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Invalidity for *Pseudomonas aeruginosa* of an Accepted Model of Bacterial Permeability to β-Lactam Antibiotics

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The accepted model for the penetration of β-lactam antibiotics into gram-negative bacteria is that proposed by Zimmermann and Rosselet (Antimicrob. Agents Chemother. 12:368–372, 1977). The model assumes (i) that diffusion of the antibiotic molecules across the outer membrane obeys Fick's law and can be characterized by a permeability constant for any given combination of organism and drug, (ii) that drug hydrolysis within the periplasm obeys Michaelis-Menten kinetics, and (iii) that a steady state is rapidly attained between drug uptake and hydrolysis. The model has allowed accurate prediction of antibiotic MICs for *Escherichia coli* strains from a knowledge of their β-lactamase production and permeability characteristics. It has been suggested that the model is inappropriate for *Pseudomonas aeruginosa*, but attempts to confirm this have been bedevilled by experimental difficulties in estimating permeability coefficients for this species. In the present study, we tested a prediction of the model that the overall resistance of *P. aeruginosa* transconjugants containing a plasmid-encoded β-lactamase should continue to depend partly on permeability. Transconjugants with PSE-4 β-lactamase were constructed in host strains with widely different levels of intrinsic, presumably impermeability-determined resistance. Contrary to the prediction of the model, all the transconjugants developed identical overall levels of resistance to substrate β-lactams, such as azlocillin and cefoperazone, irrespective of the initial levels of intrinsic resistance of the recipient strains. We conclude that the model is inappropriate for *P. aeruginosa*, and possible explanations for the organism's behavior are discussed.

It is widely accepted that β-lactamases function cooperatively with outer membrane impermeability to protect the gram-negative cell from the antibacterial actions of β-lactam drugs (10, 18–21, 24). Thus, an impermeable strain with a β-lactamase should express greater resistance to substrate drugs than a permeable strain with the same amount of the same enzyme. Zimmermann and Rosselet (24), subsequently supported by others (1, 10, 19, 20), proposed that the interplay of β-lactamase and impermeability could be represented mathematically. Transmembrane diffusion of the drug is postulated to obey Fick's law:

$$v = C(S_o - S_p) \quad (1)$$

where v is the diffusion rate, S_o and S_p are the external and periplasmic drug concentrations, respectively, and C is a permeability coefficient which is related to the cell's surface area but constant for any given combination of strain and antibiotic. Within the periplasm, which is assumed to be a sac of free solution, hydrolysis of the β-lactam molecules by the β-lactamase obeys Michaelis-Menten kinetics:

$$v^* = V_{max}S_p/(K_m + S_p) \quad (2)$$

where v^* is the hydrolysis rate and V_{max} and K_m have their normal meanings. v^* can be measured experimentally as the hydrolysis rate of the antibiotic by intact cells. This allows calculation of S_p. It is further postulated that a steady state is rapidly established, such that v and v^* equalize and S_p is constant. At steady state it may be written:

$$C(S_o - S_p) = V_{max}S_p/(K_m + S_p) \quad (3)$$

Equation 3 has been used widely to calculate values of C from a knowledge of S_p, S_o, V_{max}, and K_m (1, 4, 20, 24). Rearrangement gives:

$$S_o = S_p\{1 + [V_{max}/C(K_m + S_p)]\} \quad (4)$$

Inspection shows that S_p tends to S_o if the latter is raised sufficiently. So long, however, as $S_o >> S_p$ and $K_m >> S_p$, simplification is possible:

$$S_o \sim S_p(V_{max}/CK_m) \quad (5)$$

S_o should equal the MIC when S_p is of just sufficient magnitude to cause intolerable inactivation of the essential penicillin-binding proteins (PBPs). If we use I_p to denote this level and substitute these terms in equation 4, we find:

$$\text{MIC} = I_p\{1 + [V_{max}/C(K_m + I_p)]\} \quad (6)$$

Once again, this simplifies when I_p is much less than both the MIC and the K_m and assumes the form:

$$\text{MIC} \sim I_p(V_{max}/CK_m) \quad (7)$$

It should be appreciated that equations 6 and 7 are specific solutions of the general situation described by equations 4 and 5, respectively.

Equation 6 has been validated exhaustively for *Escherichia coli* and has proved remarkably successful for the prediction of MICs from known kinetic and permeability data (19). It has not been proven for *Pseudomonas aeruginosa*, in which the applicability of the Zimmermann and Rosselet permeability model is debated. Some authors (1, 22) have obtained experimental results suggesting that the assumptions underlying the model (and outlined in the derivation of equation 3 above) are valid for *P. aeruginosa*. Others (4, 10, 11) have disagreed, finding that measured values of the permeability constant C vary with S_o for *P. aeruginosa*, whereas they are constant for *E. coli*. The source of this discrepancy is not clear; what is certain is that the measurement of v^*—necessary as a prelude to calculation of C—is difficult with *P. aeruginosa* and is bedevilled by such factors as (i) the leakage of cellular β-lactamase during

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TABLE 1. Sources and characteristics of *P. aeruginosa* strains used as recipients

Organism	Reference or origin	Intrinsic resistance	β-Lactamase activity (U/mg of protein)[a]	MIC (μg/ml)						
				Azlocillin	Carbenicillin	Cefoperazone	Cefotaxime	Tetracycline	Nalidixic acid	Chloramphenicol
Z799/61	23	Hypersensitive	0.59	0.008	0.015	0.008	0.008	0.5	0.5	0.5
Ps50SAI$^+$	2, 16	Low	5.8	0.5	1	1	0.5	4	16	32
Ps50SAI$^-$	2, 16	Low	5.2	0.25	0.5	0.5	0.25	4	16	16
R93	Isolate	Low	13.3	2	0.5	1	1	4	2	8
PAO1	Reference strain	Moderate	9.2	4	32	4	8	8	256	32
10701	NCTC[b]	Moderate	10.0	4	32	4	8	8	256	32
M1251	Isolate	High	6.0	32	1,024	16	64	128	1,024	512
M1426	Isolate	High	4.9	128	2,048	32	128	64	1,024	512

[a] Uninduced activity, assumed to be attributable to chromosomal class I β-lactamase; 1 U of activity hydrolyzed 1 nmol of nitrocefin per min.
[b] NCTC, National Collection of Type Cultures, London, United Kingdom.

assays (4, 22), (ii) the activity of enzyme that has leaked but then been adsorbed onto the cell surface (7), and (iii) the slow settling of cells in the assay cuvette.

This study aimed to test the validity of equations 4 to 7 for *P. aeruginosa* strains without the need for experimental measurement of *C* values.

MATERIALS AND METHODS

Antibiotics and reagents. Azlocillin was obtained from Bayer UK Ltd. (Newbury, Berkshire, United Kingdom), carbenicillin was obtained from SmithKline Beecham PLC (Brockham Park, Surrey, United Kingdom), cefoperazone was obtained from Pfizer UK Ltd. (Sandwich, Kent, United Kingdom), cefotaxime was obtained from Roussel Laboratories (Wembley, Middlesex, United Kingdom), chloramphenicol was obtained from Parke-Davis (Pontypridd, Gwent, United Kingdom), and tetracycline hydrochloride was obtained from Lederle Laboratories Division (Gosport, Hampshire, United Kingdom). Other reagents were from Sigma Chemical Co., St. Louis, Mo.

Strains. The *P. aeruginosa* strains used (Table 1) were clinical isolates or laboratory organisms and were selected because they showed the widest possible range of levels of intrinsic, non-β-lactamase-mediated resistance to β-lactam and non-β-lactam antibiotics. All strains except the mutants Z799/61 and Ps50SAI$^-$ were inducible for the chromosomal class I β-lactamase that is typical of the species; Z799/61 and Ps50SAI$^-$ were basal for this enzyme, expressing the same low levels irrespective of the presence of inducers. None of the organisms produced secondary β-lactamases before introduction of the pUZ8-PSE-4 plasmid. *P. aeruginosa* PU21 *ilv leu str rif* (5), containing the recombinant pUZ8-PSE-4 plasmid (13) (coding Tc^r, Hg^r, Km^r, Sm^r, and Su^r together with PSE-4 β-lactamase), served as the universal donor in transconjugant construction.

PBP assays. PBP susceptibility was measured by direct binding assays with [^{14}C]benzylpenicillin (Amersham International, Amersham, Buckinghamshire, United Kingdom), as described previously (9).

Assay of chromosomal β-lactamase activity. The uninduced chromosomal β-lactamase activity of the strains used as recipients was quantified by spectrophotometric assay with nitrocefin as the substrate (16).

Transfer of plasmid pUZ8-PSE-4. Logarithmic-phase cultures of *P. aeruginosa* PU21(pUZ8-PSE-4) and the *P. aeruginosa* recipients were grown, with shaking, at 37°C in 10-ml volumes of Nutrient Broth No. 2 (Oxoid Ltd., Basingstoke, Hampshire, United Kingdom) and then washed in 0.1 M phosphate buffer, pH 7.0. The washed cells were resuspended and mixed together in 1-ml amounts of the same buffer. The mixture was swabbed across the surface of plates of Diagnostic Sensitivity Test agar (Oxoid). After overnight incubation, the growth was washed into 2 ml of sterile saline and streaked onto plates of Kelly and Clarkes' minimal medium (6) containing 0.5% D-glucose as the sole carbon and energy source together with one of the following antibiotics: carbenicillin (1,000 μg/ml), kanamycin (1,000 μg/ml), spectinomycin (1,000 μg/ml), and tetracycline (100 μg/ml). Presumptive transconjugants were subcultured after 24 to 48 h of incubation at 37°C and confirmed to be prototrophs and to produce PSE-4 β-lactamase, as examined by isoelectric focusing (17).

Susceptibility tests. MICs were determined on Diagnostic Sensitivity Test agar containing doubling dilutions of antibiotics. The inocula, taken from overnight cultures in nutrient broth, comprised 10^4 CFU per spot. Single-cell resistances (SCRs) were determined by spreading ca. 10^2 CFU, again from overnight nutrient broth cultures, onto Diagnostic Sensitivity Test agar plates containing doubling dilutions of antibiotics. The colonies were enumerated after overnight incubation at 37°C. The SCR was defined as the highest drug concentration to allow recovery of at least 90% of the colonies growing on the control, drug-free agar.

Kinetics of PSE-4 β-lactamase. Clarified cell sonic extracts containing PSE-4 β-lactamase were obtained from *P. aeruginosa* cultures as described previously (12). The activities of the sonic extracts against 25 to 500 μM azlocillin and benzylpenicillin and against 10 to 100 μM cefoperazone and cefotaxime were measured by spectrophotometry at 37°C. The diluent was 0.1 M phosphate buffer, pH 7.0. The assay wavelengths were 235 nm for the penicillins, 257 nm for cefotaxime, and 265 nm for cefoperazone. Measurements were made at 37°C. V_{max} and K_m values were calculated by linear regression analysis of Hanes (S/v versus S) plots of initial hydrolysis rates (v) at 10 or more different antibiotic concentrations (S).

Measurement of β-lactamase activity in intact and disrupted cells. Cultures of transconjugants were grown overnight in Antibiotic No. 3 Medium (Difco, Detroit, Mich.) containing tetracycline (50 μg/ml) to promote plasmid conservation. The cultures were then diluted 100-fold into identical but tetracycline-free media. Incubation at 37°C was continued for 4 h, with constant shaking. Subsequently, the cells were harvested at 37°C and 5,000 × *g* and resuspended at a

density of 100 mg (wet weight) per ml in 0.1 M phosphate buffer (pH 7.0) containing 0.9% NaCl. One sample from this suspension was sonicated on ice (2× 20-s bursts, amplitude 12 μm; MSE Ultrasonic Disintegrator, MSE Instruments, Crawley, Sussex, United Kingdom). The sonic extract was warmed to 37°C and mixed with 2.5 to 10 mM azlocillin in the same buffer. Hydrolysis of the drug was monitored by spectrophotometric assay at 235 nm and 37°C in 1-mm light path cuvettes. A second sample of the cell suspension was mixed with 2.5, 5, 7.5, or 10 mM azlocillin in the same buffer at 37°C. Aliquots (100 μl) were withdrawn immediately and after 10, 20, and 30 min. The cells were removed by centrifugation at 12,000 × *g* for 1 min (Eppendorf Microfuge; Eppendorf GmbH, Hamburg, Germany), and the residual azlocillin concentration was measured immediately by high-pressure liquid chromatography (HPLC) assay on an LC3 chromatograph (Pye Unicam, Cambridge, Cambridgeshire, United Kingdom), with the UV detector set at 254 nm. A reverse-phase C_{18} column (10 by 1.0 cm) enclosed in a Waters 12CM 100 radial compression module was used. The elution solvent consisted of 3.6 g of ammonium carbonate dissolved in 550 ml of water and was filled to a final volume of 1 liter with methanol. Injection was via a completely filled 20-μl fixed loop. At a flow rate of 2.0 ml/min, azlocillin had a retention time of 3.1 min and the detector response was linear over the concentration range tested. A final sample of the cell suspension was clarified by centrifugation at 12,000 × *g* for 1 min, and the β-lactamase activity, representing leaked enzyme, was measured by spectrophotometry as described above.

Validation with clarified sonic extracts showed that the azlocillin hydrolysis rate appeared to be identical regardless of whether spectrophotometry or HPLC was used as the assay system.

RESULTS

Susceptibility of recipient strains. The strains used as recipients varied widely in their susceptibilities to β-lactam and non-β-lactam antimicrobial agents (Table 1). Strains Ps50SAI$^+$ and R93 were highly susceptible to azlocillin, carbenicillin, cefoperazone, and cefotaxime and, compared with typical *P. aeruginosa* strains, were relatively susceptible to nalidixic acid, tetracycline, and (R93 only) chloramphenicol. Ps50SAI$^-$ (2, 16), the chromosomal β-lactamase-basal mutant of Ps50SAI$^+$, had a level of susceptibility to all the antimicrobial agents tested here that was identical (± 1 dilution) to that of its parent strain. Strains PAO1 and 10701 showed the levels of susceptibility and resistance to these β-lactam and non-β-lactam agents that are typical of most *P. aeruginosa* isolates, whereas strains M1251 and M1426 were unusually resistant. Cross-resistance or cross-susceptibility of this type between β-lactam and non-β-lactam agents is observed in virtually all *P. aeruginosa* isolates and is presumed to be a reflection of the level of nonspecific outer membrane (im)permeability (3, 8, 10, 11). It cannot, however, be correlated with obvious differences in porin quantity or lipopolysaccharide profile (8, 11).

The hyperpermeable mutant Z799/61 was substantially more susceptible than even strains Ps50SAI$^+$ and R93 to both β-lactam and non-β-lactam drugs.

Strain characterization. PBP profiles of all the test organisms were identical. The 50% inhibitory concentrations (i.e., those required to give 50% saturation) of [^{14}C]benzylpenicillin for individual PBPs varied by twofold or less among the strains. Likewise, the uninduced levels of β-lactamase activity, which were attributable to the chromosomal class I enzyme, showed less than threefold variation among the strains used as recipients (Table 1).

TABLE 2. Effects of plasmid pUZ8-PSE-4 on resistance to β-lactam antibiotics

Organism	SCR[a] (μg/ml)					
	Azlocillin		Cefoperazone		Cefotaxime	
	R$^-$	R$^+$	R$^-$	R$^+$	R$^-$	R$^+$
Ps50SAI$^+$	0.25	128	0.25	64	0.5	0.5
Ps50SAI$^-$	0.12	128	0.12	32	0.25	0.25
R93	1	64	0.5	32	0.5	0.25
PAO1	2	128	2	64	4	2
10701	2	64	4	32	4	2
M1251	16	128	64	64	64	32
M1426	64	128	32	64	64	16
V_{max} (% penicillin G)	49.8		6.1		<0.1	
K_m, μM (μg/ml)	22 (10)		2.5 (1.6)		>100[b] (>45)	

[a] SCRs represent the highest drug concentrations to allow growth and, in the absence of inoculum effects, should equal 0.5× the MIC. R$^-$, Without plasmid pUZ8-PSE-4; R$^+$, with plasmid pUZ8-PSE-4.
[b] >100 μM = K_i.

Susceptibilities of the transconjugants. Introduction of the pUZ8-PSE-4 plasmid raised the MICs of and SCRs to azlocillin and cefoperazone to almost the same level in all the transconjugants. Thus, the SCRs to azlocillin rose to 64 to 128 μg/ml and those to cefoperazone rose to 32 to 64 μg/ml, irrespective of the initial susceptibility or resistance of the recipient strain. These values represented a 128- to 256-fold increase in resistance in the strains with the greatest initial intrinsic susceptibility (R93 and Ps50SAI$^+$) but only a 1- to 2-fold rise in those with the greatest intrinsic resistance (M1251 and M1426). SCRs to these antibiotics of the recipients and transconjugants are shown in Table 2 and are favored over MICs as measures of resistance on the grounds that they are not subject to distortions arising from the local activity of β-lactamase leaked by dense point inocula. In the absence of such effects the SCR should equal 0.5× the MIC, and this behavior was generally observed. SCRs to and MICs of cefotaxime, which is not a substrate for PSE-4 enzyme (Table 2) (14), were unchanged or reduced by one dilution on acquisition of the pUZ8-PSE-4 plasmid. The SCRs for the transconjugant of Ps50SAI$^-$ were identical, ±1 dilution, to those for the transconjugant of Ps50SAI$^+$.

Kinetics of PSE-4 β-lactamase. The K_m and V_{max} values for the antibiotics are listed in Table 2. Azlocillin was rapidly hydrolyzed, cefoperazone was a low-V_{max}, low-K_m substrate, and cefotaxime was stable within the detection limits of the assay. Cefotaxime was found not to inhibit hydrolysis of benzylpenicillin, indicating a low affinity for PSE-4 enzyme.

Measurement of β-lactamase activity in intact and disrupted cells. The rates of hydrolysis of azlocillin by sonic extracts of the transconjugants (v_{son}) fell within a narrow range, indicating little interstrain variation in expression of the PSE-4 gene (Table 3). As would be predicted from the low K_m value, the hydrolysis rates obtained with 2.5, 5, 7.5, and 10 mM antibiotic were identical and corresponded to V_{max} (Table 3). Hydrolysis rates of 2.5 to 10 mM azlocillin by intact cells, after subtraction of the activity attributable to leaked enzyme, were from 2 to 17% of those obtained with the sonicates and also appeared to be relatively unaffected by the drug concentration employed. These hydrolysis rates

TABLE 3. Hydrolysis rates of azlocillin by intact and sonicated cells of pUZ8-PSE-4 transconjugants

Transconjugant	V_{max} (μmol/min/mg of wet cells)	Hydrolysis at S_o (mM) of[a]:							
		2.5		5		7.5		10	
		v^*/v_{son} (%)	S_p (μM)	v^*/v_{son} (%)	S_p (μM)	v^*/v_{son} (%)	S_p (μM)	v^*/v_{son} (%)	S_p (μM)
Ps50SAI$^+$	0.28	6.6	1.5	6.8	2.7	8.3	2.3	8.5	2.0
Ps50SAI$^-$	ND[b]	ND	ND	ND	ND	ND	ND	ND	ND
R93	0.35	7.9	2.0	9.9	2.4	11	2.7	11	2.7
PAO1	0.67	3.7	0.9	5.1	1.2	5.3	1.2	6.0	1.4
10701	0.43	5.4	1.3	5.2	1.2	5.3	1.2	6.5	1.5
M1251	0.34	15	3.9	16	4.1	16	4.8	17	4.6
M1426	0.50	1.7	0.4	1.7	0.4	1.5	0.3	1.5	0.3

[a] v_{son} (approximately equal to V_{max}) and v^* were determined experimentally, and S_p was calculated by using these data with equation 2. Because of the sources of error indicated in the text, the v^*/v_{son} ratios and S_p values should be considered as overestimates, not actual values.
[b] ND, Not determined.

(and the periplasmic azlocillin concentrations that they imply), derived via equation 2, are shown in Table 3. It has been mentioned already that the measurement of v^*/v_{son} for *P. aeruginosa* is problematic. Slow leakage of enzyme during assays (4, 22) or adsorption of drug or leaked enzyme onto the cell surface (4, 7) may increase the apparent value of v^*. Enzyme inactivation during sonication may reduce v_{son}. All these distortions will increase v^* relative to v_{son}, leading to overestimation of the v^*/v_{son} ratio. It is difficult to envisage any error that will have the converse effect; thus, the v^*/v_{son} ratios and S_p values in Table 3 should be taken as overestimates, not actual values. All that maters, for the present argument, is that in all cases $v^* << V_{max}$ and $S_p << K_m$ (see Discussion).

DISCUSSION

In the Introduction it was argued that equation 7 is a valid simplification of equation 6 when I_p was much less than both the K_m and MIC. These conditions were met for azlocillin in the case of the PSE-4 β-lactamase-producing *P. aeruginosa* transconjugants used in the present study. I_p can be reasonably taken as the MIC of azlocillin, i.e., 0.008 μg/ml, for the β-lactamase-deficient, hyperpermeable mutant Z799/61 (Table 1). Alternatively, the susceptibility of the primary target PBP (PBP 3 in the case of azlocillin acting against *P. aeruginosa*) can be used as an estimate of I_p (19). The 50% inhibitory concentrations of azlocillin for this PBP are reportedly in the range from 0.01 to 0.02 μg/ml (22). All these values are substantially below the K_m of PSE-4 enzyme for azlocillin (10 μg/ml) or the MICs for the recipients (0.5 to 64 μg/ml), let alone those for the transconjugants (128 to 256 μg/ml [Table 1 and 2]). Z799/61 was not used as a recipient because I_p was not much less than the MIC for this mutant in the absence of the plasmid.

The data in Table 3 also suggest that equations 5 and 7 should have been obeyed by the transconjugants. Although the S_o levels (2.5 to 10 mM [1,125 to 4,500 μg/ml]) used to measure azlocillin hydrolysis with the intact cells were 5- to 40-fold above the MICs, the v^*/v_{son} (equal to v^*/V_{max}) ratios remained low, a condition which pertains only when S_p is much less than the K_m. Thus, even at S_o values above the MIC, the assumptions ($S_p < K_m$ and $S_p << S_o$) that underpin the simplification of equation 4 to equation 5 (see Introduction) remained valid. These assumptions should therefore also be valid under the less extreme case, represented in equation 7, where S_o = MIC and $S_p = I_p$.

All this logic suggests that the *P. aeruginosa* transconjugants should have behaved in accordance with equations 5 and 7, yet they failed to do so. If equation 7 was valid, the MICs for and SCRs of the transconjugants ought still to have depended partly on the initial intrinsic resistance of the recipient strains. Azlocillin resistance ought to have been greatest for the transconjugants prepared from the recipients that had the greatest intrinsic resistance. In fact, the SCRs of and MICs for the transconjugants were virtually constant irrespective of the intrinsic resistance of the recipient hosts. Thus, the model seems invalid for *P. aeruginosa*.

Several explanations of these results can be discounted. First, the variation in intrinsic resistance among the recipients possibly was not related to impermeability. This seems unlikely. Variations in PBP susceptibility and chromosomal β-lactamase expression were discounted as sources of resistance in these organisms. Moreover, the MICs of β-lactams for the recipients correlated with those of non-β-lactam agents (Table 1), whose activities could not have been affected by PBP susceptibility or β-lactamase expression. Correlation between the MICs of penicillins, cephalosporins, quinolones, tetracyclines, and chloramphenicol is seen for the vast majority of *P. aeruginosa* isolates (3, 8, 10, 11). Similar cross-resistance is seen in carbenicillin-selected laboratory mutants, confirming that the cross-resistance depends on a single mechanism (15). It is difficult to envisage any mechanism other than outer membrane (im)permeability that could simultaneously modulate susceptibility to diverse agents, some acting inside and some acting outside the cytoplasmic membrane.

Second, our results may have been distorted by the activity of the chromosomal class I β-lactamase which was inducible in all the recipient strains except Ps50SAI$^-$. Azlocillin, though labile to this enzyme, normally fails to induce its synthesis in MIC tests; hence the activity of the enzyme does not influence the MIC obtained (16). The pUZ8-PSE-4 plasmid conceivably might alter this behavior, allowing more efficient β-lactamase induction by these drugs, perhaps particularly in the strains with the greatest permeability (i.e., R93 and Ps50SAI$^+$). The class I enzyme might then affect azlocillin susceptibility. The results with Ps50SAI$^-$ belie this suggestion. Transconjugants of this mutant, which had basal expression of its chromosomal β-lactamase, acquired identical resistance to transconjugants of its β-lactamase-inducible parent strain (Table 2).

Third, the PSE-4 β-lactamase could have been expressed at a lower level in the transconjugants of the more intrinsically resistant strains. The similarity of the v_{son} rates among the transconjugants discounts this hypothesis. Fourth, the

TABLE 4. Resistance of *P. aeruginosa* Ps50SAI and M2297 and their chromosomal β-lactamase expression mutants[a]

Mutant series	MIC (μg/ml)						
	Azlocillin	Carbenicillin	Cefoperazone	Cefotaxime	Tetracycline	Chloramphenicol	Nalidixic acid
Ps50SAI, low intrinsic resistance							
β-Lactamase-inducible parent	0.5	1	1	0.5	4	32	16
β-Lactamase-constitutive mutant	128	16	32	256	4	32	16
β-Lactamase-basal mutant	0.25	0.5	0.5	0.25	4	32	16
M2297, moderate intrinsic resistance							
β-Lactamase-inducible parent	4	32	4	8	16	64	128
β-Lactamase-constitutive mutant	128	64	64	512	16	64	128
β-Lactamase-basal mutant	4	32	4	8	16	64	128

[a] Data are from reference 16, wherein full details of the mutants' derivation and β-lactamase production characteristics are given.

pUZ8-PSE-4 plasmid might have affected permeability to β-lactams. This hypothesis was disproved by the observation that resistance to cefotaxime, which is not a substrate for PSE-4 enzyme (14) (Table 2), was unaffected by the presence of the plasmid. Fifth, the behavior of azlocillin might, for whatever reason, be unusual. The activity of cefoperazone, a weak substrate of PSE-4 enzyme, was therefore also examined against the transconjugants. The K_m of cefoperazone for PSE-4 enzyme is 2.5 μM (1.6 μg/ml) (Table 2), and I_p, estimated as for azlocillin, is 0.008 μg/ml (Table 1). Thus, equations 5 and 7 ought again to be valid. However, as with azlocillin, the resistance of the transconjugants was identical and was unrelated to the intrinsic resistance of the recipients. The behavior of azlocillin is therefore not atypical. Alternatively, the behavior of PSE-4 enzyme might be unusual. This possibility, too, can be discarded; previously (14), we have obtained mutants of *P. aeruginosa* that have high-level constitutive expression of the chromosomal class I β-lactamase. MIC data for *P. aeruginosa* Ps50SAI$^+$ (low intrinsic resistance [Table 1]) and M2297P (moderate intrinsic resistance) and their β-lactamase-constitutive mutants are reproduced and compared in Table 4. Although Ps50SAI$^+$ had much lower intrinsic resistance than M2297P, their β-lactamase-constitutive mutants were similarly resistant to β-lactam drugs. Resistance was fully lost in β-lactamase-deficient mutants that were derived from the enzyme-constitutive organisms. A final explanation of the present data would be that MICs are distorted by inoculum effects. This argument too can be discarded; SCR values, which measure the susceptibility of the individual organism, behaved analogously to the MICs.

Having discarded these explanations of the data, we must consider why *P. aeruginosa* did not behave in accordance with the model. Beyond that, we must consider why the β-lactamase should give the greatest increase in resistance to substrate β-lactams in the most permeable organisms (i.e., those with the lower intrinsic resistances, namely, strains Ps50SAI$^+$ and R93) and the least increase in the most impermeable ones (M1251 and M1426). Neither question is yet answerable. One of the more attractive explanations would be that some degree of structure or organization exists in the periplasm of *P. aeruginosa* and that this modulates both the level of intrinsic, impermeability-determined resistance expressed by a strain and the efficiency with which the β-lactamase can contact and destroy the incoming β-lactamase molecules. In the absence of direct evidence this model must, however, remain speculative. What is certainly true, however, is that *P. aeruginosa* does not conform with the model, outlined in equations 1 through 7, which has provided an excellent approximation for the behavior of *E. coli*.

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