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Reevaluation of the Factors Involved in the Efficacy of New β-Lactams against *Enterobacter cloacae*

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The roles of outer membrane permeability, β-lactamase stability, and inhibition of penicillin-binding proteins in the activity of new β-lactams against *Enterobacter cloacae* were reappraised by using several methodological improvements. Outer membrane permeability in intact cells was determined by using a high-pressure liquid chromatography (HPLC)-based technique that avoided certain possible artifacts of the traditional methods. V_{max} values were calculated from the numbers of enzyme molecules produced per cell and from catalytic constant (K_{cat}) values, which were obtained with purified β-lactamase. Minimal periplasmic antibiotic concentrations needed to inhibit bacterial cell wall synthesis were estimated from the Zimmermann-Rosselet equation. All the β-lactams tested formed relatively stable complexes with purified β-lactamase. The antibiotics that exhibited low affinity for β-lactamase apparently needed higher periplasmic concentrations to inhibit cell wall synthesis, suggesting a possible correlation between the affinity of β-lactamase and the affinity of penicillin-binding proteins for the new β-lactams. By using these estimates of outer membrane permeability, β-lactamase hydrolysis, and cell wall-inhibiting concentrations, MIC could be theoretically predicted to within 1 dilution for five β-lactams in three isogenic *E. cloacae* strains with differences in antibiotic susceptibility due to different porin or β-lactamase contents.

In recent years, several structurally modified compounds have been successively developed to improve the activity of β-lactam antibiotics. High affinity for the target enzymes, named penicillin-binding proteins (PBPs), and resistance to β-lactamase hydrolysis have been considered the most important factors contributing to the efficacy of these new β-lactams. For example, the expanded-spectrum cephalosporins were designed with these factors in mind. The addition of a methoximyl 5-aminothiazol moiety at the 7α position of the cephem ring considerably increased the β-lactamase stability of these compounds. Several authors were unable to detect any hydrolysis when expanded-spectrum cephalosporins were added to crude extracts of β-lactamases of common strains of the family *Enterobacteriaceae* (10, 27). Nevertheless, cephalosporinase enzymes exhibited high affinity (low K_m) for all of these cephalosporins (5, 16).

On the basis of these findings, Then and Angehrn (28) and subsequently several other authors (10, 32) proposed the trapping model, that is, nonhydrolytic binding to β-lactamase, to explain the resistance to expanded-spectrum cephalosporins in β-lactamase-overproducing gram-negative strains. However, Vu and Nikaido (30) pointed out that according to their calculations, the number of β-lactamase molecules produced by resistant strains of *Enterobacter cloacae* was probably insufficient to trap the number of antibiotic molecules crossing the outer membrane. Livermore (15) then established that the use of conventional hydrolysis assays had led to misleading conclusions, since the β-lactamase was subject to a 10,000-fold dilution during the preparation of crude enzyme extracts from cells. Recently, Marchou et al. (16) demonstrated experimentally that ceftriaxone was hydrolyzed at readily measurable rates by purified class I β-lactamase when the assay was performed at physiologically relevant concentrations of enzyme and substrate.

Two newer antibiotics, cefpirome and cefepime, have been classified as "fourth-generation" cephalosporins because their potencies against members of the *Enterobacteriaceae* are higher than those of the earlier broad-spectrum cephalosporins. In addition, both antibiotics remained effective against β-lactamase-overproducing gram-negative strains which were resistant to expanded-spectrum cephalosporins. Several reports (12, 13, 18, 22) ascribed this efficacy to high β-lactamase stability and low affinity for the enzyme.

In gram-negative bacterial cells, permeability also plays a key part in β-lactam activity, since the β-lactam molecules have to penetrate the outer membrane before they reach their targets at the surface of the bacterial inner membrane. A recent study (16) of the resistance to ceftriaxone in *E. cloacae* demonstrated that the activity of the β-lactam antibiotic depends on the combined effects of β-lactamase and permeability limitations. More recently, Nikaido et al. (18) found that the rates of penetration through proteoliposomes reconstituted with porins from *Escherichia coli* and *E. cloacae* were higher for cefepime and cefpirome than for ceftazidime and cefotaxime. These authors argued that the differential rates would increase in cells because of the repulsive effect of fixed anions in the periplasm acting on the negatively charged expanded-spectrum cephalosporins but not on the dipolar ionic fourth-generation compounds. However, the exact contribution of outer membrane permeability to the activity of new β-lactams could not be assessed in intact cells by using methods available at the time. Only readily hydrolyzed molecules could be assayed by the conventional method of Zimmermann and Rosselet (33), and several possible artifacts could bias the results (1). We have now developed a method based on a high-pressure liquid chromatography (HPLC) technique that allows measure-

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ment of uptake of new β-lactams in intact *E. cloacae* cells (1).

In this paper, we describe the activity of five different new β-lactam antibiotics against three isogenic *E. cloacae* strains and the relative importance of β-lactamase stability, target affinity, and outer membrane permeability properties in their efficacy.

MATERIALS AND METHODS

Chemicals and growth medium. The antibiotics employed in this study were kindly supplied as follows: ceftriaxone and carumonam, Hoffmann-La Roche, Basel, Switzerland; cefotaxime and cefpirome, Hoechst, Frankfurt, Germany; and cefepime, Bristol-Myers Co., Syracuse, N.Y. Antibiotic medium no. 3 was purchased from Difco Laboratories, Detroit, Mich.

Bacterial strains. The origin and characterization of the three *E. cloacae* 218 strains used in this study have been detailed previously (16). Briefly, strain S was a normally susceptible clinical isolate. Strain R1 was a β-lactamase-overproducing, resistant variant of strain S, selected on a ceftriaxone-gradient plate. Strain R2 was a superresistant variant of strain S, selected on ceftriaxone-gradient agar after exposure of strain S to ceftriaxone therapy in a mouse model. In addition to producing the same high level of β-lactamase as strain R1, strain R2 expressed less OmpF-like porin than R1 or S (16).

β-Lactamase studies. β-Lactamase was purified on a 3-aminophenyl boronic acid-modified Affigel 10 column (6). The activity of crude extract or purified β-lactamase was assayed as described previously (16). The number of β-lactamase molecules produced by one bacterial cell was calculated by comparing the activity of a crude extract obtained from cells with the activity of purified β-lactamase (16). Hydrolysis of β-lactams was determined at 25°C in 50 mM sodium phosphate buffer (pH 7.0) with 1.0 to 5.0 μM (depending on the stability of the substrate) purified β-lactamase and at least a 100-fold excess of the β-lactam compound.

For each antibiotic, spectrophotometric measurements of cephalosporin hydrolysis were performed at appropriate wavelengths (λ, in nanometers) at which the difference between the molar extinction coefficients (Δε, millimolar centimeter^{-1}) of the hydrolyzed and nonhydrolyzed molecule was maximum, as follows: ceftriaxone, 255 and 7.70; cefotaxime, 262 and 6.73; cefpirome, 284 and 4.75; cefepime, 280 and 5.15; and carumonam, 321 and 0.7. The linear portion of the hydrolysis progress curve was used to calculate the catalytic constant (K_{cat}) according to the relationship $V_{max} = K_{cat} \times E$. The concentration of β-lactamase per milligram of bacteria (E), e.g., in strain 218 R1, was determined as follows: (2.4×10^5 β-lactamase molecules per cell) $\times$ (3.6×10^9 cells per milligram of bacteria [dry weight]) = 8.6×10^{14} β-lactamase molecules per mg. This value divided by Avogadro's number (6.02×10^{23} molecules per mol) gives 1.44 nmol/mg. K_m values were determined at various substrate concentrations by the linear regression analysis of Lineweaver-Burk plots. K_i values were measured by using cephalothin as the substrate (initial concentration of 300 μM) by the integrated Michaelis-Menten equation method (21).

Permeability studies with intact cells. The HPLC protocol for studying β-lactam hydrolysis by intact *E. cloacae* cells has been detailed elsewhere (1). Outer membrane permeability coefficients (P) were calculated from the equation of Zimmermann and Rosselet (33), which assumes that the rate of hydrolysis of β-lactam by intact cells at equilibrium balances the rate of permeation into the cell; thus,

$$V = P \times A \times (S_o - S_p) = V_{max} \times \frac{S_p}{S_p + K_m} \qquad (1)$$

in which V is the rate of hydrolysis by intact cells (as measured directly by HPLC), $P \times A$ is equal to the C parameter of the Zimmermann-Rosselet equation (33), A is the area of cell surface per unit of weight (24), S_o is the antibiotic external concentration, V_{max} and K_m values are the Michaelis constants obtained as described above, and S_p is the periplasmic concentration of β-lactam calculated from the Michaelis-Menten relationship $S_p = (V \times K_m)/(V_{max} - V)$.

An example of a calculation of P is given below. The rate of hydrolysis (V) of 80 μM cefpirome by intact cells was 0.030 nmol $\cdot$ s^{-1} $\cdot$ mg of bacteria (dry weight)$^{-1}$. S_p was calculated to be 19 μM. From the Fick's first law equation, P was estimated to be the following: 0.030 nmol $\cdot$ s^{-1} $\cdot$ mg^{-1}/132 cm^2 mg^{-1}/[(80 − 19) nmol cm^{-3}] = 3.7×10^{-6} cm $\cdot$ s^{-1}, or 37 nm $\cdot$ s^{-1}. To calculate the minimal periplasmic concentration (S_i) needed to inhibit the targets (presumably cell wall synthesis) of β-lactams sufficiently to cause bacterial growth inhibition, we expressed S_p as a function of S_o by solving equation 1 for S_p to give the following:

$$S_p = 0.5 \times \{S_o - K_m - V_{max}/(P \times A) + [(-S_o + K_m + V_{max}/P \times A)^2 + 4 \times S_o \times K_m]^{1/2}\} \qquad (2)$$

When equation 2 was solved for the S_o equivalent to the MIC, S_p corresponded to S_i.

RESULTS

β-Lactamase studies. The three isogenic *E. cloacae* strains produced different amounts of the same (16) inducible type 1 β-lactamase: 320 molecules per cell for strain 218 S (noninduced) and 240,000 molecules per cell for strains R1 and R2. Direct measurements of K_m values of ceftriaxone, cefotaxime, and carumonam were rendered difficult because of their high affinity (low K_m) for *E. cloacae* 218 β-lactamase, so that K_i values (equal to K_m values under the conditions utilized [29]) were determined instead (Table 1). Conversely, cefepime and cefpirome exhibited low affinity (high K_m), and thus their K_m values were directly measured. The results obtained for the kinetics parameters of *E. cloacae* 218 β-lactamase were very similar to those published by Then et al. (29) for the same enzyme. Small differences in K_{cat} values could be related to the existence of a branch pathway following interaction of β-lactamase with substrate (7).

V_{max} values obtained with intact bacterial cells for readily hydrolyzed β-lactams have usually been determined with crude sonicated β-lactamase extract (20, 33). However, most of the new β-lactam compounds remained stable to hydrolysis under such conditions, probably because after sonication, β-lactamase was diluted 10,000-fold relative to its actual concentration in the periplasmic space. It is known that dilution rapidly decreases the stability and activity of β-lactamases (15). Since the physicochemical conditions in the periplasmic space, e.g., pH, viscosity, and ionic strength, are poorly understood, the actual V_{max} values in intact bacterial cells are probably not measurable. However, the variation of pH from 5 to 9, the addition of ethylene glycol to the medium, and changes in ionic strength only

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TABLE 1. Hydrolysis of β-lactams by *E. cloacae* purified β-lactamase

β-Lactam	MIC (μg/ml)[a]			Value for kinetic parameter			
	S	R1	R1/S	K_{cat}[b] (s^{-1})	V_{max}[c] (nmol/mg/s)	K_m (μM)	V_{max}/K_m
Cefotaxime	0.1	20	200	0.0381	0.0552	0.2[d]	0.28
Ceftriaxone	0.1	20	200	0.0220	0.0317	0.1[d]	0.32
Carumonam	0.02	4	200	0.0008	0.0012	0.04[d]	0.03
Cefpirome	0.05	0.5	10	0.1730	0.250	140	<0.002
Cefepime	0.05	0.5	10	0.1501	0.216	180	<0.002

[a] Results of susceptibility testing came from reference 16, with the exception of data for cefpirome and cefepime, whose MICs were determined by the same method.
[b] Catalytic constant at 25°C.
[c] V_{max} of strain 218 R1, calculated from the equation $V_{max} = K_{cat} \cdot E$ (see Materials and Methods).
[d] K_m values were obtained by indirect determination (i.e., measurement of K_i).

slightly affected the activity (V_{max}) of β-lactamase in our experiments (data not shown). These data suggested that a concentrated solution of purified β-lactamase was probably the best available model to mimic the actual conditions of the periplasm. The stability of complexes of individual β-lactams with β-lactamase was assessed by measurement of V_{max}. Measurement of K_{cat} values under these conditions demonstrated that carumonam and to a lesser extent ceftriaxone, cefotaxime, cefpirome, and cefepime formed stable complexes with β-lactamase (Table 1). Extrapolating from these data, we calculated V_{max} values from the relationship $V_{max} = K_{cat} \times E$ (see Materials and Methods). Consistently, V_{max} for cephaloridine (1,656 nmol $\cdot$ s^{-1} $\cdot$ mg^{-1}) determined in this way was higher than that obtained with crude extract (530 nmol $\cdot$ s^{-1} $\cdot$ mg^{-1}). Nevertheless, the magnitudes of our V_{max} values were similar to those of other published estimates (18, 30).

The values of V_{max}, which reflected the stability of enzyme-substrate complexes, were used to calculate the physiological efficiency of the enzyme according to the method of Pollock (23). Because of their low affinity for β-lactamase, cefepime and cefpirome exhibited very low V_{max}/K_m ratio values. This ratio, termed physiological efficiency, reflected the capability of β-lactamase to hydrolyze a given β-lactam.

Outer membrane permeability studies. Outer membrane permeability parameters (P) were calculated by a novel HPLC procedure as described previously (1) and in Materials and Methods. In strain R1, two groups of compounds demonstrating relatively rapid (cefpirome and cefepime) or slow (cefotaxime, ceftriaxone, and carumonam) uptake across the outer membrane were observed (Fig. 1; Table 2). No hydrolysis was detected for the majority of β-lactams with strain S intact cells under the experimental conditions, and thus, permeability could not be measured by the Zimmermann-Rosselet method (data not shown). All compounds exhibited 3 to 4.5 times less permeation in the porin-deficient strain R2. The ratios of the MICs for strains R1 and R2 were in good correlation with the inverse ratios of their permeability coefficients. This observation provided further evidence that our HPLC procedure allowed an accurate assessment of outer membrane permeability (1).

The profiles of β-lactam hydrolysis by intact *E. cloacae* 218 R1 cells (Fig. 1) showed that the antibiotics exhibiting higher outer membrane permeation were more rapidly hydrolyzed by intact cells. These results suggest that the rate of diffusion (V) of different β-lactams across the outer membrane of β-lactamase-overproducing strains [$V = P \times A \times (S_o - S_p)$] is directly proportional to their P values at a given external concentration (S_o). Our calculations consistently showed that the periplasmic concentrations achieved by the β-lactams were substantially lower than external concentrations (<23% for cefpirome and cefepime and <1% for all the other antibiotics).

DISCUSSION

The efficacy of β-lactam antibiotics against gram-negative bacteria has been proposed to depend on their rate of penetration across the outer membrane, their degree of resistance to β-lactamase inactivation, and their ability to inhibit the target proteins, i.e., the PBPs (19). Bacteria, by altering one of these parameters, can develop resistance against β-lactams. For example, a decrease in susceptibility

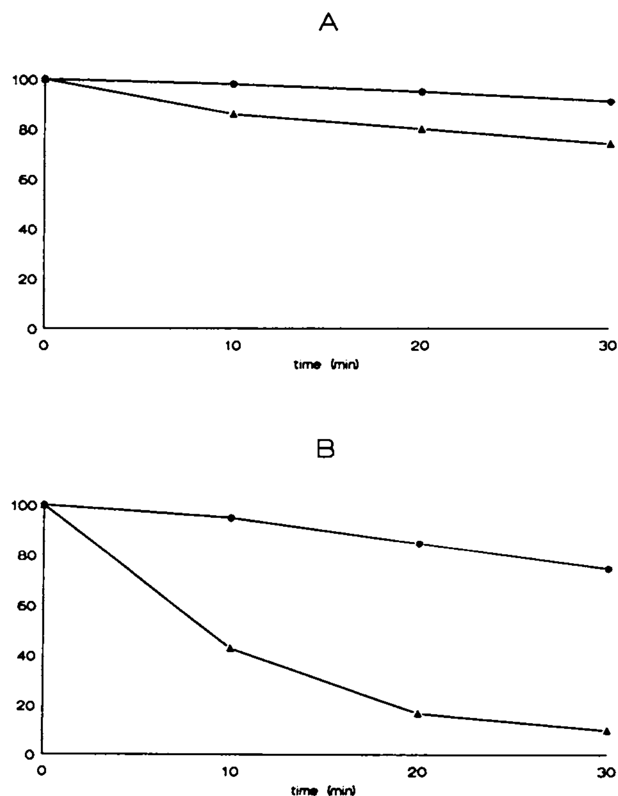

FIG. 1. HPLC assays of β-lactam hydrolysis by intact cells of *E. cloacae* 218 susceptibility variant strains R1 (▲) and R2 (●). (A) Ceftriaxone (15 μg/ml); (B) cefepime (15 μg/ml).

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TABLE 2. Outer membrane permeation (P) of β-lactams into *E. cloacae*

β-Lactam	MIC (μg/ml)[a]			P (nm/s)[b]		
	R1	R2	R2/R1	R1	R2	R1/R2
Ceftriaxone	20	100	5	5.1	1.1	4.6
Cefotaxime	20	100	5	5.6	1.2	4.7
Carumonam	4	15	4	1.9	0.6	3.2
Cefpirome	0.5	2	4	37	10	3.7
Cefepime	0.5	2	4	29	9.2	3.2

[a] Reported from reference 16, with the exception of cefpirome and cefepime MICs, which were determined by the same method.
[b] Determined from the equation of Zimmermann and Rosselet (see Materials and Methods).

TABLE 3. Parameters affecting the efficacy of β-lactams in *E. cloacae* 218 R1

β-Lactam	S_i[a] (μM)	$V(S_i)$[b] (pmol/mg/s)	TAI (10^{-5})
Cefotaxime	0.013	3.4	28
Ceftriaxone	0.017	2.5	46
Carumonam	0.009	0.2	100
Cefpirome	0.20	0.4	27,470
Cefepime	0.25	0.3	32,500

[a] Periplasmic antibiotic concentration needed for inactivating bacterial cell wall synthesis and calculated from the Zimmermann-Rosselet equation at external antibiotic concentrations equivalent to MICs.
[b] Rate of β-lactamase hydrolysis at the periplasmic antibiotic concentration corresponding to S_i.

to β-lactams in *E. cloacae* can result from either β-lactamase overproduction (14) or outer membrane permeability modifications (5). In addition, a combination of both of these mechanisms of resistance is also frequent, as reported for numerous antibiotic-resistant clinical isolates (16, 32). However, the interplay between hydrolysis by β-lactamase and restricted outer membrane permeability has rendered difficult the assessment of their respective roles in the efficacy of β-lactam antibiotics. Furthermore, technical limitations on the measurement of outer membrane permeability and the kinetic parameters of β-lactamases have provided problems in assessment of the contributions of each parameter involved in β-lactam action. The improved methods of assessment described here, combined with the use of defined variants with progressive alterations in β-lactamase levels and β-lactamase-plus-porin levels, facilitated such a study.

The antibacterial action of β-lactams results from the inhibition of the enzymatic activities of the PBPs that are involved in the final stages of peptidoglycan synthesis (9). The kinetic parameters of this interaction are not yet fully understood because of the complexity and diversity of the physiological roles of PBPs (9). To predict the periplasmic concentration needed for a β-lactam molecule to inactivate the cell wall synthesis (S_i), Nikaido and Normark (19) used literature estimates of the antibiotic concentration which inhibited by 50% the binding of radiolabeled benzylpenicillin to one of the essential PBPs, i.e., PBP 1b, PBP 2, or PBP 3, to obtain a global estimate of 0.1 μM. However, 50% inhibitory concentrations are crude estimates of the inhibition constant (K_i), and in this case, they are probably inaccurate because of the large amounts of [^{14}C]benzylpenicillin needed for the labeling of PBPs in such inhibition assays (25). Furthermore, the results have been shown to vary greatly with the conditions of such assays, the time of contact between β-lactams and the PBPs (11) and the presence of β-lactamase (2; for a review, see reference 9). This approach also oversimplifies by far the complexity of the mode of action of β-lactams. For instance, most β-lactam antibiotics bind to two or more PBPs and inhibit cell wall synthesis at different stages of bacterial growth (25). The use of 50% inhibitory concentrations for a single PBP does not take into account that the synergistic action on several PBP targets probably decreases the minimal antibiotic concentration needed for producing the same effect on a single PBP. In this study, we predicted the S_i values by calculating the β-lactam concentration achieved in the periplasmic space at an external concentration equivalent to the MIC. This value can be obtained from equation 2, assuming that the rate of β-lactam influx across the outer membrane is balanced by the rate of β-lactamase hydrolysis in the periplasm. Recently, we demonstrated that the theoretical basis of this postulate, formulated by Zimmermann and Rosselet, is rigorously correct (1). Hence, the S_i value is probably a good in vivo approximation of the minimal periplasmic β-lactam concentration needed for inhibiting the PBPs given that every parameter of equation 2 is appropriately determined.

Our calculations (Table 3) suggested that the S_i values in the strain 218 R1 for cefotaxime, ceftriaxone, and carumonam were 12- to 27-fold lower than those for cefpirome and cefepime. Interestingly, the S_i value for ceftriaxone, 0.017 μM, was very close to the 50% inhibitory concentration (0.025 μM) of *E. cloacae* PBP 3 for the same β-lactam (28). Although no study of the affinity of *E. cloacae* PBPs for new β-lactams has been published, these results suggest a possible correlation between the affinity of β-lactamase and the affinity of PBPs for the new β-lactams. The antibiotics with high S_i values exhibited high β-lactamase K_m values, while those with low S_i values showed high affinity (low K_m) for β-lactamase (Tables 1 and 3). This view is consistent with the striking similarities between two classes of enzymes (PBPs and β-lactamases). Both belong to the family of active-site serine proteases, and the comparison of the amino acid sequences of low- and high-molecular-weight PBPs and class A β-lactamases has suggested a common evolutionary origin for both enzymes (9). Considering that the three-dimensional structures of their active sites are very similar (9), one might expect the substrates exhibiting low affinity for β-lactamase to show low affinity for PBPs. Several other lines of evidence also support this view. For instance, monobactams form highly stable complexes with class I β-lactamase and very efficiently acylate the PBP 3 of gram-negative bacteria. Furthermore, PBP 5 of *E. coli* exhibits not only carboxypeptidase activity but also weak β-lactamase activity (9).

When we calculated the rate of β-lactamase hydrolysis of each compound at the concentration corresponding to the cell inhibitory concentration in the periplasm, $V(S_i)$, differences between fourth-generation cephalosporins and the other molecules were far smaller than might be expected from the enormous differences in K_m or the V_{max}/K_m ratio of these compounds (Table 3). These data suggest that the kinetic parameters describing the interaction between β-lactamase and new β-lactams are not by themselves sufficient to explain the differences in their antibacterial efficacy. However, it is clear that the much higher stability to β-lactamase hydrolysis is an important codeterminant of the antibacterial activities of new β-lactams when comparing their activities with those of earlier compounds.

The MIC of an antibiotic for a given bacterium is a simple

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expression of antibiotic activity. In the case of β-lactams and gram-negative bacteria, the MIC reflects at least the interplay of outer membrane permeability, β-lactamase stability, and PBP affinity. Zimmermann and Rosselet (33) proposed a mathematical description of this complex interaction that allowed the theoretical prediction of the MIC. Solving equation 1 for S_o gives the following:

$$S_o = S_p \left[1 + V_{max}/(P \times A) \times (K_m + S_p)\right] \quad (3)$$

When S_p is equivalent to the minimal periplasmic antibiotic concentration needed to inhibit the bacterial cell wall synthesis (S_i in this study), S_o corresponds to the MIC. Recently, Waley (31) and Nikaido and Normark (19) used rearrangements of the Zimmermann-Rosselet equation in an attempt to theoretically predict the MICs of β-lactams. The latter introduced a parameter, the target access index (TAI), defined as follows:

$$TAI = P \times A/[V_{max}/(K_m + C_{inh})] \quad (4)$$

where C_{inh} is the minimal periplasmic concentration needed to inhibit the PBPs, equivalent to S_i in this study. The parameter TAI, which is one of the terms of equation 3, reflects the probability that a drug will reach its target considering its affinity for PBPs and the effects of both outer membrane permeation and β-lactamase. According to equation 3, TAI is related to MIC by the following relationship:

$$MIC = C_{inh} (TAI^{-1} + 1) \quad (5)$$

Although TAI clearly is derived from the Zimmermann-Rosselet equation, Nikaido and Normark (19) pointed out its value in demonstrating the factors affecting the efficacy of β-lactams in gram-negative bacteria. For example, the combination of high β-lactamase stability and excellent outer membrane permeability endows cefepime and cefpirome with TAI values 300- to 1,000-fold higher than those of other β-lactams (Table 3). However, the efficacies of these two antibiotics do not increase in the same proportion since their S_i values are probably much higher than those for the other β-lactams. Our predicted MICs for strains S, R1, and R2 are in agreement with the observed values (100% within 1 antibiotic dilution) (Table 4). These results are expected for strain R1 since S_i values are precisely calculated from the observed MICs for this strain, while predicted MICs for strains S and R2 are independent estimations in which the two measured parameters (β-lactamase content and outer membrane permeability) vary separately. Furthermore, they confirm that our assumptions were probably correct and that all the parameters of equation 1 have been properly determined. Frère (8) has proposed a graphical method based on MIC extrapolations to estimate the relative importance of the different parameters involved in the efficacy of β-lactams. With the same set of *E. cloacae* strains, his estimates of S_i values (Ipl in his paper) and outer membrane permeability varied significantly from ours. However, it should be noted that this method is considerably more complicated than ours and required more than three strains to achieve an acceptable level of accuracy. Indeed, Frère's estimates for strain 218, which were based only on extrapolated data from two variants (S and R1), were expected to be relatively imprecise.

Our results may also provide guidelines for designing efficient β-lactams against gram-negative bacteria. Because of the complexity and the interplay of bacterial factors involved in β-lactam activity, improvements of a single factor may be difficult. Increased efficacy can be achieved only by the optimization of several parameters. The ideal β-lactam molecule would exhibit excellent outer membrane permeation, poor affinity for β-lactamase, high resistance to β-lactamase hydrolysis, and the ability to inhibit bacterial wall synthesis at low periplasmic concentrations. Further studies on structure-activity relationships will tell us whether some of these criteria are incompatible, as indeed the examples of cefepime and cefpirome would suggest. Nevertheless, of the aminothiazole β-lactams, these two antibiotics seem to represent an excellent compromise of all the requirements for achieving antibacterial efficiency.

TABLE 4. Predicted and observed MICs for the three susceptibility variants of *E. cloacae* 218[a]

β-Lactam	MIC (μg/ml) for:					
	S		R1		R2	
	O	P	O	P	O	P
Cefotaxime	0.1	0.1[b]	20	20	100	100
Ceftriaxone	0.1	0.1[b]	20	20	100	90
Carumonam	0.02	0.01	4	4	15	13
Cefpirome	0.05	0.1	0.5	0.5	2	1.4
Cefepime	0.05	0.1	0.5	0.5	2	1.4

[a] Predicted (P) MICs were determined from equation 3 by using S_p values equivalent to S_i (see Discussion) calculated in strain R1. O, Observed.
[b] β-Lactamase induction ratios were estimated from references 17 and 26.

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