EFFECTS OF INDUCIBLE BETA-LACTAMASE AND ANTIMICROBIAL RESISTANCE UPON THE ACTIVITY OF NEWER BETA-LACTAM ANTIBIOTICS AGAINST *PSEUDOMONAS AERUGINOSA*

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The activity of carbenicillin, ticarcillin, piperacillin, cefotaxime, moxalactam, and *N*-formimidoyl thienamycin was evaluated against 262 clinical isolates of *Pseudomonas aeruginosa*. There were 242 (92%) of the isolates that were susceptible to carbenicillin or ticarcillin by an agar dilution method. Against this population of susceptible isolates, the median MICs were 1.56 μg/ml of *N*-formimidoyl thienamycin, 3.13 μg/ml of piperacillin, 25 μg/ml of ticarcillin, 25 μg/ml of cefotaxime, 50 μg/ml of carbenicillin and 50 μg/ml of moxalactam. *N*-Formimidoyl thienamycin was the only beta-lactam antibiotic not affected by an inducible beta-lactamase detected in 24 randomly selected susceptible isolates by a disk approximation assay, while cefotaxime was inactivated to a greater extent than any of the other beta-lactam antibiotics. Resistance to carbenicillin and ticarcillin was noted in 20 isolates (8%); these were susceptible to *N*-formimidoyl thienamycin, but cross-resistance with piperacillin, cefotaxime, and moxalactam was frequent. Only four of these resistant isolates were found to have a constitutive beta-lactamase. Gentamicin resistance occurred in 51 isolates (19%) and was an independent variable of resistance to the beta-lactam drugs.

Four newer beta-lactam antibiotics are known to be active against *Pseudomonas aeruginosa*. Piperacillin (T1220) is a penicillin derivative which has markedly enhanced activity against *P. aeruginosa*, but like carbenicillin and ticarcillin, is inactivated by penicillinase[1~3]. Cefotaxime (HR 756) is the first derivative with a cephalosporin nucleus that has activity against *P. aeruginosa*[4]. Moxalactam (LY 127935) has a substituted cephalosporin nucleus and is active against many Gram-negative enteric organisms, including strains with resistance mediated by beta-lactamases[5~12]. *N*-Formimidoyl thienamycin (f-thienamycin, MK 0787) is a stable form of a novel beta-lactam antibiotic which has a fused ring nucleus and side chains that differ structurally from those of penicillin and cephalosporin compounds[13~15].

This study was designed to compare the relative activity of these beta-lactam antibiotics against a large number of recent clinical isolates of *P. aeruginosa*. The prevalence of carbenicillin resistance among clinical isolates of *P. aeruginosa* and the frequency of cross-resistance with the newer beta-lactam agents were determined. The effects of inducible or constitutive beta-lactamases in *P. aeruginosa* upon the activity of these agents were assessed. Furthermore, the influence of gentamicin resistance upon susceptibility to the beta-lactam drugs was evaluated.

Materials and Methods

Organisms

A total of 262 isolates of *P. aeruginosa* were studied. These isolates comprised all of the *P. aeruginosa* recovered from patients hospitalized at the Jackson Memorial Hospital during a three month interval. Consecutive isolates from the same patient were not included except for those recovered from

different culture sites. The isolates were identified in the clinical microbiology laboratory using standard microbiologic procedures and biochemical methods[16].

Antibiotics

The antibiotics obtained in either standard reference powders or solutions for use in this study included carbenicillin and ticarcillin (Beecham Laboratories, Bristol, Tennessee), moxalactam and tobramycin (Lilly Laboratories, Indianapolis, Indiana), piperacillin (Lederle Laboratories, Pearl River, New York), cefotaxime (Hoechst-Roussel, New Jersey), f-thienamycin (Merck, Sharp & Dohme, West Point, Pennsylvania), gentamicin (Schering Corp., Bloomfield, New Jersey), and amikacin (Bristol Laboratories, Syracuse, New York).

Susceptibility Studies

Minimal inhibitory concentrations (MICs) of these antibiotics were determined by the agar plate dilution method[17]. Serial two-fold dilutions of the antibiotics were added to MUELLER-HINTON agar to give final concentrations that ranged from 400 μg/ml to 0.01 μg/ml. The inoculum was prepared from a 4~5-hour broth culture of the organism, which was adjusted in turbidity to 10^7 CFU/ml and approximately 0.001 ml of the adjusted inoculum was applied to the plates by a STEER's replicator[18]. *P. aeruginosa* ATCC 27853 served as a control organism for the MIC determinations. The MIC was defined as the lowest concentration of antibiotic allowing either no visible growth or only one colony of growth after 18 hours of incubation at 37°C. An isolate was considered susceptible to carbenicillin/ticarcillin when its growth was inhibited at concentrations equal to or less than either 100 μg/ml of carbenicillin or 50 μg/ml of ticarcillin.

Beta-lactamase Studies

Selected isolates were tested for the presence of inducible beta-lactamase activity by a disk approximation method similar to that described by SANDERS, *et al.*[19]. Commercially prepared cefoxitin disks (30 μg) were placed on 15×100 mm plates containing approximately 15 ml of MUELLER-HINTON agar inoculated with a diluted suspension of organisms. Disks that contained 100 μg of carbenicillin, 50 μg of ticarcillin, 100 μg of piperacillin, 30 μg of cefotaxime, 30 μg of moxalactam or 10 μg of f-thienamycin were placed 12 to 13 mm from the cefoxitin disk. Four antibiotics including carbenicillin and piperacillin were tested on each plate. These plates were incubated overnight at 37°C and measurements made of the radius of the zone of inhibition and the extent that it was reduced toward the cefoxitin disk.

A chromogenic cephalosporin method was used for the detection of beta-lactamase activity[20]. Nitrocefin (Compound 87/312) was obtained from Glaxo Research Ltd., England. One-tenth ml of the cephalosporin substrate was added to each test well of a microdilution plate. A suspension of cells, taken from the periphery of the MUELLER-HINTON test plate and from around the cefoxitin disks in the center, was added to separate wells. Similarly, a 0.1 ml aliquot of overnight broth cell suspension was also tested for activity. The development of the red color within 60 minutes indicated the presence of beta-lactamase activity. Constitutive beta-lactamase activity was demonstrated if the nitrocefin test was positive on the overnight broth suspension and with organisms taken from the periphery of the MUELLER-HINTON plate. An isolate was considered to have inducible beta-lactamase activity when the nitrocefin test was positive only in organisms obtained adjacent to the cefoxitin disk.

Results

Concentrations of 100 μg/ml of carbenicillin or 50 μg/ml of ticarcillin inhibited 242 (92%) of 262 isolates of *P. aeruginosa*. This group included 11 isolates that had MICs in the susceptible range for one of these antibiotics but not the other. This population of susceptible isolates was used to obtain a set of data for each of the beta-lactam antibiotics. Each data set appeared to have a logarithmic normal distribution; the variability within these data sets was approximately similar as indicated by SDs which ranged from 0.8 to 1.2 in magnitude. Although these data sets had approximately equal variability, the MICs of the beta-lactam antibiotics against this population of susceptible *P. aeruginosa* isolates differed

considerably (Table 1). F-thienamycin was the most active beta-lactam antibiotic against carbenicillin/ticarcillin susceptible *P. aeruginosa*. Piperacillin was also very active against this population; however, the median MIC (MIC_{50}) was twice that of f-thienamycin. There was no difference between the MIC_{50} of ticarcillin and cefotaxime. These values were more than 10× that of f-thienamycin, and less than half that of carbenicillin. Susceptible isolates of *P. aeruginosa* were inhibited by moxalactam at a MIC_{50} which was similar to that obtained with carbenicillin.

Table 1. Activity of six beta-lactam antibiotics against 242 susceptible isolates of *Pseudomonas aeruginosa*.

Antibiotic	Median MIC (μg/ml)	SD (log_2)	MIC for 90% (μg/ml)
Thienamycin*	1.56(2.2)+	0.8	3.13
Piperacillin	3.13 (3.9)	1.2	12.5
Ticarcillin	25 (21)	1.0	50
Cefotaxime	25 (22)	0.9	50
Carbenicillin	50 (48)	1.1	100
Moxalactam	50 (61)	0.8	100

* *N*-Formimidoyl thienamycin.
\+ Geometric mean in parenthesis.

Twenty-four isolates of *P. aeruginosa* that were susceptible to carbenicillin were randomly selected and tested for the presence of cefoxitin-induced inactivation of the beta-lactam antibiotics by a disk approximation assay. No isolate showed constitutive beta-lactamase activity; however, all had inducible beta-lactamase activity in organisms grown around the cefoxitin disk. The mean radius of the zone of inhibition produced by each of the beta-lactam antibiotics and the mean decrease that occurred toward the cefoxitin disk are presented in Table 2. F-thienamycin was the only test antibiotic whose zone of inhibition was not diminished adjacent to the cefoxitin disk. All of the other agents showed a mean radial reduction toward the cefoxitin disk, although carbenicillin was affected to a lesser extent than ticarcillin, moxalactam or piperacillin. Cefotaxime was found to have the greatest reduction in mean radius and averaged 6.4 mm. The cefoxitin-induced radial reduction did not correlate with either the MIC or the size of the uninduced zone of inhibition for any of the beta-lactam antibiotics (correlation coefficients ≤ 0.2).

The MIC for 90% (MIC_{90}) of the susceptible isolates were used to evaluate the activity of the newer beta-lactam antibiotics against twenty (8%) of the *P. aeruginosa* isolates that were resistant to both carbenicillin and ticarcillin (Table 3). The proportions of carbenicillin resistant isolates that were susceptible to 12.5 μg/ml of piperacillin, 50 μg/ml of cefotaxime and 100 μg/ml of moxalactam were 20%, 20%, and 5%, respectively. In contrast, 18 (90%) of 20 carbenicillin resistant *P. aeruginosa*

Table 2. Inhibitory zone and cefoxitin-induced reduction in zone for 24 *Pseudomonas aeruginosa* isolates susceptible to the beta-lactam antibiotics.

Antibiotic disk (μg)	Radius of uninduced zone (mm)	Cefoxitin-induced radius reduction (mm)		
Thienamycin* (10)	11.8±1.8**	(0.5±0.5)+	p<0.05	p<0.05
Carbenicillin (100)	12.2±1.5	0.9±0.6		
Ticarcillin (50)	11.9±1.6	2.0±1.2	p<0.05	
Moxalactam (30)	11.8±1.6	2.4±1.1	p>0.05	
Piperacillin (100)	15.1±1.3	3.0±1.1		
Cefotaxime (30)	10.4±1.1	6.4±2.0	p<0.05	

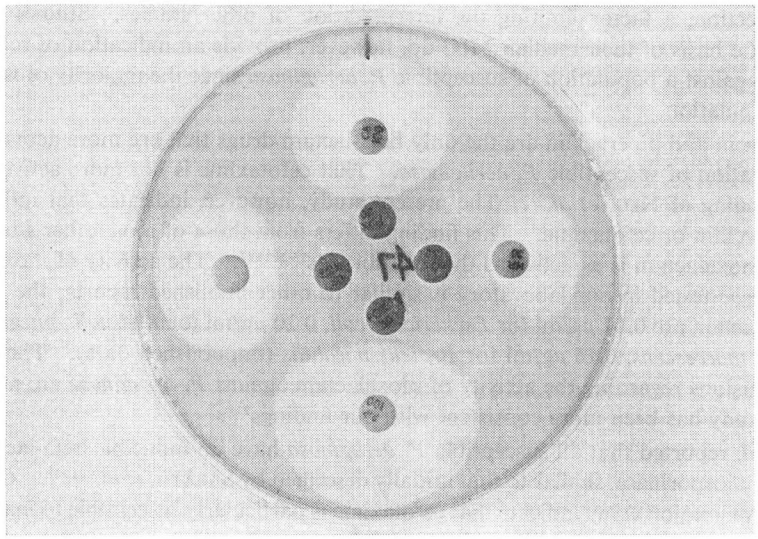
* *N*-Formimidoyl thienamycin.
** Mean±SD.
\+ Parenthesis indicate mean increase.

Table 3. Cumulative percentage of 20 carbenicillin-resistant *Pseudomonas aeruginosa* isolates inhibited by newer beta-lactam antibiotics.

Antibiotic (MIC_{90})	MIC (μg/ml)					
	3.13	6.25	12.5	25	50	100
Thienamycin (3.13)	90	95	100			
Piperacillin (12.5)			20	60	80	85
Cefotaxime (50)					20	75
Moxalactam (100)						5

Fig. 1. Cefoxitin-induced reduction in zone of inhibition for a carbenicillin resistant *Pseudomonas aeruginosa*.

The antibiotic impregnated disks arranged around the cefoxitin (FOX) disks contained 100 μg carbenicillin (CB-100), 30 μg cefotaxime (HR-30), 100 μg piperacillin (PIP-100), and 30 μg moxalactam.



were inhibited by f-thienamycin at a concentration of 3.13 μg/ml.

A constitutive beta-lactamase was demonstrated in 4 (20%) of the 20 carbenicillin resistant isolates. Three of these with constitutive beta-lactamase activity were isolates not inhibited by 100 μg/ml of piperacillin. They were also resistant to carbenicillin, ticarcillin, cefotaxime and moxalactam. These isolates produced small or no zones of inhibition to these antibiotics in the disk assay. The other isolate that caused a positive nitrocefin reaction in broth culture was also resistant to these agents, but it seemed to have an inducible component by the disk approximation method. This isolate gave relatively large zones of inhibition; however, the inhibitory zones around most of the antibiotic disks were markedly reduced adjacent to the cefoxitin disk (Fig. 1). The cefoxitin-induced radial reductions observed with this isolate were 35% for carbenicillin, 48% for ticarcillin, 48% for piperacillin, 46% for moxalactam and 78% for cefotaxime. F-thienamycin was not affected by the inducible component of this isolate. The remaining 16 isolates gave a positive nitrocefin reaction with organisms taken from around the cefoxitin disk, but not from the periphery of the plate or from broth culture. The majority of these isolates had no zones or small zones of inhibition around all of the antibiotic disks except the f-thienamycin disk. The magnitude of the cefoxitin-induced radial reduction for the isolates giving appreciable zones of inhibition was equal to or less than that noted with susceptible isolates.

Fifty-one (19%) of the 262 *P. aeruginosa* isolates were resistant to gentamicin at concentrations of 12.5 μg/ml or more. Seven (13.7%) of the gentamicin resistant isolates were not inhibited by 100 μg/ml of carbenicillin, however, this percentage was not significantly different from the percentage of gentamicin susceptible isolates that failed to be inhibited by this concentration of carbenicillin. The percentages of gentamicin resistant isolates that were also not inhibited by 6.25 μg/ml of tobramycin and 12.5 μg/ml of amikacin were 15.7% and 9.8%, respectively. Only two (22%) of the nine isolates that were resistant to more than one of these aminoglycoside antibiotics were resistant to carbenicillin.

Discussion

Most isolates of *P. aeruginosa* are susceptible to the activity of the beta-lactam antibiotics used in this study; however, differences between the activity of these agents are apparent. Differentiating a population of susceptible isolates from those that have resistance to antimicrobial activity alleviates the problem of selection, a factor limiting the interpretation of other studies. Studies which compare antibiotics on the basis of their median MIC do, however, provide an indication of relative activity of antimicrobials against a population of susceptible *P. aeruginosa* since the majority of isolates are likely to be in this population.

F-thienamycin and piperacillin are the only beta-lactam drugs that are more active than ticarcillin against a population of susceptible *P. aeruginosa*. That cefotaxime is not more active than ticarcillin confirms the finding of NEU, *et al.*[4]. The present study, however, indicates that moxalactam is less active than ticarcillin or cefotaxime. This finding differs from those of most other studies which have indicated that moxalactam is as active as these antibiotics[7~10,12]. The activity of moxalactam against *Enterobacteriaceae* tested in our laboratory is similar to other published reports; the geometric mean MICs of moxalactam are 0.17 μg/ml for *Escherichia coli*, 0.20 μg/ml for *Klebsiella pneumoniae*, 0.32 μg/ml for *Serratia marcescens*, 0.16 μg/ml for *Proteus mirabilis* (unpublished data). The reasons for the divergent conclusions regarding the activity of moxalactam against *P. aeruginosa* are not known; however, a recent study has been more consistent with our findings[21].

SYKES, *et al.* reported that all susceptible *P. aeruginosa* have an inducible beta-lactamase which is primarily a cephalosporinase, similar to that initially described by SABATH, *et al.*[22,23]. Our observations in the disk approximation assay indicate that cefotaxime is particularly susceptible to inactivation by this beta-lactamase, while f-thienamycin is evidently unaffected. Our data suggest that the inducible beta-lactamase also affects penicillin derivatives, although carbenicillin is affected to a lesser extent than either ticarcillin or piperacillin. Moxalactam appears to be susceptible to inactivation by the inducible beta-lactamase, although the reduction in moxalactam activity is less than that of cefotaxime. The expression of the inducible beta-lactamase observed in the disk approximation assay is not correlated with either the MIC or the size of inhibitory zone. This finding suggests that the presence of an inducible beta-lactamase is not a factor in these tests of *in vitro* susceptibility. Nevertheless, it could account for the rapid emergence of resistance *in vivo*.

Several plasmid-mediated beta-lactamases have been identified in carbenicillin resistant *P. aeruginosa* and the substrate profiles of these enzymes are known to differ[24,25]. Constitutive beta-lactamases found in *P. aeruginosa* are known to inactivate piperacillin to a variable extent, but not cefotaxime or moxalactam[25]. Cefotaxime is susceptible to hydrolysis by the beta-lactamases of other Gram-negative organisms[26]. Several investigations, however, indicate that moxalactam resists hydrolysis by these Gram-negative beta-lactamases[5~7,27]. The present study shows that cross-resistance between beta-lactam drugs and moxalactam does exist in *P. aeruginosa*. The finding that constitutive beta-lactamase activity accounts for antimicrobial resistance in only 20% of the carbenicillin resistant isolates is similar to that reported by KING, *et al*[28]. Therefore, it appears that permeability of the antibiotics through the cell wall is a more common mechanism for resistance to beta-lactam antibiotics than is enzymatic inactivation[29]. Our results confirm the findings of other investigations that f-thienamycin does not share cross-resistance with other beta-lactam drugs[30~32]. Strains of *P. aeruginosa* that were resistant to the

other beta-lactam drugs were as susceptible to f-thienamycin as were the carbenicillin/ticarcillin susceptible isolates.

Although the proportion of resistant isolates varies from institution to institution, resistance to gentamicin among *P. aeruginosa* has become a more frequent occurrence than resistance to carbenicillin. This may be related to the relative use of these drugs[33]; however, resistance to carbenicillin or the other beta-lactam antibiotics occurs independently of resistance to the aminoglycosides. Several studies may have failed to demonstrate cross-resistance with the newer beta-lactam drugs because aminoglycoside resistant organisms were studied[7~9].

Several factors will affect the extent that these antibiotics will be useful in the treatment of *Pseudomonas* infections. Some of the newer antibiotics may be advantageous since they are active against *P. aeruginosa* at considerably lower concentrations than the presently available agents. Resistance to the inducible beta-lactamase of *P. aeruginosa* is another consideration. The prevalence of organisms that are resistant to the available antibiotics and the lack of cross-resistance with these agents are likely to be equally important.

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