

Inoculum Effect of New β -Lactam Antibiotics on *Pseudomonas aeruginosa*

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The degree of the inoculum effect shown by the new beta-lactam antibiotics with *Pseudomonas aeruginosa* was investigated, and the antibiotics were divided into three groups based upon the observations. The group 1 antibiotics (cefotaxime, moxalactam, cefoperazone, azlocillin, piperacillin, and aztreonam) demonstrated a large inoculum effect, were poorly bactericidal, produced aberrant, elongated bacilli, and did not inhibit the increase in turbidity of high inocula during an 18-h incubation. The group 2 antibiotics (ceftazidime and ticarcillin) were slowly bactericidal, caused minimal formation of aberrant, elongated bacilli, and slowly decreased the turbidity of high inocula. The group 3 antibiotics (imipenem and gentamicin) were bactericidal, did not cause the formation of elongated bacilli, and decreased the turbidity of high inocula rapidly. Data are presented which suggest that the inoculum effect seen with the group 1 beta-lactam antibiotics is related to (i) the poor intrinsic antibactericidal activity of these antibiotics for *P. aeruginosa* at the inocula tested and (ii) failure of these antibiotics to inhibit the formation of aberrant and filamentous bacilli, which can result in increased bacterial mass and turbidity.

The phenomenon of inoculum effect is described as the significant increase seen in the MIC of an antibiotic when the inoculum size used in the testing is increased. The importance of such a phenomenon was first stressed by Luria in 1946 (11). The inoculum effect has been studied most extensively in staphylococci, in which antibiotic destruction by beta-lactamase was largely responsible for this phenomenon (3, 17). The inoculum effect has recently attracted widespread interest due in part to the recent proliferation of new beta-lactam antibiotics which demonstrate a variety of degrees of the inoculum effect (4, 6, 7, 17; K. R. Comber, K. E. Griffin, A. R. White, and R. Sutherland, Abstr. 12th Int. Congr. Chemother., Florence, Italy, p. 86-88, 1981).

For *Pseudomonas aeruginosa*, the inoculum effect with the new beta-lactam antibiotics has been particularly noticeable between inocula of 10^6 and 10^8 CFU/ml (1). Whether this increase in the MIC is due to the occurrence or development of a few resistant bacilli that can be found occasionally only among high inocula of bacteria or to the destruction of the drug with higher bacterial densities, as has been observed previously with other gram-negative rods (18), has not as yet been resolved. Both of the suggested mechanisms have been documented to occur in certain test strains and against some beta-lactam antibiotics by other investigators (6, 16). There may also be other explanations for the observed increase in antibiotic resistance when testing is done with large inocula. Our study was designed to determine the mechanism(s) of the inoculum effect with *P. aeruginosa* ATCC 27853 and an intermediately susceptible clinical isolate of *P. aeruginosa*.

MATERIALS AND METHODS

Bacterial strains. *P. aeruginosa* ATCC 27853 and a clinical isolate of *P. aeruginosa* having an intermediate susceptibility to ticarcillin (MIC = 60 μ g/ml) were subcultured onto sheep blood tryptic soy agar before use.

Antibiotics. Cefoperazone (Pfizer Inc., New York, N.Y.), ticarcillin (Beecham Laboratories, Bristol, Tenn.), azlocillin (Miles Laboratories, West Haven, Conn.), piperacillin (Lederle Laboratories, Pearl River, N.Y.), imipenem (Merck Sharp & Dohme Research Laboratories, Rahway, N.J.), aztreonam (E. R. Squibb & Sons, Inc., New Brunswick, N.J.), and gentamicin (Schering Corp., Bloomfield, N.J.) were dissolved in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) at a concentration of 1,000 μ g/ml just before use. Cefazidime test powder (Glaxo Inc., Research Triangle Park, N.C.) was mixed with 1/10 weight of sodium bicarbonate powder before dissolving in Mueller-Hinton broth.

Susceptibility methods. Methods for microdilution testing were similar to those described by Gavan and Barry (5). Serial twofold dilutions were made in microtiter trays, using 50- μ l microdiluters from a starting concentration of 1,000 μ g/ml. An equal volume of media containing two times the final inoculum density of bacteria was then added. The trays were covered, gently shaken to mix the well contents, and incubated for 18 to 48 h at 35°C in a moistureproof plastic bag. The same bacterial inoculum was similarly added to 50 μ l of media containing 10% Formalin to allow for the comparison of the visual turbidity and bacterial button sizes in the wells when the bacteria neither grew nor lysed. The appearance of the wells as compared with Formalin control wells was recorded at 18, 24, and 48 h of incubation. The densities of inocula used included 5×10^5 , 5×10^7 , and 5×10^8 CFU/ml, which were prepared from an overnight Mueller-Hinton broth culture. The initial density was adjusted to equal a no. 1 McFarland standard, which was found to correlate with ca. 10^9 CFU/ml. The final bacterial densities were determined by quantitative subculture of the entire well content. The macro-broth dilution method described by Washington and Sutter (19) was also performed, using high antibiotic concentrations and high bacterial densities as collaboration of the microtiter results.

Semiquantitative beta-lactamase assays. A method similar to that of O'Callaghan et al. (13) was used to determine the

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presence of beta-lactamase. Nitrocefin (compound 87/312; Glaxo Research Group Ltd., Greenford, England) was dissolved in Mueller-Hinton broth at a concentration of 500 µg/ml. Ten microliters of nitrocefin solution was added to each test well which had been preincubated for 6 h with bacteria in the presence or absence of antibiotics. After the addition of nitrocefin, the trays were further incubated for 30 min at 35°C before a visual reading was made of any color changes.

Assay of antibiotic destruction. To each antibiotic dissolved in Mueller-Hinton broth (1,000 µg/ml) was added either an equal volume of Mueller-Hinton broth containing two times the standard density of bacteria (5×10^5 CFU/ml) or two times a higher bacterial density (5×10^7 CFU/ml). The contents were mixed and incubated with agitation for 6 h at 35°C. The tube contents were then filtered through a 0.22-µm Millipore filter (Millipore Corp., Bedford, Mass.). The filtrates (initial drug concentrations of 500 µg/ml) were assayed for loss of the drug potency by an agar diffusion bioassay with agar seeded with a susceptible strain of *Escherichia coli*.

Quantification of resistant bacilli in a normal bacterial population. *P. aeruginosa* ATCC 27853 and the clinical isolate of *P. aeruginosa* were grown overnight on Mueller-Hinton agar. A single typical colony of each organism was suspended in Mueller-Hinton broth at bacterial densities of 10^5 , 10^7 , and 10^8 CFU/ml. Fifty microliters of each suspension containing different bacterial densities was streaked onto Mueller-Hinton agar containing one, two, three, four, and five times the MIC of the test antibiotic and incubated at 35°C. The number of visible colonies on the agar was counted after 18 to 24 h and 48 h of incubation.

Killing kinetics assay. An inoculum of 10^8 CFU of both strains of *P. aeruginosa* per ml was added to tubes with a final antibiotic concentration of 50, 100, 250, or 500 µg/ml, and the tubes were incubated at 35°C with continuous agitation. A control tube containing bacteria and no antibiotic was also included. Samples were removed at 0, 6, 12, and 24 h and were quantitatively subcultured on Mueller-Hinton agar. The experiment was also performed with four times the MIC and an inoculum of 10^5 CFU/ml.

Optical density studies. In addition to the visual assessment of the turbidity in microtiter wells and in tubes of macro-broth dilution tests, turbidity measurements were performed on organisms during exposure to 500 µg of antibiotics per ml by using a spectrophotometer at 400 nm (Spectrophotometer Junior IIA; Coleman, Maywood, Ill.).

Radionuclide studies. *P. aeruginosa* ATCC 27853 at an inoculum of 5×10^7 CFU/ml was exposed to the test antibiotic (500 µg of a beta-lactam antibiotic per ml or 10 µg of gentamicin per ml). At exposure times of 0 and 6 h, 1-ml samples were removed, and 5 µCi of mixed tritiated amino acids (New England Nuclear Corp., Boston, Mass.) was added. These samples were incubated for an additional 60 min at 35°C to allow for amino acid uptake by the viable organisms. After incubation, the bacteria from each sample were trapped on 0.22-µm Millipore filters and washed with 15 ml of Mueller-Hinton broth to remove extracellular radioactive amino acids. The filters were air dried, and the amount of radioactive amino acid uptake was determined by liquid scintillation counting.

RESULTS

Determination of MICs and inoculum effects. For *P. aeruginosa* ATCC 27853, the MICs and MBCs of the antibiotics studied are listed in Table 1. Even at the low inoculum,

aztreonam, cefoperazone, cefotaxime, moxalactam, azlocillin, and piperacillin were not bactericidal, as noted by MBCs of greater than 500 µg/ml. For ticarcillin and ceftazidime, the MBCs were within two dilutions of the MICs. When the inoculum was increased to 10^8 CFU/ml, the MICs of the first six antibiotics were markedly increased (group 1 antibiotics). The MICs of ticarcillin and ceftazidime increased but were still measureable (group 2 antibiotics), and the MICs of imipenem and gentamicin increased only slightly (group 3 antibiotics).

Determinations of MICs at high inocula (5×10^7 and 5×10^8 CFU/ml) were particularly difficult with cefotaxime, moxalactam, cefoperazone, aztreonam, azlocillin, and piperacillin. With these antibiotics, all wells showed some increased turbidity after an 18- to 24-h incubation as compared with control wells containing Formalin and the same bacterial inoculum. As expected, turbidity increased, and bacterial growth occurred in wells containing low concentrations of these beta-lactams, with a green pigment appearing in these wells (Fig. 1). In wells containing higher concentrations of these antibiotics, the contents, though not grossly cloudy, were still more turbid than the Formalin control wells, and the colony counts in these wells had decreased only minimally from that of the starting inoculum (Fig. 1). Gram stains of the well contents showed many filamentous bacillary forms. Thus, at high inocula the MIC points were not distinct with these antibiotics and only a visual approximation or "apparent" MIC could be obtained. In Fig. 2 the changes in MICs at various inoculum sizes are plotted for the test antibiotics. The MICs of piperacillin, cefoperazone, moxalactam, and aztreonam increased dramatically when the inoculum was raised from 5×10^7 to 5×10^8 CFU/ml (Fig. 2A). Similar increases were seen for cefotaxime and azlocillin (data not shown). Extended 48-h incubation of piperacillin, azlocillin, and cefoperazone, but not cefotaxime, moxalactam, and aztreonam, produced increased turbidity and green pigmentation in all wells, even when the inoculum was 5×10^5 CFU/ml. This difference observed between the antibiotics within group 1 during prolonged incubation appeared to correlate with the degree of antibiotic degradation, and this phenomenon can be observed with some group 1 antibiotics at all inoculum sizes (Table 2).

TABLE 1. MICs and MBCs of antibiotics for *P. aeruginosa* ATCC 27853

Antibiotic	MIC (µg/ml) at the following inoculum:		MBC (µg/ml) at the following inoculum:		MIC ratio ^a
	10^5 CFU/ml	10^8 CFU/ml	10^5 CFU/ml	10^8 CFU/ml	
Group 1					
Aztreonam	4	>500	>500	>500	>125
Cefoperazone	4	>500	>500	>500	>125
Cefotaxime	16	>500	>500	>500	>32
Moxalactam	16	>500	>500	>500	>32
Azlocillin	4	>500	>500	>500	>125
Piperacillin	4	>500	>500	>500	>125
Group 2					
Ticarcillin	16	64	32	256	4
Ceftazidime	2	256	8	>500	128
Group 3					
Imipenem	4	8	4	16	2
Gentamicin	2	4	2	4	2

^a MIC ratio is the ratio of the MIC obtained with an inoculum of 10^8 CFU/ml to the MIC obtained with an inoculum of 10^5 CFU/ml.

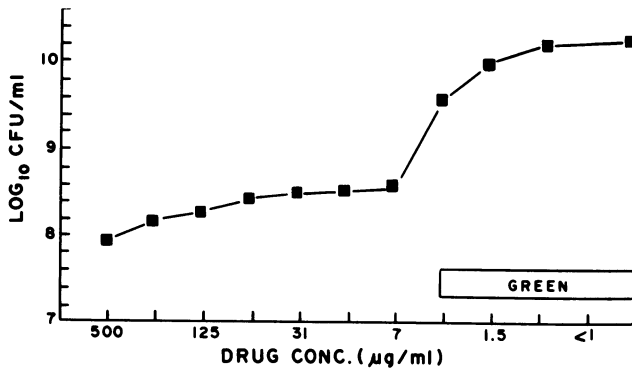


FIG. 1. Number of viable colonies of *P. aeruginosa* ATCC 27853 in wells of the microtiter tray containing twofold dilutions of cefoperazone, starting at 500 µg/ml after an 18-h incubation. An initial inoculum of 10^8 CFU/ml was used. The box shows the concentrations at which a green pigment appeared in the wells.

With ticarcillin and ceftazidime, the turbidity of the non-pigmented wells containing antibiotic concentrations higher than the apparent MIC was equal to that of the Formalin control wells during early incubation. By 24 h, these nonpigmented wells showed a marked decrease in turbidity as well as in the number of viable organisms. The MICs of these antibiotics (Fig. 2B) appeared to rise less with increasing inoculum sizes than those antibiotics designated as group 1 (Fig. 2A). Gram stains of the contents of wells with high concentrations of beta-lactams showed only a minimal formation of aberrant filamentous bacilli.

Imipenem and gentamicin demonstrated clear and distinct MIC endpoints as early as 6 h, and all wells containing antibiotic concentrations equal to or greater than this concentration appeared less turbid than the Formalin control wells. The colony counts of the well contents confirmed the rapid bactericidal activity of these antibiotics. Gram stains of the contents of those wells containing the MIC or higher of antibiotics failed to show any formation of aberrant filamentous bacilli. Increases in the size of the inoculum appeared to alter the MIC results very little (Fig. 2B). These two antibiotics are designated as group 3.

Destruction of antibiotic activity and beta-lactamase production. The filtrates obtained after incubation of 500 µg of the test beta-lactam per ml and 5×10^5 or 5×10^7 CFU of *P. aeruginosa* ATCC 27853 per ml were assayed for antimicrobial potency. No significant loss of potency was noted in those filtrates obtained after a 6-h incubation with either density of bacteria. After 18 h, a significant inactivation occurred with the higher bacterial density for cefoperazone, azlocillin, and piperacillin, although the residual concentrations were still higher than the respective MICs required to inhibit *P. aeruginosa* ATCC 27853. A smaller amount of inactivation or destruction was noted for ticarcillin than for piperacillin or azlocillin at 48 h. Only slight antibiotic destruction or inactivation was noted with cefotaxime, moxalactam, and aztreonam during the period studied (Table 2).

Development of resistant organisms. Resistant organisms were rarely detected in a 24-h broth culture with group 1 antibiotics. However, both *Pseudomonas* strains did have growth on agar plates containing cefoperazone, azlocillin, or piperacillin at 4 to 100 times the MIC if incubated for 48 h. These colonies grew slowly, and the time course of bacterial growth correlated with the time course of antibiotic destruction (Table 2). The isolated colonies on retesting required for inhibition the same initial MIC; however, incubation of $5 \times$

10^7 organisms per ml in broth containing 500 µg of the group 1 antibiotics per ml did on rare occasions produce an isolate requiring for inhibition a fourfold or higher MIC than that required by the original organism. Such resistance in these rare isolates was stable, even after 10 serial passages on agar.

No such resistant isolates were produced with imipenem, ticarcillin, or ceftazidime. After incubation of 5×10^7 CFU of organisms per ml with 10 µg of gentamicin per ml, small colonies appeared on subculture on agar containing as much as five times the MIC. These colonies were resistant on retesting and resembled the description of the previously reported small-colony variants (9).

Kinetics of killing. Killing kinetics were determined for the different antibiotics by using 10^5 CFU of bacteria per ml in Mueller-Hinton broth containing four times the MIC of the test antibiotic (determined by using an inoculum of 5×10^5 CFU/ml) or 5×10^7 CFU/ml in Mueller-Hinton broth containing 500 µg of beta-lactams per ml (Fig. 3). Group 3 agents (gentamicin or imipenem) produced the most rapid decrease in the number of viable colonies. Group 2 antibiotics (ceftazidime or ticarcillin) were less rapidly bactericidal but were able to reduce an inoculum of 10^5 by nearly 3 logs in 6 h and an inoculum of 10^7 by 3 logs in 12 h. The group 1 agents were not able to reduce an inoculum of 10^5 CFU/ml by more than 1.5 logs or an inoculum of 10^7 CFU/ml by more than 1 log. Again as in the microtiter trays, the tubes in macro-broth

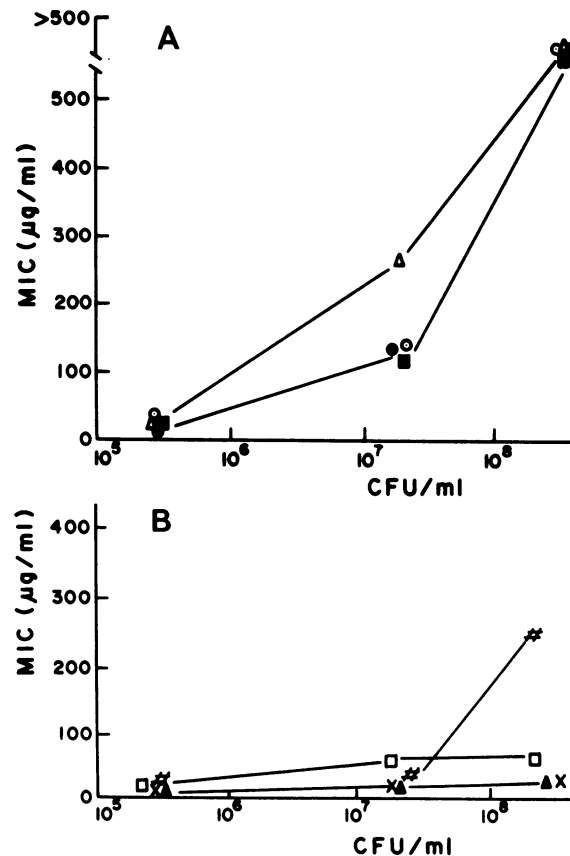


FIG. 2. Apparent MICs of seven beta-lactam antibiotics at 500 µg/ml and gentamicin at 10 µg/ml at different sizes of inoculum for *P. aeruginosa* ATCC 27853. Symbols in (A): Δ, cefoperazone; ●, piperacillin; ○, moxalactam; ■, aztreonam. Symbols in (B): #, ceftazidime; □, ticarcillin; ×, imipenem; ▲, gentamicin.

TABLE 2. Residual antibiotic activity after 18- and 48-h incubations with an inoculum of 5×10^7 CFU/ml

Antibiotic ^{a,b}	Concn (μg/ml) at 18 h		Concn (μg/ml) at 48 h	
	Without inoculum	With inoculum	Without inoculum	With inoculum
Azlocillin	400 (80) ^c	160 (32)	250 (50)	10 (2)
Aztreonam	480 (96)	470 (94)	300 (60)	300 (60)
Cefoperazone	300 (60)	32 (6)	200 (40)	0 (0)
Cefotaxime	520 (100)	526 (100)	400 (80)	300 (60)
Ceftazidime	510 (100)	460 (92)	400 (80)	300 (60)
Moxalactam	530 (100)	380 (76)	250 (50)	200 (40)
Piperacillin	280 (56)	160 (32)	250 (50)	12 (2)
Ticarcillin	400 (80)	400 (80)	300 (60)	300 (60)

^a All initial beta-lactam antibiotic concentrations were equivalent to 500 μg/ml.

^b Imipenem was not tested because of its known instability in solution.

^c Values in parentheses indicate percentage of original concentration.

dilution containing organisms and group 1 agents had an abundance of aberrant, filamentous forms. For completeness 50, 100, and 250 μg of antibiotics per ml were studied as well as 500 μg/ml for representative members of each group: cefoperazone (group 1), ceftazidime (group 2), and imipenem (group 3). No concentration-related killing was observed for any of the antibiotics tested with this series of antibiotic concentrations.

Optical density studies. The group 1 antibiotics (piperacillin and cefoperazone) failed to inhibit the development of turbidity of a high inoculum (Fig. 4). From an initial turbidity equivalent to 1.0 McFarland (correlated with an optical density of 0.3 and 10^9 CFU of *Pseudomonas* strains per ml), the tubes containing bacteria and 500 μg of cefotaxime (group 1 agent) per ml increased in turbidity to greater than an optical density of 0.9 in 6 h, an optical density equivalent

to greater than a threefold increase in the number of organisms. However, the actual number of organisms was unchanged or even slightly decreased (Fig. 3). The increase in optical density must have been due to aberrant elongation of the bacteria. Tubes containing 500 μg of group 2 antibiotics (ceftazidime or ticarcillin) per ml had a small transient rise in turbidity after 3 h of incubation, which was followed by a decrease in turbidity by 6 h. Tubes containing 10 μg of gentamicin per ml or 500 μg of imipenem per ml (group 3 agents) showed a progressive decrease in turbidity after 3 h (Fig. 4).

Radionuclide studies. Radioactive amino acids were incorporated normally into pseudomonas in the absence of antibiotics. In the presence of 500 μg of cefoperazone or piperacillin per ml (group 1), bacterial uptake of amino acids continued, even after 6 h of antibiotic exposure, indicating active metabolism by the aberrant filamentous organisms (Fig. 5). In contrast, bacteria exposed to imipenem or gentamicin had reduced uptake of radioactive mixed amino acids immediately, and after 6 h, the uptake ceased. In bacteria exposed to ticarcillin or ceftazidime (group 2), the uptake of amino acids was intermediate between that of group 1 and group 3 antibiotics (Fig. 5).

DISCUSSION

The clinical relevance of MIC results obtained with an inoculum of 10^5 CFU/ml may be questioned because most infected sites contain bacteria at a higher density (2). In principle, in vitro susceptibility testing methods should simulate those conditions encountered clinically, and the results should correlate with the clinical efficacy of the antibiotic. Attempts to perform susceptibility determinations at higher bacterial densities have produced enormously high MICs, a phenomenon known as inoculum effect, for many cell wall-active antibiotics (4, 6, 17; Comber et al., Abstr. 12th Int. Congr. Chemother., 1981).

There was poor correlation in our experiments between drug inactivation and the degree of inoculum effect. Signifi-

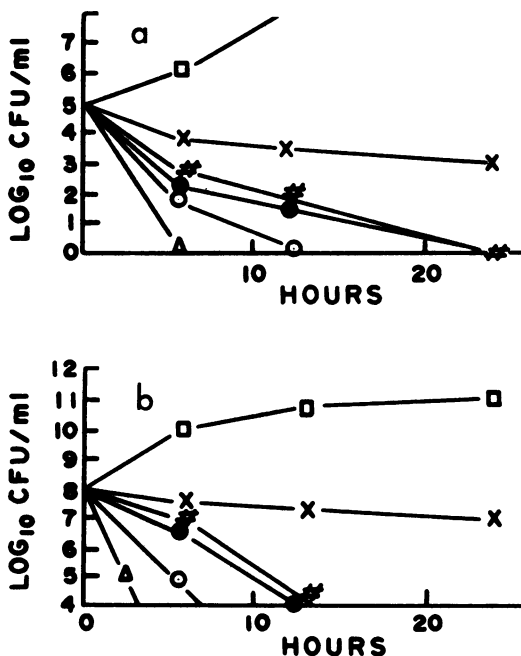


FIG. 3. Killing kinetics of *P. aeruginosa* ATCC 27853 at two different sizes of inoculum. At the lower inoculum, four times the MIC of the test antibiotic was used (a). At the higher inoculum, 500 μg of the beta-lactams per ml or four times the MIC of gentamicin was used (b). Symbols: ×, cefoperazone; #, ticarcillin; ●, ceftazidime; ○, imipenem; △, gentamicin; □, no antibiotics.

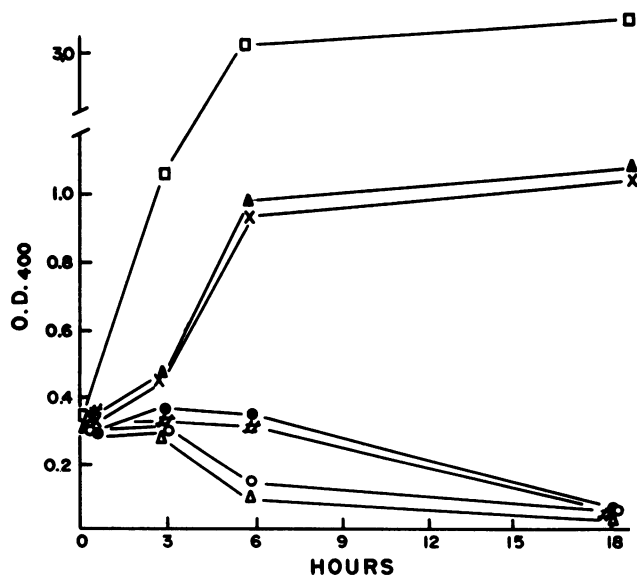


FIG. 4. Development of turbidity with time by *P. aeruginosa* ATCC 27853 during incubation with 500 μg of the beta-lactams per ml or 10 μg of gentamicin per ml. Symbols: □, no antibiotics; ▲, piperacillin; ×, cefoperazone; #, ticarcillin; ●, ceftazidime; ○, imipenem; △, gentamicin. O.D.₄₀₀, Optical density at 400 nm.

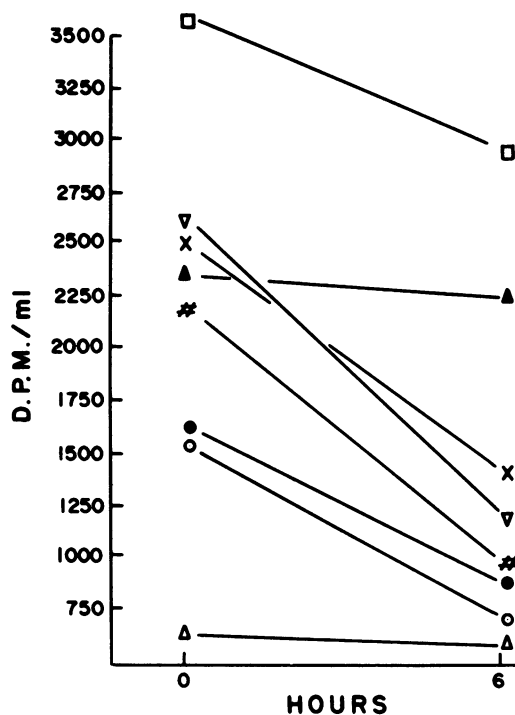


FIG. 5. Amino acid incorporation into *P. aeruginosa* at a density of 10^8 CFU/ml in the presence and in the absence of 500 μ g of the beta-lactams per ml or 10 μ g of gentamicin per ml. Symbols: □, no antibiotics; ▲, piperacillin; ×, cefoperazone; ▽, cefotaxime; #, ticarcillin; ●, ceftazidime; ○, imipenem; △, gentamicin. D.P.M., Disintegrations per minute.

cant drug destruction occurred by 18 h of incubation for only the penicillins and cefoperazone. Even for those drugs which were significantly inactivated (cefoperazone, azlocillin, and piperacillin), the residual antibiotic concentrations at 18 h were considerably higher than the apparent MIC required to inhibit the organism at an inoculum of 10^5 CFU/ml. However, for other agents (cefotaxime, moxalactam, and aztreonam) there was little inactivation with prolonged incubation, and this stability was expected from results of other investigators in which cefotaxime, moxalactam, and aztreonam have been demonstrated to be beta-lactamase stable (12). The inoculum effect was very large for all of these group 1 agents (azlocillin, aztreonam, cefoperazone, cefotaxime, moxalactam, and piperacillin). Just as antibiotic destruction or inactivation cannot be used to explain the inoculum effect, neither can the development of in vitro resistance by the test organism. The bacteria which persisted in the culture broth containing high concentrations of these group 1 beta-lactam antibiotics were not resistant upon retesting with the standard inoculum size.

In our investigation, the observed magnitude of inoculum effect appears to depend on the type of antipseudomonal activity demonstrated by the antibiotic. Three types of antibiotic activity were consistently seen. In type 1 activity, little or no decrease in CFU per milliliter was seen even at high antibiotic concentrations, and there was progressive formation of giant aberrant and filamentous forms (Fig. 4). The turbidity which developed despite high antibiotic concentrations was apparently due to an increase in mass of aberrant bacilli. An abrupt increase in the magnitude of the inoculum effect was particularly noticeable between an inoculum size of 10^7 and 10^8 CFU/ml in group 1 antibiotics. This may be due to the fact that 10^7 CFU/ml has a turbidity

just below our visual detection limits, and any small increase in bacterial mass would result in frank turbidity and in a reading of bacterial "growth." Antibiotics in this group included certain third-generation cephalosporins (moxalactam, cefotaxime, cefoperazone), certain antipseudomonal penicillins (piperacillin, azlocillin), and a new monobactam (aztreonam). With type 2 activity, as typified by ceftazidime and ticarcillin (group 2 antibiotics), slow bactericidal activity was observed. The colony counts decreased 3 logs or more in 12 h from an initial inoculum of 10^5 or 10^8 CFU/ml. Although some aberrant bacterial forms were briefly noted, no significant increase in turbidity occurred at any time points examined, and the magnitude of the inoculum effect was small (Fig. 4). With type 3 activity, as typified by imipenem and gentamicin (group 3 antibiotics), the colony counts decreased by 3 logs in 6 h, no aberrant filamentous bacterial forms were seen, and no inoculum effect was observed.

An apparent explanation for the production of the elongated aberrant forms by some beta-lactams may be that the antibiotic binds to penicillin-binding protein 3 (PBP 3) but not to PBP1 and 2. Recent work on ceftazidime, an antibiotic which we find to have type 2 activity with *P. aeruginosa*, confirms the existence of a transient period in which aberrant filamentous bacilli are formed, followed by a reduction in colony counts (8). This sequence of events is associated with sequential ceftazidime binding to PBP 3 and 1. It was found that PBP 3 was bound by ceftazidime at a lower antibiotic concentration than PBP 1, which when bound would itself result in bacterial lysis (8).

At all inocula, but most noticeably at large inocula, antibiotics with type 1 activity did not demonstrate a real MIC on close comparison with a Formalin control well, apparently because of the development of filamentous forms. Although there was an apparent cessation of bacterial multiplication as determined by quantitative subcultures, the continued uptake of amino acids and the observed elongation of bacilli indicate that the bacterial cells were still metabolically active. The data suggest that an increase in the total bacterial mass from bacterial elongation occurred even though the count of viable bacteria may actually have shown a slight decline. It is obvious that agents of this group would produce a wide gap between the apparent inhibitory and bactericidal antibiotic concentrations when tested at the standard inoculum. Indeed, we found the MBC level could not be achieved by group 1 antibiotics.

Consideration of the type of activity displayed by an antipseudomonal antibiotic may well be clinically important. Results of treatment of *P. aeruginosa* pyelonephritis in mice appear to support the view that antibiotics which are rapidly bactericidal are more effective (A. S. Beale, K. R. Comber, and R. Sutherland, Abstr. 12th Int. Congr. Chemother., p. 668-669, 1981). Similar results have been demonstrated in *P. aeruginosa* sepsis or pneumonia in animal models (10, 14, 15). Although the exact meaning of the observations in vitro and in animals is unclear, the data as a whole suggest that if inoculum effect should have clinical significance, then the differences observed in the type of activity between the three groups of beta-lactams may also be clinically significant.

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LITERATURE CITED

1. Bodey, G. P., V. Fainstein, and A. M. Hinkle. 1981. Comparative in vitro study of new cephalosporins. *Antimicrob. Agents Chemother.* **20**:226-230.
2. Bryant, R., A. L. Rashad, J. A. Mazza, and D. Hammond. 1980. Beta-lactamase activity in human pus. *J. Infect. Dis.* **142**:594-601.
3. Chapman, S., and R. Steigbiegel. 1983. Staphylococcal β -lactam antibiotics: *in vitro* and *in vivo* evaluation. *J. Infect. Dis.* **147**:1078-1082.
4. Corrado, M. L., S. H. Landesman, and C. E. Cherubin. 1980. Influence of inoculum size on activity of cefoperazone, cefotaxime, moxalactam, piperacillin, and *N*-formimidoyl thienamycin (MK0787) against *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.* **18**:893-896.
5. Gavan, T. L., and A. L. Barry. 1980. Microdilution test procedures, p. 459-462. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*. American Society for Microbiology, Washington, D.C.
6. Greenwood, D., and A. Eley. 1982. A turbidity study of the response of select strains of *Pseudomonas aeruginosa* to eight antipseudomonas β -lactam antibiotics. *J. Infect. Dis.* **145**:110-117.
7. Gwynn, M. N., and G. N. Rolinson. 1980. Selection of variants of *Pseudomonas aeruginosa* resistant to beta-lactam antibiotics. *Infection* **8**:73-80.
8. Hayes, M. V., and D. C. Orr. 1983. Mode of action of ceftazidime: affinity for the penicillin-binding proteins of *Escherichia coli* K12, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. *J. Antimicrob. Chemother.* **12**:119-126.
9. Li, K., J. J. Farmer, III, and A. Coppola. 1974. A novel type of resistant bacteria induced by gentamicin. *Trans. N.Y. Acad. Sci.* **36**:396-441.
10. Lumish, R. M., and C. W. Norden. 1976. Therapy of neutropenic rats infected with *Pseudomonas aeruginosa*. *J. Infect. Dis.* **133**:538-544.
11. Luria, S. E. 1946. A test for penicillin sensitivity and resistance in staphylococcus. *Proc. Soc. Exp. Biol. Med.* **61**:46-51.
12. Neu, H. 1982. Mechanisms of bacterial resistance to antimicrobial agents, with a particular reference to cefotaxime and other β -lactam compounds. *Rev. Infect. Dis.* **4**(Suppl.):S288-S299.
13. O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler. 1972. Novel method for detection of β -lactamases by using a chromogenic cephalosporin substrate. *Antimicrob. Agents Chemother.* **1**:283-288.
14. Pennington, J. E., C. E. Johnson, and R. Plott. 1982. Third-generation cephalosporin treatment of pneumonia due to *Pseudomonas aeruginosa* in guinea pigs. *J. Infect. Dis.* **146**:567.
15. Pennington, J. E., and R. M. Stone. 1979. Comparison of antibiotic regimens for treatment of experimental pneumonia due to *Pseudomonas*. *J. Infect. Dis.* **140**:881-889.
16. Rosdahl, V. T., and N. Rosdahl. 1971. Minimum inhibitory concentrations of carbenicillin against *Pseudomonas aeruginosa*. *Chemotherapy* **16**:18-28.
17. Sabath, L. D., C. Garner, C. Wilcox, and M. Finland. 1975. Effect of inoculum and of beta-lactamase on the anti-staphylococcal activity of thirteen penicillins and cephalosporins. *Antimicrob. Agents Chemother.* **8**:344-349.
18. Sanders, C. C., and W. E. Sanders, Jr. 1983. Emergence of resistance during therapy with the newer beta-lactam antibiotics: role of inducible beta-lactamases and implications for the future. *Rev. Infect. Dis.* **5**:639-648.
19. Washington, J. A., II, and V. L. Sutter. 1980. Dilution susceptibility test: agar and macro-broth dilution procedures, p. 453-458. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), *Manual of clinical microbiology*. American Society for Microbiology, Washington, D.C.