ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1995, p. 308–313 Vol. 39, No. 2
0066-4804/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Development of Test Panel of β-Lactamases Expressed in a Common *Escherichia coli* Host Background for Evaluation of New β-Lactam Antibiotics

PATRICIA A. BRADFORD[1]† AND CHRISTINE C. SANDERS[1,2]*

Center for Research in Anti-Infectives and Biotechnology[2] and Department of Medical Microbiology,[1] Creighton University, Omaha, Nebraska 68178

Received 14 July 1994/Returned for modification 29 August 1994/Accepted 10 November 1994

A test panel of 35 different β-lactamases expressed in a common *Escherichia coli* host was created to compare the effect that each β-lactamase had on susceptibility to various β-lactam antibiotics. A comparison of the MICs obtained with this panel generally reflected differences in the substrate profiles of the various β-lactamases examined. In addition, several strains of the panel were subjected to selection with porin-specific bacteriophages to obtain mutants lacking either the OmpC or OmpF porin protein. A mutation in either OmpC or OmpF did change the susceptibilities of certain strains expressing β-lactamase to certain β-lactam antibiotics. However, the loss of a single porin did not predictably alter susceptibility to any given β-lactam drug. This panel of strains producing various β-lactamases was found to be a useful tool for comparing the effects of different β-lactamases and outer membrane permeability upon susceptibility to β-lactam drugs.

The production of β-lactamase is the dominant mechanism for bacterial resistance to β-lactam antibiotics. Both the level of β-lactamase activity and the substrate profile of the enzyme play a role in determining bacterial resistance conferred by the enzyme in gram-negative bacteria (20, 44, 50, 54). In addition, the outer membrane of gram-negative bacteria is known to act as a permeability barrier for various antibiotics, including the β-lactams. The combination of external antibiotic concentration, permeability, and β-lactamase activity determines the periplasmic free drug concentration and, consequently, susceptibility or resistance to the drug (31, 40, 41). The appearance of clinical isolates of the family *Enterobacteriaceae* that have a high level of resistance to β-lactam antibiotics has been shown, in some instances, to be the result of the interaction between β-lactamase production and decreased permeability. For example, the combination of an extended-spectrum β-lactamase and altered outer membrane proteins was responsible for high-level resistance to ceftazidime in a clinical isolate of *Escherichia coli* producing TEM-12 (63).

Because of the importance of β-lactamase and permeability in resistance to β-lactam antibiotics, the present study was undertaken in an attempt to develop a test panel of *E. coli* isolates that would allow an assessment of the effects of various β-lactamases and altered permeability on susceptibility to β-lactam antibiotics. First, a test panel consisting of many different plasmid-mediated β-lactamases expressed in a common host background was constructed. Subsequently, several strains expressing β-lactamase were subjected to selection with porin-specific bacteriophage, resulting in the isolation of mutants lacking either the OmpC or the OmpF porin protein. The resulting panel was then used to (i) compare the effects of these β-lactamases on susceptibilities to various β-lactam antibiotics and (ii) assess the influence of the combination of β-lactamase expression and a change in outer membrane proteins on susceptibility to β-lactam antibiotics.

* Corresponding author. Phone: (402) 280-1881. Fax: (402) 280-1225.

† Present address: American Cyanamid, Medical Research Division, Pearl River, NY 10965.

(This work was presented in part at the 11th Interdisciplinary Meeting on Anti-Infectious Chemotherapy, 5 to 6 December 1991, Paris, France, [6] and the 92nd General Meeting of the American Society for Microbiology 26 to 30 May 1992, New Orleans, La. [5].)

MATERIALS AND METHODS

Creation of the test panel. A panel of different plasmid-mediated β-lactamases expressed in a common host background was created in *E. coli* C600N, a nalidixic acid-resistant derivative of *E. coli* C600 (3) (kindly provided by R. V. Goering). The plasmids encoding the β-lactamases used in the study are listed in Table 1. The plasmids were transferred to the recipient by either conjugation or transformation by previously described methods, selecting for resistance to both nalidixic acid and ampicillin (3, 4, 12, 49). The presence of a single β-lactamase of the correct isoelectric point in the strains of the test panel was confirmed by isoelectric focusing (55, 62).

E. coli C600N panel strains constitutively producing high-levels of group 1 β-lactamase (Ecl-C) and producing a high basal level and hyperinducible group 1 β-lactamase (Ecl-Hy) were spontaneous mutants of the wild-type inducible strain (Ecl-I) containing pBP131 with the *ampR ampC* region cloned from *Enterobacter cloacae* 14 (27). An overnight culture of the strain inducibly expressing the group 1 enzyme (Ecl-I) was diluted in fresh Luria-Bertani (LB) broth (Difco Laboratories, Detroit, Mich.) and was grown to the mid- to late log phase. Serial 10-fold dilutions of the cells were made in sterile saline, and 0.1 ml was plated onto LB agar plates containing 8, 16, and 32 μg of cefotaxime (2× to 8× the MIC). Mutants were selected and tested for constitutive β-lactamase production by induction studies (52, 53).

Selection with bacteriophage. Spontaneous mutants of nine strains of the *E. coli* C600N panel lacking either the OmpC or the OmpF porin protein were selected by using porin-specific bacteriophages. Bacteriophage SS4 is specific for the OmpC of *E. coli* K-12 derivatives, whereas bacteriophage K20 is specific for OmpF (56). Phages were the kind gift of C. A. Schnaitman. Fresh phage lysates were diluted to 10^{-3} in LB broth. A total of 20 μl of overnight culture and 10 μl of diluted phage were added to a melted soft agar of nutrient or Trypticase soy agar, and the contents were mixed and poured over the surface of a dry agar plate. Following incubation, spontaneous mutants appear as colonies that grow in the presence of phage. Resistant colonies were subcultured and subjected to reselection with phage. A confluent lawn of bacteria should be obtained from strains that have lost a specific porin. The complete loss of a specific outer membrane protein was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis in the presence of 8 M urea as described previously (1, 33).

Susceptibility tests. All susceptibility tests were performed by standard procedures (40). Broth microdilution tests were performed with a final inoculum of 10^4 CFU per well. Microdilution trays containing serial twofold dilutions of antibiotic were prepared by using the MIC 2000 apparatus (Dynatech Laboratories, Chantilly, Va.). The MICs of the following antibiotics were determined: ticarcillin and potassium clavulanate (Beecham Laboratories, Bristol, Tenn.),

TABLE 1. β-Lactamases expressed by strains in the *E. coli* C600N test panel

β-Lactamase	Plasmid	Received from:	Reference
TEM-1	R6K	A. Medeiros	10
TEM-1 (high)	pUC19		64
TEM-2	RP1	A. Medeiros	35
TEM-3	pCFF04	J. Sirot	59
TEM-4	pUD16	G. Paul	43
TEM-5	pCFF14	J. Sirot	60
TEM-7		L. Gutmann	17
TEM-8	HM12G	C. Mabilat	34
TEM-9		A. Harris	61
TEM-10	pJPQ100	J. Quinn	46
TEM-12	pDAW402	D. Weber	63
SHV-1	R1010	A. Medeiros	48
SHV-2	pBP60	B. Wiedemann	25
SHV-3	pUD18	A. Philippon	22
SHV-4	pUD21	A. Philippon	7
SHV-5	pAFF2	L. Gutmann	16
SHV-6	pSLH47	G. Arlet	2
OXA-1	RGN238	G. Jacoby	9
OXA-2	R1818 (R46)	A. Medeiros	8
OXA-3	pIP55	G. Jacoby	35
OXA-4	pMG203	A. Medeiros	38
OXA-5	pMG54	A. Medeiros	38
OXA-6	pUZ8-pMG39	A. Medeiros	38
OXA-7	pMG202	A. Medeiros	38
PSE-1	pMG217	G. Jacoby	37
PSE-2	R140	G. Jacoby	32
PSE-3	Rms149	G. Jacoby	18
PSE-4	pMON705	G. Jacoby	15
SAR-1	pUK657	S. Amyes	47
ROB-1	pMON401	G. Jacoby	30
HMS-1	R997	A. Medeiros	36
CARB-4	pMON1024	G. Jacoby	45
LCR-1	pMK20:Tn1412	G. Jacoby	29, 58
L-1	pMON01	R. Levesque	11
MIR-1	pMLC28Kpn1-8	A. Medeiros	42
ampC	pBP131	G. Korfmann	27

aztreonam and cefepime (Bristol-Meyers-Squibb, Princeton, N.J.), ceftazidime (Glaxo Group Research Ltd., Greenford, England), cefotaxime and cefpirome (Hoechst-Roussel Pharmaceuticals Inc., Sommerville, N.J.), cefixime, piperacillin, and tazobactam (Lederle Laboratories, Pearl River, N.Y.), ampicillin, cefoperazone, and sulbactam (Pfizer Inc., New York, N.Y.), and amoxicillin (Sigma Chemical Company, St. Louis, Mo.). Ampicillin-sulbactam, amoxicillin-clavulanate, and cefoperazone-sulbactam combinations were tested at 2:1 ratios. Serial twofold dilutions of ticarcillin, each containing 2 μg of clavulanate per ml, and serial twofold dilutions of piperacillin, each containing 4 μg of tazobactam per ml, were tested. The MIC was defined as the lowest concentration preventing growth after 18 h of incubation at 35°C. Results for most antibiotics were interpreted by using the criteria approved by the National Committee for Clinical Laboratory Standards (39). For cefoperazone-sulbactam the criteria of Jones et al. (23) were used, for cefepime the criteria of Fuchs et al. (13) were used, and for cefpirome the criteria of Jones et al. (24) were used. When comparing MIC results, a significant change in MIC was defined as a fourfold increase or decrease in the MIC.

RESULTS

Effect of type of β-lactamase on susceptibility. Broth microdilution MICs were determined for all strains of the *E. coli* C600N test panel (Table 2). In general, the MICs of the β-lactam antibiotics tested followed the reported substrate profiles for the various β-lactamases. The presence of any β-lactamase raised the MIC of ampicillin to ≥128 μg/ml and the MIC of ticarcillin to ≥32 μg/ml. Sulbactam restored susceptibility to ampicillin in strains expressing TEM-3, TEM-4, TEM-8, TEM-9, TEM-10, TEM-12, OXA-2, OXA-3, PSE-1, PSE-3, and ROB-1 β-lactamases. Clavulanate restored susceptibility to amoxicillin only in the strain expressing PSE-3. Clavulanic acid restored susceptibility to ticarcillin in strains expressing OXA-2, LCR-1, and the inducible group 1 β-lactamase (Ecl-I).

All of the TEM, SHV, and PSE β-lactamases raised the MIC of piperacillin to >128 μg/ml with the exception of the MICs for strains expressing TEM-5 and PSE-1, which were moderately susceptible to piperacillin (Table 2). Tazobactam did not restore susceptibility to piperacillin in strains expressing TEM-1 or TEM-2; however, the MICs of the combination were 2 to 8 μg/ml when they were determined for strains expressing TEM-3 through TEM-12. Among strains expressing SHV β-lactamases, tazobactam restored susceptibility to piperacillin only in those strains expressing SHV-2 and SHV-3. All of the strains expressing PSE enzymes were susceptible to the combination. Strains expressing TEM-1, TEM-2, SHV-1, SHV-2, SHV-3, SHV-5, SHV-6, PSE-2, HMS-1, and CARB-4 β-lactamases were resistant to cefoperazone (MICs, 64 to >128 μg/ml). Sulbactam did not restore susceptibility to cefoperazone in strains expressing TEM-3, SHV-6, HMS-1, or CARB-4.

For strains which were susceptible to the β-lactam–β-lactamase inhibitor combinations, susceptibility was not as great as that of the β-lactamase-negative strain, C600N (Table 2). In general, the MICs of these antibiotics for strains producing a TEM β-lactamase were lower than those for strains producing an SHV enzyme. In addition, MICs for strains producing the extended-spectrum β-lactamases (TEM-3 through TEM-12, SHV-2 through SHV-6) were lower than those for strains producing the parental enzymes TEM-1, TEM-2, or SHV-1. Although the group 1 enzymes are not highly susceptible to the β-lactamase inhibitors, the inhibitors did lower the MICs of the companion drug 2- to 32-fold. However, susceptibility was not restored for any of the combinations with the strains expressing the group 1 enzyme constitutively (Ecl-C) or hyperinducibly (Ecl-Hy) (Table 2).

For the extended-spectrum β-lactam drugs, the presence of TEM-1, TEM-2, and SHV-1 β-lactamases did not significantly affect the MICs of cefotaxime or aztreonam. However, the MICs of ceftazidime and cefpirome in the presence of TEM-2 and SHV-1 were four- to eightfold higher than the MICs obtained for *E. coli* C600N. For strains expressing an extended-spectrum β-lactamase (TEM-3 through TEM-12, SHV-2 through SHV-6) the MICs of cefotaxime, ceftazidime, aztreonam, cefepime, and cefpirome were increased up to 4,096-fold. The MICs of cefotaxime were greater than the MICs of ceftazidime for four strains, while the MICs of ceftazidime were greater than the MICs of cefotaxime for eight strains. The MICs of aztreonam were greater than the MICs of either ceftazidime or cefotaxime for the strains expressing the SHV-5 β-lactamase. The extended-spectrum β-lactamases increased the MICs of cefepime and cefpirome 4- to 128-fold above those obtained in tests with the host strain, C600N.

The presence of any of the group 1 β-lactamases (MIR-1, Ecl-I, Ecl-C, and Ecl-Hy) significantly increased the MICs of cefotaxime, ceftazidime, and aztreonam, regardless of the level of enzyme expression (Table 2). In contrast, only high-level expression of group 1 β-lactamase was sufficient to significantly increase the MICs of cefepime or cefpirome.

Effect of a change in outer membrane protein on susceptibility. Mutants lacking either OmpC, OmpF, or both OmpC and OmpF were selected by using porin-specific phages with strains from the C600N panel expressing TEM-1, TEM-4, TEM-9, SHV-1, SHV-4, MIR-1, Ecl-I, and Ecl-C β-lactamases. The MICs of the various β-lactam drugs determined for these strains are given in Table 2. There was no general trend for the loss of one porin correlating with a change in the MIC.

TABLE 2. MICs of various β-lactam antibiotics for the *E. coli* C600N test panel

Enzyme	Porin	MIC (μg/ml)[a]														
		AM	AMC	SAM	PIP	P/T	TIC	TIM	CFP	C/S	CFM	CTX[b]	CAZ[b]	ATM	PIME[b]	ROME[b]
None	WT[c]	4	8	4	2	2	8	4	≤0.06	0.125	0.5	≤0.06	0.125	≤0.06	≤0.06	≤0.06
None	OmpC⁻	4	8	8	2	2	4	4	≤0.06	0.125	0.25	≤0.06	0.25	≤0.06	≤0.06	≤0.06
None	OmpF⁻	8	8	4	2	2	16	8	0.5	0.5	0.5	≤0.06	0.25	0.125	≤0.06	≤0.06
TEM-1	WT	>128	16	128	>128	32	>128	128	128	8	0.5	≤0.06	0.25	≤0.06	≤0.06	0.125
TEM-1	OmpC⁻	>128	16	64	>128	32	>128	128	>128	4	0.5	≤0.06	0.5	≤0.06	≤0.06	0.125
TEM-1	OmpF⁻	>128	32	128	>128	16	>128	128	64	16	0.5	≤0.06	0.5	0.125	0.125	0.25
TEM-1 (high)	WT	>128	32	>128	>128	64	>128	128	128	32	0.25	≤0.06	0.25	0.125	0.25	0.25
TEM-2	WT	>128	64	>128	>128	64	>128	128	>128	16	0.5	≤0.06	0.5	0.125	0.125	0.25
TEM-3	WT	>128	16	4	>128	2	>128	64	32	64	4	32	16	4	0.5	2
TEM-4	WT	>128	16	8	>128	4	>128	128	32	1	16	64	16	16	2	8
TEM-4	OmpC⁻	>128	16	8	>128	8	>128	64	32	1	8	64	8	8	4	8
TEM-4	OmpF⁻	>128	16	8	>128	4	>128	128	64	1	16	128	32	16	4	32
TEM-4	OmpC⁻ OmpF⁻	>128	16	16	>128	16	>128	128	128	4	32	32	16	16	8	32
TEM-5	WT	>128	16	16	64	8	>128	32	16	2	32	4	64	2	2	2
TEM-7	WT	>128	32	16	>128	8	>128	128	16	1	1	0.125	32	1	2	2
TEM-8	WT	>128	16	8	>128	8	>128	64	16	0.5	8	2	128	8	2	2
TEM-9	WT	>128	16	8	>128	8	>128	128	16	0.5	8	2	>128	8	2	4
TEM-9	OmpC⁻	>128	16	8	>128	8	>128	32	16	1	8	2	>128	8	4	8
TEM-9	OmpF⁻	>128	16	8	>128	4	>128	128	32	0.5	16	8	>128	32	16	16
TEM-9	OmpC⁻ OmpF⁻	>128	16	8	>128	4	>128	128	32	2	16	4	>128	16	8	16
TEM-10	WT	>128	16	8	>128	8	>128	128	8	1	16	1	>128	128	2	2
TEM-12	WT	>128	32	8	>128	8	>128	128	16	1	0.5	≤0.06	16	0.5	2	4
SHV-1	WT	>128	32	>128	>128	128	>128	128	>128	16	0.25	≤0.06	1	≤0.06	0.125	0.25
SHV-1	OmpC⁻	>128	32	>128	>128	128	>128	128	>128	32	1	≤0.06	1	1	0.5	1
SHV-1	OmpF⁻	>128	>128	>128	>128	128	>128	128	128	32	1	≤0.06	1	0.25	0.25	1
SHV-1	OmpC⁻ OmpF⁻	>128	32	>128	>128	32	>128	128	>128	8	0.25	≤0.06	1	≤0.06	≤0.06	0.125
SHV-2	WT	>128	16	16	>128	16	>128	128	64	8	2	16	8	4	4	8
SHV-3	WT	>128	16	32	>128	16	>128	32	128	4	4	64	4	4	4	4
SHV-4	WT	>128	16	16	>128	32	>128	128	32	8	>128	32	>128	>128	4	4
SHV-4	OmpC⁻	>128	8	8	>128	16	>128	128	8	2	64	32	>128	>128	0.5	1
SHV-4	OmpF⁻	>128	8	8	>128	32	>128	64	64	4	128	64	>128	128	1	2
SHV-5	WT	>128	64	64	>128	32	>128	128	128	16	0.5	0.25	0.25	1	0.25	1
SHV-6	WT	>128	>128	>128	>128	>128	128	128	>128	64	4	1	32	0.5	2	8
OXA-1	WT	>128	64	64	>128	64	>128	128	1	1	0.5	0.5	0.25	0.125	2	2
OXA-2	WT	>128	16	8	32	2	>128	8	4	0.5	0.5	≤0.06	1	≤0.06	≤0.06	≤0.06
OXA-3	WT	>128	32	8	32	4	>128	64	8	1	1	≤0.06	0.5	0.125	≤0.06	0.125
OXA-4	WT	>128	64	32	128	32	>128	128	0.5	0.5	0.25	≤0.06	0.125	≤0.06	0.5	1
OXA-5	WT	128	16	16	4	1	>128	32	1	1	0.5	≤0.06	0.125	≤0.06	≤0.06	≤0.06
OXA-6	WT	>128	16	32	32	8	>128	128	8	8	≤0.06	≤0.06	0.25	0.5	≤0.06	≤0.06
OXA-7	WT	>128	64	64	128	32	>128	128	32	16	0.5	≤0.06	0.125	1	0.125	0.5
PSE-1	WT	>128	32	8	32	1	>128	128	8	0.5	0.25	≤0.06	0.5	≤0.06	≤0.06	≤0.06
PSE-2	WT	>128	32	32	>128	1	>128	128	>128	1	0.5	≤0.06	0.25	≤0.06	≤0.06	≤0.06
PSE-3	WT	>128	8	8	>128	4	>128	64	8	0.5	0.5	≤0.06	0.25	≤0.06	≤0.06	≤0.06
PSE-4	WT	>128	32	64	>128	16	>128	128	32	8	0.25	≤0.06	0.25	≤0.06	0.125	≤0.06
SAR-1	WT	>128	16	32	>128	8	>128	64	16	2	0.5	≤0.06	0.125	≤0.06	≤0.06	≤0.06
ROB-1	WT	>128	16	8	>128	2	>128	128	32	1	0.5	≤0.06	8	0.125	0.25	0.5
HMS-1	WT	>128	32	128	>128	32	>128	128	>128	64	0.5	≤0.06	0.25	≤0.06	≤0.06	≤0.06
CARB-4	WT	>128	32	64	>128	64	>128	128	>128	32	0.25	≤0.06	0.25	≤0.06	0.25	≤0.06
LCR-1	WT	>128	32	32	8	2	64	16	2	1	0.25	0.125	0.25	≤0.06	0.125	0.125
L-1	WT	>128	>128	128	128	>128	>128	128	>128	>128	8	16	64	≤0.06	≤0.06	1
MIR-1	WT	>128	64	64	64	32	>128	128	>128	8	>128	128	64	64	0.25	0.5
MIR-1	OmpC⁻	>128	128	64	64	32	>128	128	128	16	>128	>128	64	64	1	2
MIR-1	OmpF⁻	>128	128	32	32	16	>128	128	64	8	>128	32	64	32	0.125	0.25
(Ecl-I)	WT	>128	128	64	16	4	32	16	4	1	64	4	2	0.5	≤0.06	≤0.06
(Ecl-I)	OmpC⁻	>128	128	64	32	16	128	64	8	4	128	8	16	2	≤0.06	≤0.06
(Ecl-I)	OmpF⁻	>128	128	64	16	2	64	32	8	4	>128	8	4	1	≤0.06	0.125
(Ecl-I)	OmpC⁻ OmpF⁻	>128	128	64	8	4	32	8	2	1	64	8	1	0.5	≤0.06	≤0.06
(Ecl-C)	WT	>128	128	64	>128	64	>128	128	>128	32	>128	128	>128	8	1	8
(Ecl-C)	OmpC⁻	>128	64	128	128	64	128	128	>128	16	>128	64	128	16	2	64
(Ecl-C)	OmpF⁻	>128	>128	64	>128	32	>128	128	>128	32	>128	32	>128	16	4	64
(Ecl-C)	OmpC⁻ OmpF⁻	>128	>128	64	128	32	128	128	128	32	>128	128	>128	16	2	64
(Ecl-Hy)	WT	>128	64	32	128	64	128	128	128	16	>128	128	128	32	1	16

[a] MICs were determined in broth microdilution tests with 10^4 CFU per well (39). AM, ampicillin; AMC, amoxicillin-clavulanate; SAM, ampicillin-sulbactam; PIP, piperacillin; P/T, piperacillin-tazobactam; TIC, ticarcillin; TIM, ticarcillin-clavulanate; CFP, cefoperazone; C/S, cefoperazone-sulbactam; CFM, cefixime; CTX, ceftotaxime; CAZ, ceftazidime; ATM, aztreonam; PIME, cefepime; ROME, cefpirome.

[b] Some data were published previously (51).

[c] WT, wild-type porins (both OmpC and OmpF porin proteins were present).

For example, for the strain expressing TEM-9, the loss of OmpC resulted in a fourfold decrease in the MIC of ticarcillin-clavulanate, while the loss of OmpF resulted in a fourfold or greater increase in the MICs of cefotaxime, aztreonam, cefepime, and cefpirome (Table 2). In general, penicillins were less affected by changes in outer membrane proteins, whereas cefpirome was most often affected.

DISCUSSION

Previous studies have used a test panel of β-lactamase-producing *E. coli* to study the activities of various enzymes against β-lactam antibiotics (19, 21). However, those studies included a limited number of types of β-lactamase, and the same host strain was not used throughout all analyses. The test panel presented in the current study was more extensive in its construction in that it included representatives of each of the major groups of β-lactamases, i.e., cephalosporinases, broad-spectrum enzymes, extended-spectrum β-lactamases, enzymes hydrolyzing carbenicillin or cloxacillin, and metallo-β-lactamases. In addition, the panel included mutants of the same strain that differed only in their outer membrane proteins or in the expression of the same enzyme, i.e., the group 1 β-lactamase and the TEM-1 β-lactamase. Therefore, with this panel it was possible to assess the influence of the type of β-lactamase, the level of expression for certain enzymes, and permeability, alone and in combination, upon susceptibility to β-lactam antibiotics.

The results of our study with the porin mutants supported and expanded upon those of Jacoby and Sutton (21) and Jacoby and Carreras (19). Those earlier studies also showed that variations in outer membrane porins had relatively little effect on the level of β-lactamase-mediated resistance. These results may at first seem surprising in light of the high level of resistance that a combination of a change in outer membrane proteins and β-lactamase production can contribute to clinical isolates (50, 54, 63). The lack of any consistent effect seen in these test panels may be due, in part, to the fact that these mutant strains were selected under phage pressure and may therefore behave differently from clinical isolates selected under antibiotic pressure. Also, since the same porin mutant may arise from mutations in either structural or control genes, this may explain the lack of a consistent effect as well.

It should be noted that this and the previous test panels (19, 21) consisted of laboratory strains of *E. coli*. The introduction of β-lactamases into these strains and the selection of mutants altered in enzyme expression or outer membrane permeability may not accurately predict the actual level of resistance which may be seen when the same change occurs in a clinical isolate of *E. coli* because other factors influencing susceptibility to β-lactam antibiotics may differ in clinical isolates. Nevertheless, such test panels can be very useful in assessing whether a specific factor, taken alone or in combination with others, will influence susceptibility to a given β-lactam antibiotic. However, since the plasmids encoding the various β-lactamases were not the same, differences in plasmid copy number and promoter efficiency could be responsible for some of the differences in susceptibility observed across the various enzymes.

A number of interesting observations were made with the test panel used in the current investigation. First, β-lactamase inhibitors did not always restore susceptibility to their companion drugs in tests with strains producing enzymes highly susceptible to the inhibitors. This supports reports of clinical isolates resistant to inhibitor-drug combinations because of the hyperproduction of inhibitor-susceptible enzymes (52, 54). Second, the diminished impacts of extended-spectrum β-lactamases on the activities of cefepime and cefpirome in comparison with their effects on older expanded-spectrum β-lactam antibiotics were apparent in tests with the panel. Whether this predicts the clinical success of the new drugs for the treatment of infections caused by strains producing extended-spectrum β-lactamases awaits human trials. However, it should be noted that the acquisition of certain extended-spectrum β-lactamases did diminish the activities of cefepime and cefpirome.

There were also several interesting observations that were made with the porin mutants examined in the present study. First, the activities of both cefepime and cefpirome were diminished by the loss of OmpC, OmpF, or both porins in strains constitutively producing the group 1 β-lactamase. These drugs are reported to be more active against hyperproducers of this β-lactamase than are other cephalosporins in part because they diffuse into the bacterial cell at a faster rate (14, 26, 28, 41, 51, 53, 57). Therefore, it was not surprising to see an increase in the level of resistance to these compounds caused by a decrease in permeability in strains expressing high levels of group 1 β-lactamase. However, this effect was not seen in the strain producing the MIR-1 enzyme. The difference between the strains producing MIR-1 and the group 1 β-lactamase constitutively may lie either in differences between the enzymes themselves, the lack of *ampD* in the latter strain, or both factors. Overall, results of the study with the outer membrane protein mutants suggest that while a loss of either OmpC or OmpF did change the susceptibilities of certain strains expressing β-lactamase to specific β-lactam antibiotics, the loss of a single porin did not predictably alter the susceptibility of a strain containing β-lactamase to any given β-lactam drug.

ACKNOWLEDGMENTS

We thank all of the investigators listed in Table 1 who so generously contributed strains to the study.

This work was sponsored in part by Pfizer/Roerig, N.Y., Lederle Laboratories, and Bristol-Meyers-Squibb.

REFERENCES

1. **Ames, G. F.** 1974. Resolution of bacterial proteins by polyacrylamide gel electrophoresis on slabs. J. Biol. Chem. **249:**634–644.
2. **Arlet, G., M. Rouveau, D. Bengoufa, M. H. Nicolas, and A. Philippon.** 1991. Novel transferable extended-spectrum β-lactamase (SHV-6) from *Klebsiella pneumoniae* conferring selective resistance to ceftazidime. FEMS Microbiol. Lett. **81:**57–62.
3. **Bachmann, B. J.** 1972. Pedigrees of some mutant strains of *Escherichia coli* K-12. Bacteriol. Rev. **36:**525–557.
4. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1523.
5. **Bradford, P. A., and C. C. Sanders.** 1992. The impact of β-lactamase in combination with a change in outer membrane protein on susceptibility to β-lactam antibiotics, abstr. A-142, p. 24. *In* Abstracts of the 92nd General Meeting of the American Society for Microbiology 1992. American Society for Microbiology, Washington, D.C.
6. **Bradford, P. A., C. C. Sanders, and E. S. Moland.** 1991. An isogenic panel to study β-lactamase expression in a common host background, abstr. 339. *In* Program of the 11th Interdisciplinary Meeting on Anti-Infectious Chemotherapy. French Society of Microbiology, Section of Antimicrobial Agents, Paris.
7. **Buré, A., P. Legrand, G. Arlet, V. Jarlier, G. Paul, and A. Philippon.** 1988. Dissemination in five French hospitals of *Klebsiella pneumoniae* serotype K25 harbouring a new transferable enzymatic resistance to third generation cephalosporins and aztreonam. Eur. J. Clin. Microbiol. Infect. Dis. **7:**780–782.
8. **Dale, J. W., and J. T. Smith.** 1971. The purification and properties of the β-lactamase specified by the resistance factor R-1818 in *Escherichia coli* and *Proteus mirabilis*. Biochem. J. **123:**493–500.
9. **Dale, J. W., and J. T. Smith.** 1974. R-factor-mediated β-lactamases that hydrolyze oxacillin: evidence for two distinct groups. J. Bacteriol. **119:**351–356.
10. **Datta, N., and P. Kontomichalou.** 1965. Penicillinase synthesis controlled

by infectious R factors in Enterobacteriaceae. Nature (London) **208:**239–244.

11. **Dufresne, J., G. Vézina, and R. Levesque.** 1988. Cloning and expression of the imipenem-hydrolyzing β-lactamase operon from *Pseudomonas maltophilia* in *Escherichia coli*. Antimicrob. Agents Chemother. **32:**819–826.
12. **El Solh, N., J. Allignet, R. Bismuth, B. Buret, and J. Fouace.** 1986. Conjugative transfer of staphylococcal antibiotic resistance in the absence of detectable plasmid DNA. Antimicrob. Agents Chemother. **30:**161–169.
13. **Fuchs, P. C., R. N. Jones, A. L. Barry, and C. Thornsberry.** 1986. Tentative disk diffusion susceptibility interpretive criteria for BMY-28142, a new cephalosporin. J. Clin. Microbiol. **23:**634–636.
14. **Fung-Tome, J., T. J. Dougherty, F. J. DeOrio, V. Simich-Jacobson, and R. E. Kessler.** 1989. Activity of cefepime against ceftazidime- and cefotaxime-resistant gram-negative bacteria and its relationship to β-lactamase levels. Antimicrob. Agents Chemother. **33:**498–502.
15. **Furth, A. J.** 1974. Purification and properties of a constitutive β-lactamase from *Pseudomonas aeruginosa* strain Dalgleish. Biochim. Biophys. Acta **377:** 431–443.
16. **Gutmann, L., B. Ferrè, F. W. Goldstein, N. Rizk, E. Pinto-Schuster, J. F. Acar, and E. Collatz.** 1989. SHV-5, a novel SHV-type β-lactamase that hydrolyzes broad spectrum cephalosporins and monobactams. Antimicrob. Agents Chemother. **33:**951–956.
17. **Gutmann, L., M. D. Kitzis, D. Billot-Klein, F. Goldstein, G. Tran Van Nhieu, T. Lu, J. Carlet, E. Collatz, and R. Williamson.** 1988. Plasmid-mediated β-lactamase (TEM-7) involved in resistance to ceftazidime and aztreonam. Rev. Infect. Dis. **10:**860–866.
18. **Hedges, R. W., and G. A. Jacoby.** 1980. Compatibility and molecular properties of plasmid Rms149 in *Pseudomonas aeruginosa* and *Escherichia coli*. Plasmid **3:**1–6.
19. **Jacoby, G. A., and I. Carreras.** 1990. Activities of β-lactam antibiotics against *Escherichia coli* strains producing extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **34:**858–862.
20. **Jacoby, G. A., and A. A. Medeiros.** 1991. More extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **35:**1697–1704.
21. **Jacoby, G. A., and L. Sutton.** 1985. β-Lactamases and β-lactam resistance in *Escherichia coli*. Antimicrob. Agents Chemother. **28:**703–705.
22. **Jarlier, V., M. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in Enterobacteriaceae: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
23. **Jones, R. N., A. L. Barry, R. R. Packer, W. W. Gregory, and C. Thornsberry.** 1987. In vitro antimicrobial spectrum, occurrence of synergy, and recommendations for dilution susceptibility testing concentrations of the cefoperazone-sulbactam combination. J. Clin. Microbiol. **25:**1725–1729.
24. **Jones, R. N., A. L. Barry, and C. Thornsberry.** 1986. Cefpirome (HR810) disk diffusion susceptibility tests: confirmation of interpretive criteria using cefotaxime and cefoperazone-resistant strains and effects of blood supplemented media. Diagn. Microbiol. Infect. Dis. **4:**345–349.
25. **Kliebe, C., B. A. Nies, J. F. Meyer, R. M. Tolxdorff-Neutzling, and B. Wiedemann.** 1985. Evolution of plasmid-coded resistance to broad spectrum cephalosporins. Antimicrob. Agents Chemother. **28:**302–307.
26. **Knapp, C. C., and J. A. Washington.** 1989. Activity of cefepime, ceftazidime, and ceftizoxime against mutants of Enterobacteriaceae and *Pseudomonas aeruginosa* derepressed for class 1 β-lactamase. J. Antimicrob. Chemother. **24:**1011–1012.
27. **Korfmann, G., and B. Wiedemann.** 1988. Genetic control of β-lactamase production in *Enterobacter cloacae*. Rev. Infect. Dis. **10:**793–799.
28. **Lattrell, R., J. Blumbach, W. Duerckheimer, H.-W. Fehlhaber, K. Fleischmann, E. Schrinner, W. Schwab, K. Seeger, G. Seibert, and M. Wieduwilt.** 1988. Synthesis and structure-activity relationships in the cefpirome series. J. Antibiot. **41:**1374–1417.
29. **Levesque, R. C., and G. A. Jacoby.** 1988. Molecular structure and interrelationships of multiresistance β-lactamase transposons. Plasmid **19:**21–29.
30. **Levesque, R. C., A. Medeiros, and G. A. Jacoby.** 1987. Molecular cloning and DNA homology of plasmid-mediated β-lactamase genes. Mol. Gen. Genet. **206:**252–258.
31. **Livermore, D. M.** 1988. Permeation of β-lactam antibiotics into *Escherichia coli*, *Pseudomonas aeruginosa*, and other gram-negative bacteria. Rev. Infect. Dis. **10:**691–698.
32. **Livermore, D. M., J. P. Maskell, and J. D. Williams.** 1984. Detection of PSE-2 β-lactamase in enterobacteria. Antimicrob. Agents Chemother. **25:** 268–272.
33. **Lugtenberg, B., J. Meijers, R. Peters, P. van der Hoek, and L. V. Alphen.** 1975. Electrophoretic resolution of the "major outer membrane protein" of *Escherichia coli*. FEBS Lett. **58:**254–258.
34. **Mabilat, C., and P. Courvalin.** 1990. Development of "oligotyping" for characterization and molecular epidemiology of TEM β-lactamases in members of the family *Enterobacteriaceae*. Antimicrob. Agents Chemother. **34:** 2210–2216.
35. **Matthew, M., and R. W. Hedges.** 1976. Analytical isoelectric focusing of R factor-determined β-lactamases: correlation with plasmid compatibility. J. Bacteriol. **125:**713–718.
36. **Matthew, M., R. W. Hedges, and J. T. Smith.** 1979. Types of β-lactamase determined by plasmids in gram negative bacteria. J. Bacteriol. **138:**657–662.
37. **Matthew, M., and R. B. Sykes.** 1977. Properties of the beta-lactamase specified by the *Pseudomonas* plasmid RPL11. J. Bacteriol. **132:**341–345.
38. **Medeiros, A., M. Cohenford, and G. A. Jacoby.** 1985. Five novel plasmid-determined β-lactamases. Antimicrob. Agents Chemother. **27:**715–719.
39. **National Committee for Clinical Laboratory Standards.** 1992. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
40. **Nichols, W. W.** 1988. Towards a fundamental understanding of the MIC of β-lactam antibiotics. J. Antimicrob. Chemother. **22:**275–283.
41. **Nikaido, H., W. Liu, and E. Y. Rosenberg.** 1990. Outer membrane permeability and β-lactamase stability of dipolar ionic cephalosporins containing methoxyimino substituents. Antimicrob. Agents Chemother. **34:** 337–342.
42. **Papanicolaou, G., A. A. Medeiros, and G. A. Jacoby.** 1990. Novel plasmid-mediated β-lactamase (MIR-1) conferring resistance to oxyimino- and α-methoxy β-lactams in clinical isolates of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **34:**2200–2209.
43. **Paul, G. C., G. Gerbaud, A. Bure, A. M. Philippon, B. Pangon, and P. Courvalin.** 1989. TEM-4, a new plasmid-mediated β-lactamase that hydrolyzes broad-spectrum cephalosporins in a clinical isolate of *Escherichia coli*. Antimicrob. Agents Chemother. **33:**1958–1963.
44. **Philippon, A., R. Labia, and G. Jacoby.** 1989. Extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **33:**1131–1136.
45. **Philippon, A. M., G. C. Paul, A. P. Thabaut, and G. A. Jacoby.** 1986. Properties of a novel carbenicillin-hydrolyzing β-lactamase (CARB-4) specified by an IncP-2 plasmid from *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **29:**519–520.
46. **Quinn, J. P., D. Miyashiro, D. Sahm, R. Flamm, and K. Bush.** 1989. Novel plasmid-mediated β-lactamase (TEM-10) conferring selective resistance to ceftazidime and aztreonam in clinical isolates of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **33:**1451–1456.
47. **Reid, A. J., and S. G. B. Aymes.** 1986. Plasmid penicillin resistance in *Vibrio cholerae*: identification of a new β-lactamase SAR-1. Antimicrob. Agents Chemother. **30:**245–247.
48. **Roupas, A., and J. S. Pitton.** 1974. R factor-mediated and chromosomal resistance to ampicillin in *Escherichia coli*. Antimicrob. Agents Chemother. **5:**186–191.
49. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
50. **Sanders, C. C.** 1992. β-Lactamases of gram-negative bacteria: new challenges for new drugs. Clin. Infect. Dis. **14:**1089–1099.
51. **Sanders, C. C.** 1993. Cefepime: the next generation? Clin. Infect. Dis. **17:** 369–379.
52. **Sanders, C. C., J. P. Iaconis, G. P. Bodey, and G. Samonis.** 1988. Resistance to ticarcillin-potassium clavulanate among clinical isolates of the family *Enterobacteriaceae*: role of PSE-1 β-lactamase and high levels of TEM-1 and SHV-1 and problems with false susceptibility in disk diffusion tests. Antimicrob. Agents Chemother. **32:**1365–1369.
53. **Sanders, C. C., and W. E. Sanders, Jr.** 1986. Type I beta-lactamases of gram negative bacteria: interaction with beta-lactam antibiotics. J. Infect. Dis. **154:**792–800.
54. **Sanders, C. C., and W. E. Sanders, Jr.** 1992. β-Lactam resistance in gram-negative bacteria: global trends and clinical impact. Clin. Infect. Dis. **15:**824–839.
55. **Sanders, C. C., W. E. Sanders, Jr., and E. S. Moland.** 1986. Characterization of β-lactamases in situ on polyacrylamide gels. Antimicrob. Agents Chemother. **30:**951–952.
56. **Schnaitman, C. A., and G. A. McDonald.** 1984. Regulation of outer membrane protein synthesis in *Escherichia coli* K-12: deletion of ompC affects expression of the omp F protein. J. Bacteriol. **159:**555–563.
57. **Siebert, G., M. Limbert, I. Winkler, and T. Dick.** 1983. The antibacterial activity in vitro and β-lactamase stability of the new cephalosporin HR 810 in comparison with five other cephalosporins and two aminoglycosides. Infection **11:**275–279.
58. **Simpson, I. N., S. J. Plested, M. J. Budin-Jones, J. Lees, R. W. Hedges, and G. A. Jacoby.** 1983. Characterization of a novel plasmid-mediated β-lactamase and its contribution to β-lactam resistance in *Pseudomonas aeruginosa*. FEMS Microbiol. Lett. **19:**23–27.
59. **Sirot, D., J. Sirot, R. Labia, A. Morand, P. Courvalin, A. Darfeuille-Michaud, R. Perroux, and R. Cluzel.** 1987. Transferable resistance to third-generation cephalosporins in clinical isolates of *Klebsiella pneumoniae*: identification of CTX-1, a novel β-lactamase. J. Antimicrob. Chemother. **20:**323–334.
60. **Sirot, J., R. Labia, and A. Thabaut.** 1987. *Klebsiella pneumoniae* strains more resistant to ceftazidime than to other third-generation cephalosporins. J. Antimicrob. Chemother. **20:**611–612.
61. **Spencer, R. C., P. F. Wheat, T. G. Winstanley, D. M. Cox, and S. J. Plested.**

1987. Novel β-lactamase in a clinical isolate of *Klebsiella pneumoniae* conferring unusual resistance to β-lactam antibiotics. J. Antimicrob. Chemother. **20:**919–921.
62. **Vecoli, C., F. E. Prevost, J. J. Ververis, A. A. Medeiros, and J. G. P. O'Leary.** 1983. Comparison of polyacrylamide and agarose gel thin-layer isoelectric focusing for the characterization of β-lactamases. Antimicrob. Agents Chemother. **24:**186–189.
63. **Weber, D. A., C. C. Sanders, J. S. Bakken, and J. P. Quinn.** 1990. A novel chromosomal TEM derivative and alterations in outer membrane proteins together mediate selective ceftazidime resistance in *Escherichia coli*. J. Infect. Dis. **162:**460–465.
64. **Yanisch-Perron, C., J. Vieira, and J. Messing.** 1985. Improved M13 phage cloning vectors and host strains: nucleotide sequences of the M13mp18 and pUC19 vectors. Gene **33:**103.