Activities of β -Lactam Antibiotics against *Escherichia coli* Strains Producing Extended-Spectrum β -Lactamases

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Seven extended-spectrum β -lactamases related to TEM and four enzymes derived from SHV-1 were transferred to a common *Escherichia coli* host so that the activity of a variety of β -lactams could be tested in a uniform genetic environment. For most derivatives, penicillinase activity was 10% or less than that of strains making TEM-1, TEM-2, or SHV-1 β -lactamase, suggesting that reduced catalytic efficiency accompanied the broader substrate spectrum. Despite this deficit, resistance to aztreonam, carumonam, cefdinir, cefepime, cefixime, cefmenoxime, cefotaxime, cefotiam, ceftazidime, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, and E1040 was enhanced. For strains producing TEM-type enzymes, however, MICs of carumonam, cefepime, cefmenoxime, cefotiam, ceftazidime, ceftibuten, and ceftizoxime were 8 μ g/ml or less. Susceptibilities of cefmetazole, cefotetan, ceftazidime, flomoxef, imipenem, meropenem, moxalactam, temocillin, FCE 22101, and Sch 34343 were unaffected. FCE 22101, imipenem, meropenem, and Sch 34343 were inhibitory for all strains at 1 μ g/ml or less. In *E. coli* an *OmpF*⁻ porin mutation in combination with an extended-spectrum β -lactamase enhanced resistance to many of these agents, but generally by only fourfold. Hyperproduction of chromosomal AmpC β -lactamase increased resistance to 7- α -methoxy β -lactams but not that to temocillin. When tested at 8 μ g/ml, clavulanate was more potent than sulbactam or tazobactam in overcoming resistance to ampicillin, while cefoperazone-sulbactam was more active than ticarcillin-clavulanate or piperacillin-tazobactam, especially against TEM-type extended-spectrum β -lactamases.

Extended-spectrum β -lactamases were recognized for their ability to provide resistance to cefotaxime, ceftazidime, and other broad-spectrum cephalosporins and monobactams as well as to many older β -lactam antibiotics (for a review, see reference 22). Some of the enzymes are related to TEM-1 or TEM-2 by one or more mutations that alter the configuration of the active site, while others arose from SHV-1. Because they often occur in clinical isolates that are resistant to other useful agents, therapeutic options can be limited. The purpose of this study was to evaluate a variety of newer parenteral and oral β -lactam antibiotics for their activities against a set of strains producing 11 different extended-spectrum enzymes encoded on plasmids transferred to a common *Escherichia coli* host. The influence of an *ompF* porin mutation which affects outer cell membrane permeability was also investigated.

MATERIALS AND METHODS

Strains. Table 1 describes the *E. coli* strains and the plasmids used in this study. For convenience, plasmids that were unnamed by the investigators who described them have been given pMG designations. Plasmids determining each of the extended-spectrum β -lactamases were introduced into *E. coli* C600, JF568, or JF703 by conjugation (11), except for pNU104, which was inserted by transformation (15).

Antibiotics. Antibiotics were obtained from the following sources: Abbott Laboratories (cefmenoxime), Beecham Laboratories (amoxicillin, potassium clavulanate, temocillin, ticarcillin), Bristol-Myers Co. (cefepime), Eisai Co., Ltd. (E1040), Farmitalia Carlo Erba (FCE 22101), Fujisawa Pharmaceuticals Co., Ltd. (cefdinir or FK482), Hoechst-Roussel Pharmaceuticals Inc. (cefotaxime, ceftazidime), Hoffmann-La Roche (carumonam, ceftriaxone), ICI Pharmaceuticals Group (cefotetan, meropenem or SM7338), Le-

derle Laboratories (ceftazidime, cefuroxime, moxalactam), Eli Lilly & Co. (ceftazidime, cefuroxime, moxalactam), Merck Sharp & Dohme (cefoxitin, imipenem), Pfizer Inc. (cefoperazone, sulbactam), Schering-Plough Corp. (ceftibuten, Sch 34343), Shionogi & Co., Ltd. (flomoxef), Sigma Chemical Co. (ampicillin), Smith Kline & French Laboratories (ceftizoxime), E. R. Squibb & Sons (aztreonam), Takeda Chemical Industries, Ltd. (cefotiam), and The Upjohn Co. (cefmetazole, cefpodoxime).

Susceptibility testing. MICs were determined by agar dilution by using Trypticase soy agar plates (BBL Microbiology Systems, Cockeysville, Md.) containing graded concentrations of antibiotics and inocula of 10^4 to 10^5 organisms per spot that were applied with a replica-plating device. Plates were incubated overnight at 37°C.

β -Lactamase assays. Enzyme activity in sonic extracts was assayed colorimetrically by the neocuproine-copper procedure of Cohenford et al. (7), with 584 μ M benzylpenicillin used as the substrate. Each β -lactamase assay was repeated at least three times to provide an average value. One unit of activity was defined as the amount of enzyme that hydrolyzed 1 μ mol of benzylpenicillin per min at 25°C and pH 7.0. Protein was measured by the technique of Bradford (3) so that the β -lactamase specific activity could be calculated.

RESULTS

Plasmids determining extended-spectrum β -lactamases and TEM-1, TEM-2, or SHV-1 were introduced into *E. coli* C600 as a common host to provide a uniform genetic background for susceptibility testing. Table 2 shows the penicillinase activities of the C600 strains that produced the various enzymes. All the extended-spectrum β -lactamases related to SHV-1 and most of those related to TEM-1 or TEM-2 had lower β -lactamase activities than those of their progenitors. Despite this deficiency, for strains producing these enzymes the MIC of ampicillin was 500 μ g/ml or more.

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TABLE 1. *E. coli* strains and plasmids used in this study

Strain or plasmid	Properties ^a	Reference
<i>E. coli</i>		
C600	<i>leuB6 thi-1 thr-1</i>	1
JF568	<i>aroA357 his-53 ilv-22 metB65 purE41</i>	18
JF703	As JF568 plus <i>ompF254 aroA</i> ⁺	18
Plasmids		
R1	Ap(TEM-1) Cm Km Sm Su	10
RP4	Ap(TEM-2) Km Tc	10
pCFF04	Ap(TEM-3) Ak Km Su Tc Tm	5
pUD16	Ap(TEM-4) Gm Km Sm Su Tc Tm Tp	21
pCFF14	Ap(TEM-5) Km Sm Su Tc	5
pMG226 ^b	Ap(TEM-6) Cm Km Sm	2
pIF100	Ap(TEM-7) Ak Gm Km Su Tc Tm	9
pMG228 ^c	Ap(TEM-9) Cm Gm Sm Su Tc Tm Tp	24
pCFF34	Ap(CAZ-2) Ak Km Su Tc Tm	5
R1010	Ap(SHV-1) Cm Km Sm Su	10
pMG229 ^d	Ap(SHV-2) Sm Su Tc Tp	12
pUD18	Ap(SHV-3) Ak Gm Km Sm Su Tc Tm Tp	17
pUD21	Ap(SHV-4) Ak Gm Km Sm Su Tc Tm Tp	4
pAFF2	Ap(SHV-5) Gm Sm Su Tc	8
pNU104	Ap(AmpC) Km	14

^a Antibiotic resistance abbreviations: Ak, amikacin; Ap, ampicillin; Cm, chloramphenicol; Gm, gentamicin; Km, kanamycin; Sm, streptomycin; Su, sulfonamide; Tc, tetracycline; Tm, tobramycin; Tp, trimethoprim.

^b Plasmid not named in original publication. Originated in *E. coli* HB 251.

^c Plasmid not named in original publication. Supplied in *K. pneumoniae* 2633E.

^d Plasmid not named in original publication. Originated in *K. pneumoniae* 86-1.

The MIC of ampicillin returned toward that for the R⁻ strain in the presence of the β -lactamase inhibitors clavulanate, sulbactam, or tazobactam. Of the three inhibitors, clavulanate was the most effective when all inhibitors were tested at 8 μ g/ml. Sulbactam and tazobactam were least efficient against strains producing SHV-2, SHV-3, SHV-4, TEM-5, or TEM-6. Table 2 also shows the MICs of ticarcillin-clavulanate, cefoperazone-sulbactam, and piperacillin-tazobac-

tam. Of these combinations, cefoperazone-sulbactam was the most potent, although MICs for strains producing SHV-2, SHV-3, or SHV-4 remained at 16 μ g/ml or greater.

Table 3 shows MICs for these strains when they were tested against nine broad-spectrum cephalosporins. The presence of an extended-spectrum β -lactamase increased MICs of cefepime, cefmenoxime, cefotaxime, cefotiam, ceftirome, ceftazidime, ceftizoxime, ceftriaxone, and E1040. The highest MICs were observed with ceftazidime. In general, MICs for the derivatives that made SHV-type enzymes were higher than they were for those that produced TEM-derived β -lactamases. For the set of TEM-type enzymes, MICs of cefepime, cefmenoxime, cefotiam, and ceftirome were the lowest, with none being greater than 8 μ g/ml. However, for at least one strain that produced SHV-type β -lactamase, MICs of each of these four cephalosporins were 32 μ g/ml or greater.

Table 4 shows the results of MIC tests with five cephalosporins that can be administered orally. The extended-spectrum β -lactamases increased resistance to cefdinir, cefixime, cefpodoxime, ceftibuten, and cefuroxime. MICs of ceftibuten were least affected, but for the strain which produced SHV-4 the MIC of ceftibuten was 64 μ g/ml.

Strains producing extended-spectrum enzymes also had enhanced resistance to the monobactams aztreonam and carumonam (Table 5). MICs of carumonam were lower than those of aztreonam, but for a strain that made SHV-4, the MIC of carumonam was 128 μ g/ml. In contrast, Table 5 shows that MICs of the penems FCE 22101 and Sch 34343 and the carbapenems imipenem and meropenem were not affected by the production of these β -lactamases. Meropenem was the most potent of the agents tested, with MICs of 0.03 μ g/ml or less for all strains; but MICs of imipenem, FCE 22101, and Sch 34343 were no greater than 1 μ g/ml.

Table 6 provides MICs of cephamycins and other 7- α -methoxy compounds. MICs of cefmetazole, cefotetan, cefoxitin, temocillin, moxalactam, and flomoxef were virtually unchanged by the production of extended-spectrum enzymes.

TABLE 2. Susceptibilities of *E. coli* C600 derivatives producing extended-spectrum β -lactamases to β -lactam- β -lactamase inhibitor combinations

β -Lactamase type	Activity ^a	MIC (μ g/ml)					
		Ampicillin ^b				Ticarcillin + clavulanate ^c	Piperacillin + tazobactam ^c
		Alone	With clavulanate	With sulbactam	With tazobactam		
R ⁻	10	4	8	4	8	4	2
TEM-1	611	1,000	8	64	8	16	2
TEM-2	20,400	$\geq 16,000$	64	≥ 256	128	256	256
TEM-3	271	1,000	8	8	8	32	2
TEM-4	233	1,000	8	8	8	32	2
TEM-5	60	1,000	8	32	16	16	2
TEM-6	948	2,000	16	64	32	64	8
TEM-7	719	4,000	16	8	16	32	4
TEM-9	313	2,000	8	8	8	64	2
CAZ-2	18	1,000	8	8	8	32	2
SHV-1	9,940	$\geq 16,000$	16	≥ 256	≥ 256	64	256
SHV-2	832	$\geq 16,000$	16	≥ 256	128	32	≥ 256
SHV-3	552	$\geq 16,000$	16	≥ 256	64	16	≥ 256
SHV-4	171	$\geq 16,000$	8	≥ 256	32	128	≥ 256
SHV-5	75	500	8	8	8	32	4

^a β -Lactamase specific activity expressed in milliunits per milligram of protein.

^b Ampicillin was tested alone or in the presence of 8 μ g of clavulanate, sulbactam, or tazobactam per ml.

^c The concentration of β -lactamase inhibitor was a constant 8 μ g/ml.

TABLE 3. Susceptibilities of C600 derivatives to broad-spectrum parenteral cephalosporins

β -Lactamase	MIC (μ g/ml)								
	Cefepime	Cefmenoxime	Cefotaxime	Cefotiam	Cefpirome	Ceftazidime	Ceftizoxime	Ceftriaxone	E1040
R ^{-a}	0.125	0.125	0.125	0.25	0.125	0.25	0.125	0.125	0.125
TEM-1	0.125	0.125	0.125	0.25	0.125	0.25	0.125	0.125	0.25
TEM-2	0.5	0.125	0.125	1	0.5	0.5	0.125	0.125	1
TEM-3	4	8	32	4	4	64	16	16	4
TEM-4	4	8	32	4	4	32	16	32	4
TEM-5	2	8	4	4	4	128	2	16	4
TEM-6	4	4	1	4	2	128	0.5	8	4
TEM-7	8	0.5	0.5	0.5	4	64	0.25	1	8
TEM-9	8	2	2	0.5	4	128	2	4	16
CAZ-2	4	2	2	0.5	4	128	2	4	8
SHV-1	0.5	0.125	0.125	1	0.5	1	0.125	0.125	2
SHV-2	16	32	64	64	32	32	16	64	8
SHV-3	16	32	64	32	32	32	16	64	16
SHV-4	32	32	128	64	32	128	64	64	16
SHV-5	4	4	64	8	2	128	64	16	4

^a *E. coli* C600 with or without plasmids encoding the indicated β -lactamases.

To evaluate the contribution of outer membrane permeability to the level of resistance produced by these β -lactamases, plasmids producing TEM-3 and TEM-9 were introduced into *E. coli* JF568 and JF703, which differ in production of the major outer membrane porin OmpF (18). A plasmid encoding the *E. coli ampC* gene (14) was inserted into the same pair of strains to mimic naturally occurring isolates of other genera that hyperproduce chromosomal β -lactamase. Table 7 shows the results of MIC tests with these strains. Even without added β -lactamase, for the OmpF⁻ mutant MICs of many β -lactams were fourfold higher, although this was not the case for penems or carbapenems. Similarly, for OmpF⁻ strains producing TEM-3, TEM-9, or extra AmpC β -lactamase, MICs of certain β -lactams were somewhat higher, but generally by no more than a factor of four. Hyperproduction of AmpC provided resistance to cephamycins, monobactams, some cephalosporins, and β -lactamase inhibitor-ampicillin combinations. AmpC overproducers were also resistant to combinations of piperacillin-tazobactam and ticarcillin-clavulanate, but MICs of cefoperazone-sulbactam were 4 μ g/ml or less (data not shown). Antibiotics with MICs of 8 μ g/ml or less against

the entire set of strains included cefepime, cefmenoxime, cefpirome, imipenem, meropenem, and Sch 34343.

DISCUSSION

Mutations that are responsible for extending the β -lactamase spectrum increase the enzyme affinity for otherwise nonhydrolyzable substrates, but they do so at some cost in catalytic efficiency (22). The amount of plasmid-determined β -lactamase produced by a strain is proportional to the plasmid copy number (25) and is influenced by variations in promoter efficiency (6). For natural isolates of *E. coli*, TEM-1 production can vary over more than a 60-fold range (19). Plasmids encoding the β -lactamases used in this study were 80 kilobases or larger in size and would be expected to have low and comparable copy numbers. Assuming a promoter efficiency similar to that of SHV-1, less than 10% as much β -lactamase activity was produced by strains making SHV-2, SHV-3, SHV-4, and especially SHV-5. Similarly, TEM-3 and TEM-7, which are derived from TEM-2 (22), had less than 10% as much enzyme activity as did a TEM-2 producer. For strains producing extended-spectrum β -lac-

TABLE 4. Susceptibilities of C600 derivatives to cephalosporins that can be administered orally

β -Lactamase	MIC (μ g/ml)				
	Cefdinir	Cefixime	Cefpodoxime	Ceftibuten	Cefuroxime
R ⁻	0.5	0.5	1	1	8
TEM-1	0.5	0.5	1	1	8
TEM-2	1	1	1	1	16
TEM-3	8	8	64	1	128
TEM-4	32	16	128	2	256
TEM-5	16	64	32	4	64
TEM-6	1	8	16	2	16
TEM-7	1	1	16	1	16
TEM-9	2	16	32	2	16
CAZ-2	2	8	32	2	16
SHV-1	1	1	2	1	16
SHV-2	16	8	256	4	128
SHV-3	32	8	256	4	256
SHV-4	64	256	≥ 256	64	≥ 256
SHV-5	16	64	128	16	64

TABLE 5. Susceptibilities of C600 derivatives to monobactams, penems, and carbapenems

β -Lac- tamase	MIC (μ g/ml)					
	Aztreo- nam	Caru- monam	FCE 22101	Sch 34343	Imi- penem	Mero- penem
R ⁻	0.125	0.25	1	1	0.5	0.03
TEM-1	0.125	0.25	1	0.5	0.25	0.03
TEM-2	0.25	0.25	1	0.5	0.25	0.03
TEM-3	16	1	1	1	0.5	0.03
TEM-4	16	2	1	1	0.25	≤ 0.015
TEM-5	8	8	1	1	0.25	0.03
TEM-6	64	2	1	1	0.25	≤ 0.015
TEM-7	2	0.5	1	1	0.5	0.03
TEM-9	128	8	1	1	0.5	≤ 0.015
CAZ-2	16	4	1	1	0.25	≤ 0.015
SHV-1	0.5	0.25	1	1	0.5	≤ 0.015
SHV-2	32	4	1	1	0.5	0.015
SHV-3	32	4	1	1	0.25	≤ 0.015
SHV-4	256	128	1	0.5	0.25	≤ 0.015
SHV-5	256	32	1	0.5	0.25	0.03

TABLE 6. Susceptibilities of C600 derivatives to cephamycins and other 7- α -methoxy compounds

β -Lactamase	MIC (μ g/ml)					
	Cefmetazole	Cefotetan	Cefoxitin	Temocillin	Moxalactam	Flomoxef
R ⁻	4	0.5	16	16	0.25	0.25
TEM-1	4	0.5	16	16	0.25	0.25
TEM-2	4	1	16	16	1	0.25
TEM-3	4	2	16	32	2	0.25
TEM-4	2	1	16	32	2	0.25
TEM-5	4	1	16	16	2	0.25
TEM-6	2	1	16	32	2	0.25
TEM-7	2	1	16	32	1	0.25
TEM-9	4	1	16	32	2	0.25
CAZ-2	4	2	16	32	2	0.25
SHV-1	4	1	16	16	1	0.25
SHV-2	4	1	16	16	4	0.25
SHV-3	4	2	16	32	4	0.25
SHV-4	4	4	16	64	4	0.5
SHV-5	2	1	8	16	4	0.25

tamases related to TEM-1, enzyme activity varied from levels greater than those in TEM-1 strains (TEM-6, TEM-9) to levels of 10% or less (TEM-5, CAZ-2). Further studies comparing the activities of purified enzymes are needed to refine these estimates.

Benzylpenicillin was chosen as the reference for the β -lactamase assay since activities with other substrates are

often expressed relative to benzylpenicillin activity. It should be noted, however, that little relationship was evident between ampicillin MICs and β -lactamase activities (Table 2), as was true also in an earlier study in which β -lactamase was assayed with nitrocefin (11).

Despite low β -lactamase production by some strains, all conferred resistance to broad-spectrum cephalosporins, supplementing more limited data that have been provided by other investigators (5, 9, 13, 16, 23). The cephalosporins that were affected included cefixime, cefotaxime, ceftazidime, ceftizoxime, ceftriaxone, and cefuroxime, which are in use, as well as investigational agents such as cefdinir, cefepime, cefmenoxime, cefotiam, cefpirome, cefpodoxime, ceftibuten, and E1040. However, for strains producing TEM-type enzymes, MICs of cefepime, cefmenoxime, cefotiam, cefpirome, and ceftibuten were 8 μ g/ml or less. Resistance to the monobactams aztreonam and carumonam was also enhanced by these enzymes, but for TEM-type β -lactamases, MICs of carumonam were 8 μ g/ml or less.

7- α -Methoxy β -lactams such as cefmetazole, cefotetan, cefoxitin, temocillin, moxalactam, and flomoxef lost none of their activity against strains that made either TEM- or SHV-related β -lactamases. However, with the exception of temocillin, resistance to other members of this group was enhanced both by the OmpF⁻ porin mutation and by hyperproduction of chromosomal AmpC β -lactamase (Table 7). The failure of cefoxitin to cure a patient infected with a *Klebsiella pneumoniae* strain producing TEM-3 β -lactamase has been reported recently (20). A cefoxitin-resistant deriv-

TABLE 7. Influence of OmpF⁻ mutation on β -lactam resistance

β -Lactam	MIC (μ g/ml) for the following β -lactamases with or without OmpF ^a :							
	R ⁻		TEM-3		TEM-9		AmpC	
	+	-	+	-	+	-	+	-
Ampicillin	4	4	500	500	1,000	2,000	2,000	4,000
Ampicillin + clavulanate ^b	8	8	8	8	8	8	≥ 256	≥ 256
Ampicillin + sulbactam ^b	4	4	4	4	4	4	≥ 256	≥ 256
Ampicillin + tazobactam ^b	8	8	8	8	8	8	≥ 256	≥ 256
Aztreonam	0.06	0.25	4	8	32	64	16	64
Carumonam	0.06	0.25	0.125	1	2	4	32	64
Cefdinir	0.25	1	2	8	1	2	≥ 64	≥ 64
Cefepime	≤ 0.03	0.125	0.25	2	2	4	0.125	0.5
Cefixime	0.5	0.5	1	4	8	8	≥ 64	≥ 64
Cefmenoxime	0.06	0.25	2	4	1	2	1	4
Cefmetazole	1	4	1	4	1	4	64	≥ 64
Cefotaxime	≤ 0.06	0.125	2	8	1	2	8	16
Cefotetan	0.125	0.5	0.125	2	0.25	1	16	≥ 64
Cefotiam	0.25	0.5	1	4	0.5	1	128	64
Cefoxitin	4	16	4	16	4	16	256	≥ 256
Cefpirome	0.06	0.125	0.5	4	4	8	0.125	0.25
Cefpodoxime	1	2	32	64	64	32	≥ 64	≥ 64
Ceftazidime	0.125	0.25	8	16	128	256	32	64
Ceftibuten	0.25	1	0.5	2	1	2	256	≥ 256
Ceftizoxime	≤ 0.03	0.125	2	8	1	1	32	32
Ceftriaxone	≤ 0.03	0.125	4	16	2	4	4	4
Cefuroxime	8	16	32	≥ 64	16	16	≥ 256	≥ 256
E1040	0.125	0.125	1	4	4	16	0.25	1
FCE 22101	1	1	1	1	1	1	8	16
Flomoxef	0.125	0.25	0.125	0.25	0.25	0.25	32	32
Imipenem	0.5	0.125	0.5	0.125	1	0.25	0.5	0.5
Meropenem	0.015	≤ 0.0075	0.015	≤ 0.0075	0.015	≤ 0.0075	0.015	0.015
Moxalactam	0.25	1	0.25	2	0.25	2	4	16
Sch 34343	0.5	0.5	0.5	0.5	0.5	0.5	1	2
Temocillin	8	16	16	16	16	32	8	32

^a *E. coli* JF568 (OmpF⁺) or JF703 (OmpF⁻) was used as the plasmid host.

^b β -Lactamase inhibitors were used at concentrations of 8 μ g/ml.

ative appeared that had diminished production of two outer membrane proteins that were thought to be porins, thus indicating that cephamycins should be used with caution to treat such patients.

Another therapeutic option is to combine a β -lactamase-susceptible antibiotic with a β -lactamase inhibitor. Together with ampicillin, clavulanate at a fixed concentration of 8 μ g/ml provided lower MICs, especially with SHV-type enzymes, than did sulbactam or tazobactam. For TEM-type enzymes cefoperazone-sulbactam was a more potent combination than was either ticarcillin-clavulanate or piperacillin-tazobactam. With TEM-3 or TEM-9, an OmpF⁻ porin mutation did not enhance resistance to β -lactamase inhibitor combinations.

Of all the agents tested, the most potent were the penems FCE 22101 and Sch 34343 and the carbapenems imipenem and meropenem. Each was effective at MICs of 1 μ g/ml or less against strains that produced extended-spectrum β -lactamases, and their activities were unaffected by the OmpF⁻ mutation. Clinical experience with these compounds in treating infections caused by strains that produce extended-spectrum β -lactamases is awaited with interest.

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