In the case of drugs that are primarily

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[1]Rashad, A.L. Personal Communication. Feb. 1971

bacteriostatic the levels required for killing the organism are extremely high, much higher than the levels required for inhibition. Such high levels are not normally attained *in vivo*.

Our tests utilizing MH agar and MH agar enriched with blood showed that the addition of blood to the medium produced little or no influence on carbenicillin MICs or on diameters of inhibition zones. The changes observed in MICs were within twofold variations. Likewise, the differences in the diameters of the zones of inhibition were in the range of ± 2 mm. These results show that fastidious microorganisms such as *Streptococci* and *Hemophilus* can be readily tested against carbenicillin by the agar dilution and agar diffusion methods utilizing MH medium enriched with blood or chocolatized blood. Our results also indicate that carbenicillin is not inactivated to any significant degree by blood. This is in agreement with the findings of Smith and Finland (41). These workers reported that enriching the medium with 50% serum did not significantly alter the carbenicillin MICs determined by the broth dilution test.

Antibiotics can be bound to serum proteins, particularly albumin. The degree of binding varies with different drugs. Antibiotics may be arbitrarily classified into three main groups. The protein binding of the first group is either low (less than 30%) or nil. Examples of these are kanamycin, ampicillin, and cephaloridine. The second group includes antibiotics which are moderately bound to proteins (30-70%). Examples of these are chloramphenicol, penicillin G, nafcillin, and carbenicillin. Several investigators have shown that

approximately 50-55% of carbenicillin is bound to serum proteins (1, 17, 19, 38). The third group are those antibiotics which exhibit a high degree of protein binding (greater than 70%). Examples are sulfonamide, cloxacillin, and novobiocin. The latter drug is one of the most highly protein-bound antibiotics known. As much as 99% of novobiocin is bound to serum proteins. The binding of antibiotics to proteins is significant both to the treating physician and to the laboratory worker. From the clinical point of view it is important to recognize that the bound portion of the antibiotic is inactive and incapable of diffusing into tissues (36). From the laboratory point of view a knowledge of the degree of protein binding is important when agar dilution or agar diffusion tests are performed with media enriched with blood. It appears that the addition of 5% blood to MH medium produces little or no change in the endpoints when dealing with antibiotics that exhibit little or moderate degrees of binding. On the other hand, antibiotics that are highly protein-bound tend to produce higher MIC values and smaller zones of inhibition when tested on media enriched with blood.

The data reported in this thesis show that the carbenicillin discs are stable when stored at -60°C or -20°C. The potency of the discs deteriorated when they were stored at room temperature or at 37°C particularly for those discs kept without a dessicant. Smith and Finland (41) have also provided evidence indicating that carbenicillin is a stable drug. Of the currently available penicillin and cephalosporin group of drugs, ampicillin, methicillin and cephalothin are

relatively unstable. Discs impregnated with these drugs are apt to deteriorate on storage and it is recommended that they be stored at -60°C with dessicant.[2] The instability of antibiotics poses a problem for the clinical laboratory. A drop in the potency of a disc with prolonged storage leads to smaller zones of inhibition and this may lead to erroneous interpretations of results of the susceptibility test. Lynn (24) in a report on the pharmaceutical aspects of semi-synthetic penicillins stated that the most important factor governing the stability of carbenicillin in the solid state is moisture content. He recommends that carbenicillin should be stored in the refrigerator. The storage of carbenicillin at higher temperatures, particularly in the presence of moisture, allows decarboxylation to take place with the production of benzyl penicillin.

Our results clearly show that certain bacterial strains are capable of inactivating carbenicillin in vitro. Sixteen of 68 strains investigated showed a maximum degree of inactivation and 12 exhibited minimum inactivation. This inactivation is probably due to the ability of the organisms to produce carbenicillinase. We did not attempt to isolate or characterize the enzyme. Several investigators, however, have shown that certain microorganisms are capable of producing carbenicillinase. Jack, Sykes and Richmond (16) have characterized in the Enterobacteriaceae four main types of beta-lactamases that are active against the penicillins and cephalosporins. Among the Pseudomonads, however, they identified three types of beta-lactamases. One

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[2]Sherris, J.C. Personal Communication. Feb. 1971

of these was an enzyme which was predominantly active against carbenicillin. Lowbury et al. (22) have also shown that certain carbenicillin-resistant strains of Pseudomonas aeruginosa were capable of producing an enzyme that inactivated the antibiotic. Similar results have been reported by other investigators (31, 38).

We found that resistance to carbenicillin was not always associated with the ability of bacteria to inactivate the antibiotic. Thirteen strains with MIC values of 250 µg/ml or greater failed to inactivate carbenicillin in vitro. These data support the view that resistance to carbenicillin is mediated by at least two mechanisms. One type of resistance is due to the production of carbenicillinase. The second type appears not to be dependent on carbenicillinase. We do not know its nature or the underlying mechanism. It is conceivable that this may be an innate type of resistance that is genetically determined. We recognized two main groups of enteric organisms in our study. One group was capable of inactivating carbenicillin and this is presumably due to the production of carbenicillinase. The second group failed to inactivate the antibiotic and it is not unlikely that such strains may possess an innate type of resistance. The Pseudomonas and Pseudomonas-like organisms such as EO-1 that we tested showed no evidence of inactivation of carbenicillin. Lowbury et al. (22), however, encountered two types of carbenicillin-resistant Pseudomonas; one type produced a beta-lactamase that inactivated the antibiotic and a second type that produced no detectable carbenicillinase. These investigators were able to show that these two types of Pseudomonas

belonged to different serotypes and had different phage patterns.

Our studies with the Staphylococcus aureus showed that the penicillin-resistant strains inactivated carbenicillin whereas the penicillin-sensitive strains did not. These results are in agreement with findings of other investigators who reported that carbenicillin is susceptible to staphylococcal beta-lactamase (6, 23).

Resistance of microorganisms to antibiotics creates a major problem for the treating physician. Furthermore, new antimicrobial agents will have to be continuously sought to replace drugs to which resistance has developed over the years. When a given antibiotic is first introduced it is usually highly active against some bacterial species and inactive against others. With continued usage of the drug, the initially susceptible population may remain sensitive or, as frequently happens, resistant variants start to appear. Emergence of resistance is usually due to selection of naturally occurring resistant mutants. This is usually the outcome of widespread usage of the antibiotic resulting in the elimination of susceptible organisms in the population. It is important, therefore, that indiscriminate use of antibiotics be abandoned.

Crofton (7), in an interesting review of the principles of chemotherapy of bacterial infections, classified drug resistance into three main groups: (a) natural drug resistance, (b) acquired drug resistance, and (c) transferred or "infectious" drug resistance.

Natural drug resistance occurs in organisms which have not been exposed to the drug. This type of resistance may be characteristic

of an entire species or it may be confined to particular strains of a certain species. It may be due to (a) the absence of the metabolic process which is affected by the drug in question, (b) a structural peculiarity such as the absence of the cell wall in mycoplasmata which renders them resistant to penicillin, or (c) the production of enzymes which destroy the drug as in the production of penicillinase by Staphylococci.

Acquired drug resistance is a term used to indicate that a strain of bacteria infecting a patient and originally sensitive to a particular drug has become resistant to that drug during treatment. This is usually an outcome of selection of spontaneously occurring resistant variants.

Genes determining drug resistance may be located on the bacterial chromosome or on extrachromosomal genetic elements. The latter are known as plasmids or resistance factors (R-factors). Infectious drug resistance refers to the transfer of genetic material conferring resistance from a resistant to a sensitive species or strain. There are three main mechanisms by which genetic material may be transferred (48). One mechanism is conjugation which consists in the transfer of genetic material by physical contact between individual bacterial cells by means of a cytoplasmic bridge or "pilus". A second mechanism is transduction which consists in carrying of genetic material from a resistant to a sensitive strain by means of bacteriophage. The third mechanism is transformation which consists of the transfer of parts of the chromosomal DNA into the cell. Of the three mechanisms listed

above, transformation plays little or no role under natural conditions though it can be demonstrated in the laboratory. Conjugation plays a major role in the transfer of R-factors among gram-negative rods particularly the Enterobacteriaceae (29, 48). Transduction, on the other hand, plays a very important role in the transfer of R-factors in Staphylococci (30, 32, 48).

Resistance to carbenicillin may be natural, acquired or transferred. Klebsiella is an example of an organism which is naturally resistant to the drug. Over 90% of the Klebsiella strains tested in our laboratory were resistant to carbenicillin in spite of the fact that the drug has never been used in our hospital except in one instance. Acquired resistance to carbenicillin has been shown to occur both in vivo and in vitro. Hoffman and Bullock (14) used carbenicillin in the treatment of 17 patients with serious Pseudomonas infections. Initial in vitro susceptibility tests showed that the organisms were sensitive to the antibiotic (MICs less than 125 µg/ml). They found that five cases relapsed with highly resistant Pseudomonas aeruginosa (MICs 125 µg/ml to greater than 500 µg/ml). Marks and Eickhoff (27) have similarly reported the emergence of carbenicillin-resistant Pseudomonas strains during therapy in four patients. Jack et al. (16) were able to transfer beta-lactamase genes responsible for the inactivation of carbenicillin between a carbenicillin-resistant strain of Pseudomonas aeruginosa and a sensitive Escherichia coli strain. The initially susceptible Escherichia coli which did not produce carbenicillinase became resistant to the antibiotic and synthesized a beta-lacta-

mase. Similar results have also been reported by Neu and Swarz (31).

Despite the suitability of the agar dilution technique for large-scale testing, the agar diffusion method continues to be the one most commonly employed in the hospital laboratory because of its simplicity. The results reported in this thesis confirm that when such tests are made under standard conditions the sizes of inhibition zones can be related to results of dilution tests and quantitative information is obtained. The regression lines we plotted show an approximately linear relationship between Log MIC and zone size over a wide range of concentrations. This is consistent with findings reported by other investigators for a variety of other antimicrobial agents (27, 39, 40, 46).

Interpretation of the results of susceptibility tests is usually based primarily on the relationship of the MIC of the organism to blood levels attained with commonly used dose schedules. With carbenicillin blood levels as high as 125 µg/ml can be attained with a high dose (30-40 gm/day). This is the dose routinely employed for treating Pseudomonas infections. Infections caused by organisms other than Pseudomonas are usually treated with a low dose of carbenicillin (6-8 gm/day). Blood levels obtained with such a dose usually do not exceed 16 µg/ml.(19, 20, 27, 41)

MIC values corresponding to the attainable blood levels were taken as break points on the regression lines. The corresponding diameters of zones of inhibition represent the limits by means of which susceptibility of the Pseudomonas and other organisms can be determined.

A Pseudomonas strain sensitive to carbenicillin (MIC = 62.5 or less) exhibits a zone of inhibition measuring 18 mm or more when tested by the agar diffusion method using a 100 µg disc. Organisms other than Pseudomonas such as Escherichia coli which are susceptible to carbenicillin (MIC = 15.6 µg/ml or less) give rise to inhibition zones measuring 25 mm or greater around the 100 µg disc.

The criteria we developed for determining the in vitro susceptibility to carbenicillin by the agar diffusion method have been used in our service laboratory since January 1, 1971. Our compiled data for 1562 bacterial strains tested against carbenicillin by the agar diffusion technique clearly show that the majority of the Pseudomonas, Proteus mirabilis, Proteus morganii and Proteus rettgeri were highly susceptible. It was interesting to find that the Proteus vulgaris, unlike other indol positive Proteus organisms, was more resistant to carbenicillin. Of the 6 strains tested by the agar diffusion method only 1 strain was susceptible. These results are in agreement with our determination of the susceptibility of 11 other strains by the broth dilution method. None was found susceptible to carbenicillin. One strain was intermediate (MIC = 31.2 µg/ml) and the remaining 10 strains were resistant (MICs = 62.5 or greater). It would be of interest to confirm these observations with a larger number of Proteus vulgaris. If confirmed it would seem appropriate that high doses of carbenicillin, similar to those used in Pseudomonas infections, should be employed for treating Proteus vulgaris infections. In this case the criteria used for determining the in vitro susceptibility of

Proteus vulgaris should be the same as those utilized for Pseudomonas.

Our data have also shown that carbenicillin is highly active against a variety of gram-positive organisms such as Streptococcus, Pneumococcus and non-penicillinase producing Staphylococcus aureus. It is unlikely that carbenicillin would be used clinically for treating infections caused by gram-positive organisms such as those mentioned above. This is because penicillin, a less expensive drug, still remains highly active against these bacteria. Furthermore, the wide use of carbenicillin may result in the emergence of resistant strains. Presently the use of carbenicillin is recommended for treatment of serious infections caused by Pseudomonas and indol positive Proteus (6, 28, 40, 42, 46). The available drugs used in the treatment of such infections have a high degree of toxicity to the kidney. Before carbenicillin was introduced, the treatment of patients with impaired renal function and suffering from life-threatening infections due to Pseudomonas or indol positive Proteus presented a major problem for the clinician. Agents such as polymyxin, colistin, kanamycin or gentamicin must be given with extreme caution to such patients and blood levels must also be carefully monitored during therapy in order to avoid toxic effects.

During the last three years extensive clinical trials have been carried out for determining the effectiveness of carbenicillin in the treatment of a variety of bacterial infections. Duma, Warner and Utz (8) used it in 14 patients with pneumonia due to Pseudomonas aeruginosa. They found that the drug was effective in 75% of the infections.

Swarz and Neu (45) have similarly shown that carbenicillin was highly effective in the treatment of 24 serious Pseudomonas pulmonary infections. No major toxicity was encountered and the pneumonias showed excellent response without superinfection. Bodey, Rodriguez and Whitecar (4) utilized carbenicillin for treatment of severe infections in leukemic patients. Of 9 septicemic episodes treated, the response was good in 8 patients and partial in 1. Similar results have also been reported by Stratford (44). Labowitz and Holloway (20) found that carbenicillin was effective in the treatment of 23 out of 24 patients suffering from serious gram-negative infections of the blood stream, urinary tract and lung. Likewise, Standiford et al. (42) observed striking clinical responses in 4 of 5 patients with significant, persistent infections caused by Pseudomonas and in a patient with a mixed infection due to Proteus rettgeri and a Providencia strain. Van Rooyen et al. (47) evaluated the efficiency of carbenicillin in the control of Pseudomonas infection in four burn patients. The drug was found to exert a marked effect in suppressing the growth of Pseudomonas aeruginosa and so arrested the development of septicemia. Boxerbaum et al. (5) reported that carbenicillin was useful in treatment of cystic fibrosis patients suffering from Pseudomonas infections. Several other investigators have also found that carbenicillin was useful in the treatment of infections of the urinary tract, respiratory tract and wounds (6, 13, 21, 26, 35, 43).

Marks and Eickhoff (27) used carbenicillin for treating 45 patients with gram-negative infections, 38 of which were caused by Pseudomonas.

Therapy was successful in 10 patients, resulted in improvement in 19 and was considered a failure in 16 patients. These investigators found that carbenicillin was most effective in treating Pseudomonas urinary tract infections, although the relapse rate was high. Sixty-one percent of the patients, however, who developed bacteriologic relapses, had obstructive uropathy. These workers also reported that the results of therapy for bacteremia, pneumonia and other foci of infection were far less satisfactory than urinary tract infections. Hoffman and Bullock (14) evaluated the clinical effectiveness of carbenicillin in 17 patients with serious Pseudomonas infections and 3 patients with other gram-negative bacillary infections. Although most patients showed improvement only 6 were cured. The initial pathogenic species was not eradicated from 13 patients and 5 relapses due to Pseudomonas aeruginosa were observed.

The available clinical evidence, therefore, indicates that carbenicillin is an effective antibiotic for the treatment of Pseudomonas infections provided that the causative strain is susceptible. Treatment failures and/or relapses can be attributed to the following: (a) infections caused by a resistant organism, (b) emergence of carbenicillin-resistant variants during therapy and (c) presence of an underlying disorder such as obstructive uropathy, etc. It is imperative that susceptibility of the infecting strain be determined before carbenicillin is used in therapy.

concentrations of 15.6 µg/ml or less and the other half was highly resistant with MIC values of 500-1000 µg/ml. The Proteus and Providencia tended to exhibit a bimodal distribution with two distinct populations, a susceptible one and a highly resistant one.

Criteria were established for determining susceptibility to carbenicillin by the agar diffusion test using 100, 50, and 30 µg discs. Two criteria were utilized, one for Pseudomonas and a second one for the other organisms. A Pseudomonas strain with a zone of inhibition of 18 mm or greater around the 100 µg disc is susceptible. In other bacterial species a zone size of 25 mm or greater is indicative of susceptibility. The established criteria for determining susceptibility to carbenicillin by the agar diffusion method were introduced in the service laboratory on January 1, 1971. The analysis of the susceptibility of 1562 bacterial isolates tested in the service laboratory showed that it was similar to the pattern obtained with the 305 other strains tested by the dilution technique.

MICs for 223 bacterial strains were determined both by the agar dilution and broth dilution tests. There was good correlation between the MICs obtained by the two methods. Minimum bactericidal concentrations (MBCs) were determined for 249 bacterial strains and compared to the MICs. The MBC was equal to the MIC in approximately 90% of the strains. These findings support the conclusion that carbenicillin is a bactericidal agent.

The influence of altering the inoculum size and prolonging the incubation time on results of the dilution tests was investigated.

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