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Use of an Isogenic *Escherichia coli* Panel To Design Tests for Discrimination of β-Lactamase Functional Groups of *Enterobacteriaceae*

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A study was designed to determine if an isogenic panel of *Escherichia coli* strains containing many different β-lactamases could be used for the preliminary screening of a large number of β-lactam agents to identify which might be most useful in the development of a definitive test for specific β-lactamases found among the members of family *Enterobacteriaceae*. The susceptibilities of 46 strains, comprising the isogenic panel, to expanded-spectrum cephalosporins, cephamycins, and aztreonam were determined in the presence and absence of β-lactamase inhibitors in broth microdilution tests. The results indicated that strains producing extended-spectrum β-lactamases (ESBLs) could be distinguished from strains producing other Bush-Jacoby-Medeiros functional group 2 or group 1 β-lactamases. For strains producing group 1 β-lactamases, cefpodoxime and ceftazidime MICs were ≥4 μg/ml and addition of clavulanate did not reduce the MICs more than fourfold. For strains producing group 2 enzymes other than ESBLs, cefpodoxime and ceftazidime MICs were ≤2 μg/ml. With a single exception (ceftazidime for the strain producing SHV-3), among strains producing ESBLs, cefpodoxime and ceftazidime MICs were ≥4 μg/ml and addition of clavulanate reduced the MICs by more than eightfold. Cephamycins could also be used to discriminate between strains producing group 1 β-lactamases and ESBLs, since only the former required cefotetan concentrations as high as 8 μg/ml or cefoxitin concentrations of >16 μg/ml for inhibition. Other cephalosporins provided some discrimination between the various β-lactamase producers, although they were not as reliable as either cefpodoxime or ceftazidime. These results indicate the utility of an isogenic panel for identification of candidate drugs among many for further testing with clinical isolates of the family *Enterobacteriaceae* to determine the best agents for detection of specific β-lactamases in this family.

The single most-prevalent mechanism responsible for resistance to β-lactam antibiotics among clinical isolates of the family *Enterobacteriaceae* is the production of β-lactamase (20). Until recently, resistance mediated by β-lactamases was readily detected by clinical microbiology laboratories through the use of a variety of routine antimicrobial susceptibility tests. Unfortunately, the appearance of new forms of certain β-lactamases, the extended-spectrum β-lactamases (ESBLs), and new plasmid derivatives of the AmpC β-lactamase has made detection of resistance in routine susceptibility tests unreliable (22). Therefore, it has become necessary to develop new tests specifically for the detection of these enzymes, which may produce hidden, but clinically relevant, resistance to newer cephalosporins and aztreonam.

A number of new tests for the detection of ESBLs among clinical isolates of the family *Enterobacteriaceae* are currently under development (7, 10, 15, 19, 23, 25). Some of these methods involve the use of single drugs as indicators of the presence or absence of ESBLs, while others involve testing of drugs with and without β-lactamase inhibitors. Regardless of the type of test involved, each could be performed with any one of several agents among the many β-lactam antibiotics clinically available today. However, testing of all possible candidate drugs would not be feasible.

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Therefore, a study was designed to determine if an isogenic panel of *Escherichia coli* strains containing many diverse β-lactamases could be used for the preliminary screening of a large number of β-lactam agents to identify which might be most useful in the development of a more definitive test for specific β-lactamases found among the members of the family *Enterobacteriaceae*. A group of expanded-spectrum cephalosporins and a monobactam were tested, with and without β-lactamase inhibitors, as candidates for differentiation of ESBLs from other functional group 2 enzymes, and two cephamycins were included for differentiation of functional group 1 β-lactamases (5, 6). The isogenic panel examined consisted of a single *E. coli* host into which plasmids encoding various β-lactamases had been introduced (3). This test panel has been expanded to include recently described enzymes. Forty-six strains were selected from the complete panel for use in this study. The strains selected produced β-lactamases of groups 1, 2b, 2be, 2c, and 2d of the Bush-Jacoby-Medeiros classification scheme (6). Testing in this panel eliminates the confounding influences of intrinsic susceptibility differences between various host organisms. This makes it possible to examine directly the effect of specific β-lactamases on the results obtained. A potential disadvantage of using this defined panel is that all testing is done in a laboratory strain of a single species; hence, results obtained with such a panel may not be the same as those obtained with clinical isolates of the same or a different species. Therefore, this initial study was used to identify candidate drugs and/or inhibitor-drug combinations which could then be tested against large numbers of clinical isolates to evaluate their utility in tests performed by clinical laboratories.

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MATERIALS AND METHODS

Strains. The test panel consisted of 46 strains of *E. coli* C600N, a nalidixic acid-resistant mutant of strain C600 (*thr-1 leuB6 lacY1 supE44 rfbD1 thi-1 tonA21* λ^-), containing β-lactamase-encoding plasmids (3). β-Lactamases expressed by the panel organisms are listed in Table 1 and include enzymes representing functional groups 1, 2b, 2be, 2c, and 2d (6). Plasmids were introduced into the C600N host strain through standard transformation (electroporation or chemical transformation) or conjugation techniques (18). Strains were stored at −70°C until used and were grown on Luria-Bertani agar, Miller (Difco, Detroit, Mich.), containing ampicillin (20 μg/ml) where necessary for plasmid maintenance. For quality control purposes, *E. coli* ATCC 25922 was also included in the testing.

Susceptibility testing. Antibiotic susceptibility testing was performed according to standard National Committee for Clinical Laboratory Standards microdilution methods (13), using dehydrated investigational panels prepared by Dade MicroScan, Inc. (Sacramento, Calif.). Drugs contained in the panels were cefpodoxime, ceftazidime, cefotaxime, ceftriaxone, aztreonam, cefoxitin, and cefotetan. Cefoxitin and cefotetan were tested in the concentration range 0.12 to 16 μg/ml, alone and in combination with sulbactam (8 μg/ml). The remaining drugs were tested in the range 0.06 to 8 μg/ml or 0.12 to 16 μg/ml, alone and in combination with sulbactam (8 μg/ml) or clavulanate (1, 2, or 4 μg/ml).

RESULTS

Ranges of MICs for the drugs when tested alone against the test panel are shown in Table 2. Cefpodoxime was the only agent that by itself was able to discriminate between all strains producing groups 1 or 2be enzymes and strains producing other group 2 β-lactamases, though ceftazidime was nearly as effective. Cefpodoxime and ceftazidime MICs were ≥4 μg/ml only in tests with strains producing group 1 or 2be β-lactamases. A ceftazidime MIC of 0.5 μg/ml was obtained for the strain producing SHV-3, thus yielding its only MIC range overlap. MIC ranges for aztreonam and the other expanded-spectrum cephalosporins overlapped for all groups, although generally higher MICs were obtained in tests with strains producing β-lactamases of functional group 1 or 2be (Table 2). The only other clear separation that was possible with single-drug testing was the identification of group 1-producing strains on the basis of cefoxitin or cefotetan MICs (Table 2). Cefoxitin MICs of >16 μg/ml and cefotetan MICs of ≥8 μg/ml were obtained only in tests with strains producing group 1 β-lactamases.

Individual drugs yielded wide ranges of MICs within enzyme groups 1 and 2be, through the number of nonconforming enzymes differed depending on the drug tested. All group 1 enzyme producers yielded cephalosporin MICs of ≥16 μg/ml except for ceftazidime in strains producing AmpC(i) (MIC, 0.5 to 1 μg/ml) and cefotaxime and ceftriaxone in strains producing AmpC(i) (MIC, 0.5 to 1) or FOX-1 (MIC, 2 μg/ml). Aztreonam MICs for group 1-producing strains were split, with LAT-1-, MIR-1-, and AmpC(hy)-producing strains having MICs of ≥16 μg/ml and the others yielding low values (FOX-1-producing strains, 0.5 μg/ml; AmpC(i)-producing strains, 0.25 μg/ml; and AmpC(c)-producing strains, ≤0.12 μg/ml). Group 2be producers also generated cefpodoxime and ceftazidime MICs that were generally ≥16 μg/ml, with the following exceptions: the cefpodoxime MIC for the SHV-6-producing strain was 4 to 8 μg/ml, while that for the TEM-12- and TEM-43-producing strains was 8 μg/ml; the ceftazidime MIC for the SHV-2-producing strain was 4 to 8 μg/ml, and that for the SHV-3-producing strains was 0.5 μg/ml. Cefotaxime and ceftriaxone MICs for the group 2be producers were predominantly <16 μg/ml. The cefotaxime MICs for the group 2be-producing strains were as follows: for the SHV-6 and TEM-12 producers, 0.25 μg/ml; for the TEM-7 and TEM-43 producers, 0.5 μg/ml; for the TEM-10 producer, 1 μg/ml; for the TEM-28 producer, 2 μg/ml; for the SHV-3 and TEM-8 producers, 4 μg/ml; for the TEM-5 and TEM-8 producers, 8 μg/ml; and for the SHV-2 producers, 8 to 16 μg/ml. The ceftriaxone MICs for the group 2be-producing strains were as follows: for the TEM-12 producer, 0.25 μg/ml; for the SHV-6 and TEM-43 producers, 0.5 μg/ml; for the TEM-7-producing strain, 1 μg/ml; for the TEM-10 producer, 2 μg/ml; for the TEM-8, TEM-43, and SHV-3 producers, 4 μg/ml; and for the SHV-2-producing

TABLE 1. β-Lactamases and plasmids included in the *E. coli* test panel

β-Lactamase	Plasmid	Received from:	Reference
Group 1			
AmpC(i)[a]	pBP131	G. Korfmann	3
AmpC(c)[a]		G. Korfmann	3
AmpC(hy)[a]		G. Korfmann	3
MIR-1	pMLC28Kpn1-8	A. Medeiros	3
LAT-1	pHP15	L. Tzouvelekis	24
FOX-1		F. Baquero	9
Group 2b			
TEM-1	R6K	A. Medeiros	3
TEM-1 (high)	pUC19		3
TEM-2	RP1	A. Medeiros	3
SHV-1	R1010	A. Medeiros	3
ROB-1	pMON401	G. Jacoby	3
HMS-1	R977	A. Medeiros	3
TLE-1		G. Jacoby	11
Group 2be			
TEM-3	pCFF04	J. Sirot	3
TEM-4	pUD16	G. Paul	3
TEM-5	pCFF14	J. Sirot	3
TEM-7		L. Gutmann	3
TEM-8	HM12G	C. Mabilat	3
TEM-10	pJPQ100	J. Quinn	3
TEM-12	pDAW402	D. Weber	3
TEM-28	pCLL3412	P. Bradford	2
TEM-42	pAZ331	P. Mugnier	12
TEM-43	pCLL3416	P. Bradford	26
SHV-2	pBP60	B. Weidemann	3
SHV-3	pUD18	A. Philippon	3
SHV-4	pUD21	A. Philippon	3
SHV-6	pSLH47	G. Arlet	3
SHV-7	pCLL3410	P. Bradford	4
PER-1	pPZ1	P. Nordmann	14
Group 2c			
PSE-1	pMG217	G. Jacoby	3
PSE-2	R140	G. Jacoby	3
PSE-3	Rms149	G. Jacoby	3
PSE-4	pMON705	G. Jacoby	3
SAR-1	pUK657	S. Aymes	3
CARB-4	pMON1024	G. Jacoby	3
Group 2d			
OXA-1	RGN238	G. Jacoby	3
OXA-2	R1818 (R46)	A. Medeiros	3
OXA-3	pIP55	G. Jacoby	3
OXA-4	pMG203	A. Medeiros	3
OXA-5	pMG54	A. Medeiros	3
OXA-6	pUZ8-pMG39	A. Medeiros	3
OXA-7	pMG202	A. Medeiros	3
OXA-12	pCLL2222	B. Rasmussen	1
LCR-1	pMK20:Tn1412	G. Jacoby	3

[a] AmpC enzymes are cloned from *Enterobacter cloacae*. (i), Expressed inducibly; (c), expressed constitutively; (hy), expressed hyperinducibly.

TABLE 2. MIC ranges obtained in tests with strains producing β-lactamases of various functional groups

Drug	MIC (μg/ml) by functional group[a]				
	1	2be	2b	2c	2d
Cefpodoxime	16–>16	4–>16	0.5–1	0.5	0.5–2
Ceftazidime	0.5–>16	0.5–>16	0.25–1	0.25	<0.12–1
Cefotaxime	0.5–>16	0.25–>16	<0.12–0.5	<0.12	<0.12–0.5
Ceftriaxone	0.5–>16	0.25–>16	<0.12–0.5	<0.12	<0.12–0.5
Aztreonam	<0.12–>16	0.25–>16	<0.12–0.5	<0.12	<0.12–1
Cefoxitin	>16	2–8	4	4	2–8
Cefotetan	8–>16	<0.12–2	0.25–0.5	<0.12–2	<0.12–2

[a] Bush-Jacoby-Medeiros classification (6).

strains, 8 to 16 μg/ml. Aztreonam MICs for group 2be producers were ≥16 μg/ml except for the strains producing SHV-3 and -6 (0.25 μg/ml), TEM-12 (1 μg/ml), SHV-2 (2 to 4 μg/ml), TEM-7 (4 μg/ml), and TEM-3 and -43 (8 μg/ml). These results indicated that two of the tested drugs, ceftazidime and cefpodoxime, produced markedly fewer nonconforming results and might yield more-reliable discriminations among large groups of enzymes.

The influence of the addition of a β-lactamase inhibitor on the MIC, expressed as the fold reduction in MIC compared to the value for each drug when tested alone, for strains producing group 1 or 2be enzymes is presented in Table 3. The use of cefpodoxime in combination with 1, 2, or 4 μg of clavulanate/ml allowed the separation of strains producing group 1 β-lactamases from those producing group 2be enzymes. Cefpodoxime MICs in tests with only the latter strains were reduced eightfold or more by clavulanate. Similar separations of strains producing group 1 and 2be β-lactamases were possible with ceftazidime plus 1 or 2 μg of clavulanate/ml, cefotaxime plus 1 μg of clavulanate/ml, or aztreonam plus 1 or 2 μg of clavulanate/ml. Addition of sulbactam did not allow discrimination between these two functional groups with any of the drugs tested (Table 3). Addition of β-lactamase inhibitors to the drugs did not improve discrimination between strains producing other group 2 β-lactamases (data not shown).

The other cephalosporins examined in this study provided some differentiation between strains producing different β-lactamases, though not as reliably as cefpodoxime or ceftazidime. Although ceftriaxone MICs were <4 μg/ml in tests with strains producing TEM-7, -10, or -12 or SHV-6, addition of clavulanate at 2 μg/ml did cause more than a fourfold reduction in all cases except that of the TEM-12 producer (Table 3). Cefotaxime MICs were <4 μg/ml in tests with strains producing TEM-7, -10, -12, -28, or -43 or SHV-6, but addition of clavulanate at 2 μg/ml reduced cefotaxime MICs by more than fourfold except in tests with the strains producing TEM-12 and SHV-6. Therefore, cefpodoxime or ceftazidime, alone or in combination with clavulanate, allowed the most reliable separations of the test strains, followed by ceftriaxone and cefotaxime (in that order).

DISCUSSION

This study examined the ability of individual drugs or drug-inhibitor combinations to distinguish between strains of an isogenic test panel producing enzymes of functional groups 1 and 2. Initial screening of the seven primary β-lactam drugs alone allowed us to select two cephalosporins, cefpodoxime and ceftazidime, which were most capable of discriminating between the different functional groups. Either of these drugs was generally capable of dividing the panel strains into two categories, those producing an enzyme of either group 1 or 2be and those producing other group 2 β-lactamases. When used alone, however, these agents were not capable of further discrimination within these categories. Given that the group 1 (AmpC-type) and group 2be (ESBL) enzymes are of greater clinical concern than the other enzymes tested, we then focused on methods to discriminate these two groups from each other. Both testing in combination with β-lactamase inhibitors and independent testing with cephamycins were found to effectively discriminate between these two groups.

The best separations were produced by testing with cefpodoxime alone or in combination with clavulanate at 2 μg/ml. Cefpodoxime MICs of less than 4 μg/ml were observed only in tests with strains elaborating β-lactamases of group 2b, 2c, or 2d. No other test further distinguished between these three groups. Cefpodoxime MICs of ≥4 μg/ml were observed only in tests with strains elaborating β-lactamases of group 1 or 2be. These two groups could then be distinguished from each other either by the presence of elevated MICs in tests with cephamycins (group 1 enzymes) or by the eightfold or greater reduction in cefpodoxime MICs by clavulanate at 2 μg/ml (group 2be enzymes). Clavulanate was not tested in combination with cephamycins because these drugs were included to aid in the

TABLE 3. Influence of addition of β-lactamase inhibitors on MICs obtained in tests with strains producing functional group 1 or 2be β-lactamases

Drug	Fold reduction in MIC following combination of drug with[a]:							
	Clavulanate at:						Sulbactam at 8 μg/ml; functional group:	
	1 μg/ml		2 μg/ml		4 μg/ml			
	Functional group:		Functional group:		Functional group:			
	1	2be	1	2be	1	2be	1	2be
Cefpodoxime	0–4×	8–64×	0–4×	16–128×	0–4×	8–128×	0–128×	8–128×
Ceftazidime	0–2×	8–128×	0–2×	8–128×	0–4×	4–128×	0–32×	8–128×
Cefotaxime	0–2×	4–256×	0–4×	4–256×	0–4×	2–128×	2–32×	4–128×
Ceftriaxone	0–4×	4–256×	0–4×	4–256×	0–4×	2–128×	2–32×	2–128×
Aztreonam	0–2×	4–256×	0–2×	4–256×	0–2×	2–128×	0–16×	2–128×
Cefoxitin	NT[b]	NT	NT	NT	NT	NT	0–16×	2–16×
Cefotetan	NT	NT	NT	NT	NT	NT	0–64×	2–16×

[a] Functional groups per Bush-Jacoby-Medeiros classification (6).
[b] NT, not tested.

detection of group 1 enzymes and clavulanate is an ineffective inhibitor of these β-lactamases.

Although the screening of candidate drugs and drug-inhibitor combinations and the interpretation of the results are simplified by the use of an isogenic background, this process also has its shortcomings. The host strain used in these studies is a laboratory strain of *E. coli* and is intrinsically more susceptible to many antibacterial agents than its clinical counterparts. Additionally, the intrinsic β-lactam susceptibilities of other clinically important species of the family *Enterobacteriaceae* producing ESBLs and AmpC-type enzymes are quite different from those of the *E. coli* C600N host. Thus, results from testing in this isogenic background may not be directly applicable to strains encountered in the clinical laboratory. This may be especially true for *Enterobacteriaceae* species that are not intrinsically susceptible to cefpodoxime, the single most-useful β-lactam drug in tests with this *E. coli* panel. For this reason, it was important to identify additional drugs that functioned relatively well in the test panel.

Ceftazidime and ceftazidime-clavulanate were essentially as reliable as cefpodoxime and cefpodoxime-clavulanate for discrimination of enzymes from the three general groups (functional group 1, group 2be, and other group 2 enzymes). Using the same criteria outlined for cefpodoxime above, the only enzyme not easily discriminated by ceftazidime and ceftazidime-clavulanate was SHV-3. This shortcoming may be of limited importance since SHV-3 is not a prevalent ESBL. Ceftriaxone and cefotaxime, with and without clavulanate, were also able to provide some discrimination, although they were much less reliable than ceftazidime or cefpodoxime. The ability of the cephamycins to distinguish between strains producing group 1 and 2be β-lactamases in this study may be of limited value in tests with clinical strains. Isolates of *Klebsiella pneumoniae* that produce ESBLs may undergo porin changes that produce resistance to cephamycins (16, 17). Furthermore, many *Enterobacteriaceae* species that produce ESBLs, like *Enterobacter*, *Citrobacter*, and *Serratia* species, are intrinsically resistant to the cephamycins. Thus, the major use of this test panel of *E. coli* was in the identification of the best candidate drugs for further testing. The validity of this approach awaits evaluation in a similar study with clinical isolates (21).

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