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SHV-5, a Novel SHV-Type β-Lactamase That Hydrolyzes Broad-Spectrum Cephalosporins and Monobactams

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SHV-5 (pI 8.2), a novel broad-spectrum β-lactamase encoded by a ca. 150-kilobase plasmid, was found in *Klebsiella pneumoniae* 160. SHV-5 β-lactamase caused decreased susceptibility to most penicillins, cephalosporins, and monobactams, except imipenem and compounds which have a C6 or C7 α-methoxy substituent. β-Lactamase inhibitors (clavulanic acid, sulbactam, and tazobactam) inhibited its activity and showed a synergistic effect when associated with different hydrolyzable β-lactam compounds. Hybridization studies suggested that this enzyme may be related to, or derived from, the SHV enzyme. Increased MICs of cephamycins and temocillin associated with a decreased synergistic effect of the inhibitors on *K. pneumoniae* 160 might be linked to a decrease in two outer membrane proteins.

Recently, plasmid-mediated β-lactamases, derived from either TEM-1 or TEM-2 (2, 6, 15, 23, 26) or from SHV (3, 12, 16, 17), which cause resistance to broad-spectrum cephalosporins and to aztreonam have been described. Among those, two derivatives of the TEM β-lactamases (i.e., TEM-5 [CAZ-1] and TEM-7) were responsible for higher resistance to ceftazidime (6, 23) and aztreonam (6) than to cefotaxime when present in *Escherichia coli*. In this work, we have studied a new plasmid-mediated β-lactamase, presumably derived from the SHV-type enzymes, which in contrast to SHV-2 and SHV-3 (12, 15, 16) is responsible for higher MICs of ceftazidime and monobactams than of other broad-spectrum cephalosporins.

MATERIALS AND METHODS

Bacterial strains and growth conditions. *Klebsiella pneumoniae* 160 containing SHV-5 was isolated in September 1987 from blood cultures obtained from a patient with peritonitis treated with cefotaxime and amikacin at San Juan de Dias Hospital in Santiago, Chile. The strain was resistant to penicillins, cephalosporins, monobactams, aminoglycosides (except gentamicin), tetracycline, chloramphenicol, and sulfonamides. *E. coli* BM694 (18) and *K. pneumoniae* 2222 (9) were used as recipients for plasmids encoding different β-lactamases. The plasmid harboring the SHV-5 gene (pAFF1) was not self-transferable to *E. coli*. However, the gene encoding SHV-5 could be incorporated into plasmid R135 (30), introduced into *K. pneumoniae* 160, and conjugated into *E. coli* BM694 after selection on cefotaxime (2 μg/ml) plus nalidixic acid (20 μg/ml). R135 containing the SHV-5 gene was denominated pAFF2. pAFF3 was obtained from pAFF2 by cloning a ca. 3.8-kilobase (kb) *Bam*HI fragment containing the SHV-5 gene into the *Bam*HI site of pACYC184 (4).

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MIC determinations and antibiotics. MICs were determined at 37°C by a dilution method on Mueller-Hinton agar with a Steers-type replicator device and an inoculum of 10^5 CFU per spot. MICs were read after an 18-h incubation at 37°C. Antimicrobial agents were kindly provided as follows: amoxicillin, clavulanic acid, temocillin, and ticarcillin, Beecham Laboratories; ceftazidime and cephalothin, Glaxo Pharmaceuticals, Ltd.; carumonam, Hoffmann-La Roche Inc.; cefotetan and SM 7338, ICI Pharmaceuticals, Inc.; piperacillin, Lederle Laboratories; cefamandole, cephalexin, and moxalactam, Eli Lilly & Co.; cefoxitin and imipenem, Merck Sharp & Dohme-Chibret; sulbactam, Pfizer Inc.; cefotaxime, Hoechst-Roussel Pharmaceuticals Inc.; cefixime, Pharmuka SF.; cefetamet and ceftriaxone, Roche SA.; aztreonam and tigemonam, E. R. Squibb & Sons; tazobactam, Taiho Pharmaceuticals.

β-Lactamase assays. β-Lactamases were obtained from the clinical isolate or from transconjugants after ultracentrifugation of sonified cells. Analytical isoelectric focusing was performed by the method of Matthew et al. (22). Kinetic analyses of SHV-type β-lactamases were performed with soluble extracts from transconjugants and repeated at least once, with little variation. β-Lactamase activity was determined spectrophotometrically in sodium phosphate buffer (pH 7, 10 mM) at 30°C with a double-beam spectrophotometer (model 550S; The Perkin Elmer Corp.). One unit of β-lactamase activity was defined as the amount of enzyme hydrolyzing 1 μmol of nitrocefin per min at 30°C. The wavelengths of maximal absorption differences between hydrolyzed and nonhydrolyzed compounds were as previously described (10, 20, 25, 29). K_i values for inhibitors and monobactams with different β-lactamases were determined by using cephaloridine as the substrate. K_m and K_i values were calculated by computerized linear regression analysis of Woolf-Augustinsson-Hofstee plots (V versus V/S). The K_i values were calculated from initial linear hydrolysis rates within 20 s after addition of the enzyme to premixed sub-

TABLE 1. MICs of β-lactam antibiotics for strains harboring different plasmid-mediated SHV-type β-lactamases

Antibiotic	MIC (μg/ml) for:						
	K. pneumoniae 160(pAFF1)[a,b,c]	*K. pneumoniae* 2222[a]	*K. pneumoniae* 2222(pAFF2)[a,b]	*E. coli* BM694	*E. coli* BM694 (p453)[a]	*E. coli* BM694[d]	*E. coli* BM694 (pAFF2)[b]
Amoxicillin	>2,048	32	>2,048	2	>2,048	2,048	>2,048
Ticarcillin	>1,024	64	>1,024	4	>1,024	>1,024	>1,024
Piperacillin	256	16	128	1	512	128	128
Cephalexin	128	2	32	2	8	8	64
Cephalothin	512	4	256	4	16	256	256
Cefamandole	256	1	128	1	8	32	64
Cefotaxime	16	0.06	8	0.06	0.12	4	8
Ceftriaxone	8	0.03	8	0.03	0.06	4	8
Cefetamet	4	0.12	1	0.25	0.25	1	4
Cefixime	256	0.06	32	0.25	0.5	2	128
Ceftazidime	128	0.12	64	0.12	0.25	4	64
Aztreonam	256	0.12	64	0.06	0.12	4	128
Tigemonam	>128	0.25	128	0.5	1	4	>128
Carumonam	32	0.06	8	0.06	0.06	0.25	16
Cefoxitin	32	4	4	4	4	4	4
Moxalactam	1	0.12	0.25	0.06	0.125	0.25	0.25
Cefotetan	2	0.12	0.25	0.25	0.25	0.5	0.5
Imipenem	0.12	0.12	0.12	0.25	0.12	0.25	0.25
SM 7338	0.015	0.015	0.015	0.015	0.015	0.015	0.015
Temocillin	16	2	4	8	8	8	8

[a] Carries β-lactamase SHV-1.
[b] Carries β-lactamase SHV-5.
[c] Carries β-lactamase TEM-1.
[d] Carries an unnamed plasmid transferred from *K. pneumoniae* 2144 (provided by H. Knothe) which codes for β-lactamase SHV-2.

strate and inhibitor. The concentration required to inhibit 50% of the β-lactamase activity was measured after 10 min of preincubation by using 100 μM cephaloridine as the substrate and identical amounts of enzyme activities.

Outer membrane proteins. Outer membrane proteins were extracted from crude membranes containing 70 μg of protein by using 0.3% *N*-laurylsarcosine as previously described (9) and separated on a polyacrylamide gel (12%) containing sodium dodecyl sulfate.

Isolation of plasmids and hybridization of DNA. Plasmid DNA was extracted by the procedure of Kado and Liu (14). A fragment of ca. 1.4 kb containing the gene for SHV-3 (12; M. H. Nicolas, V. Jarlier, A. Philippon, and S. T. Cole, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 478, 1988) was used as a probe. DNA was labeled with [^{32}P]ATP by using the Multiprime system of Amersham, and hybridization was carried out under stringent conditions (19) after transfer to nitrocellulose sheets by the method of Southern (28). Restriction enzyme digestions of DNA, ligation, and transformation were performed as previously described (21).

RESULTS

Antibiotic susceptibilities. MICs of different β-lactam antibiotics for the clinical isolate *K. pneumoniae* 160 and for *K. pneumoniae* 2222 or *E. coli* BM694 transconjugants producing the novel β-lactamase SHV-5 or other plasmid-mediated SHV-type β-lactamases are presented in Table 1. All strains producing SHV-5 showed decreased susceptibility to amoxicillin, ticarcillin, piperacillin, all monobactams (aztreonam, carumonam, and tigemonam), and most cephalosporins tested. MICs of ceftazidime, aztreonam, and tigemonam were 8- to 16-fold higher than those of cefotaxime. In the transconjugants, no increase or only a moderate increase was observed in the MICs of the C7 α-methoxy cephalosporins (cefoxitin, moxalactam, and cefotetan) and of temocillin, which has a C6 α-methoxy substituent. The same was true for the MICs of imipenem and the new carbapenem SM 7338.

Compared with the *K. pneumoniae* transconjugant, the clinical isolate *K. pneumoniae* 160 displayed increased MICs of most of the β-lactam antibiotics tested, including those with a C6 or C7 α-methoxy substituent.

Characterization of the β-lactamase SHV-5. The clinical isolate and the transconjugants produced a β-lactamase with a pI of approximately 8.2 (Fig. 1). *K. pneumoniae* 160 also produced the chromosome-mediated β-lactamase SHV-1 and the TEM-1 β-lactamase. The substrate profile of SHV-5 was compared with that of SHV-1 and that of SHV-2, the

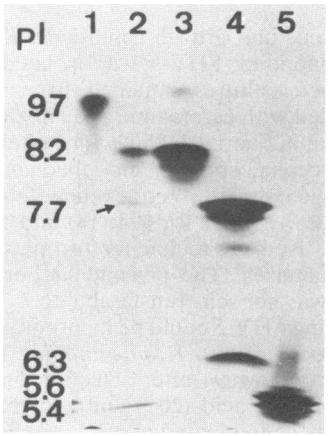

FIG. 1. Analytical isoelectric focusing of β-lactamases. Lanes: 1, cephalosporinase of *Enterobacter cloacae* HBR (pI 9.7); 2, SHV-5 (pI 8.2), SHV-1 (pI 7.7 [arrow]), and TEM-1 (pI 5.4) extracted from *K. pneumoniae* 160; 3, SHV-5 extracted from a transconjugant; 4, SHV-1 (pI 7.7) and TEM-3 (CTX-1; pI 6.3); 5, TEM-1 (pI 5.4) and TEM-2 (pI 5.6).

TABLE 2. Substrate profiles of SHV-1, SHV-2, and SHV-5 enzymes

β-Lactamase[a]	Rate of hydrolysis[b]										
	AMP	MEZ	PIP	CER	CTN	CTX	CTZ	AZT	CRU	TIG	OXA
SHV-1	235	210	84	52	8	<0.5	<0.5	<0.5	<0.5	<0.5	<0.5
SHV-2	184	252	79	118	49	17	1	1	1	>0.5	1
SHV-5	290	381	134	143	43	25	11	1	13	5	10

[a] Specific activities: SHV-1, 3,750 mU/mg; SHV-2, 474 mU/mg; SHV-5, 406 mU/mg; in each case, 35 mU was used.
[b] Rate of hydrolysis at 100 μM relative to that of penicillin G. Abbreviations: AMP, ampicillin; MEZ, mezlocillin; PIP, piperacillin; CER, cephaloridine; CTN, cephalothin; CTX, cefotaxime; CTZ, ceftazidime; AZT, aztreonam; CRU, carunoman; TIG, tigemonam; OXA, oxacillin.

first described extended-spectrum β-lactamase (16). In contrast to SHV-1, SHV-5 and SHV-2 showed a substantial hydrolysis rate (Table 2) for cefotaxime. SHV-5 showed higher hydrolysis rates than SHV-2 for ceftazidime, carumonam, tigemonam, and oxacillin. In the presence of oxacillin or clavulanic acid (100 μM), the activity of SHV-5 was inhibited by 65 and 100%, respectively. The K_m values of the different antibiotics (Table 3) for SHV-5 and SHV-2 were lower than those for SHV-1. The hydrolytic efficiency of SHV-5 for ceftazidime was higher than that of SHV-2. The K_i values of the different monobactams for SHV-5 were 10- to 100-fold lower than for SHV-2 and more than 1,000-fold lower than for SHV-1.

Analysis of outer membrane proteins. Since no hydrolysis of cefoxitin, moxalactam, cefotetan, or temocillin was obtained with SHV-5 (data not shown) and since the transconjugants producing SHV-5 caused no increase in the MICs of these antibiotics, we thought that a decreased outer membrane permeability (9, 24) could be the cause of the resistance of these antibiotics in *K. pneumoniae* 160. Examination of the outer membrane proteins (Fig. 2) showed that, compared with the susceptible *K. pneumoniae* 2222 strain, there was a disappearance of the ca. 41-kilodalton (kDa) outer membrane protein. A decrease in quantity of about 50% (determined by scanning of the electropherogram) of the ca. 40-kDa outer membrane protein was also observed, while the amount of the OmpA protein (36 kDa) (9, 24) remained unchanged. The 41- and the 40-kDa outer membrane proteins are the putative porins of *K. pneumoniae* (9, 24).

Synergy with β-lactamase inhibitors. Instead of evaluating the decrease in MICs of different β-lactam antibiotics at fixed inhibitor concentrations, we chose to evaluate the concentration of inhibitor necessary to decrease the MICs of the different hydrolyzed β-lactam antibiotics to arbitrary MICs corresponding to levels easily achieved in serum. The concentrations of clavulanic acid needed to restore the arbitrary MICs of the different antibiotics were always lower than those of sulbactam or tazobactam (Table 4). In combination with amoxicillin, higher concentrations of the different inhibitors were necessary to restore the arbitrary MICs for *E. coli* BM694 producing plasmid-mediated β-lactamase SHV-1 than for *K. pneumoniae* 2222 producing the same, but chromosome-encoded, enzyme. This is probably due to the higher quantity of β-lactamase (>50-fold) which is produced when it is plasmid-mediated. Interestingly, higher quantities of the inhibitors were necessary to restore the arbitrary MICs of the different β-lactam antibiotics for *K. pneumoniae* 160 than for the transconjugants producing SHV-5. Although additional small quantities of TEM-1 were present (Fig. 2), this circumstance alone does not explain the requirement of higher quantities of inhibitor for the restoration of the arbitrary MICs, in particular those of cefotaxime, ceftazidime, and aztreonam, which are not hydrolyzed by TEM-1. In the reverse experiment, with 4 μg of inhibitor per ml, susceptibility was completely restored for transconjugants containing SHV-5 but was only partially restored for *K. pneumoniae* 160. For example, in the presence of 4 μg of clavulanic acid, sulbactam, or tazobactam per ml, the MICs of ceftazidime decreased to 0.12, 0.5, and 0.25 μg/ml, respectively, for *K. pneumoniae* 2222 producing SHV-5 and to 0.5, 8, and 2 μg/ml, respectively, for *K. pneumoniae* 160. K_i values of tazobactam for SHV-5 were slightly lower than those of sulbactam and clavulanic acid (Table 5). In contrast, the I_{50} concentration of clavulanic acid for SHV-5 was 10- to 100-fold lower than those of tazobactam and sulbactam (Table 5). This would explain the lower quantities of clavulanic acid needed to restore the

TABLE 3. Kinetics of hydrolysis of β-lactam antibiotics by different plasmid-mediated β-lactamases

Antibiotic	Kinetics of hydrolysis by:					
	SHV-1		SHV-2		SHV-5	
	K_m (μM)	V_{max}/K_m	K_m (μM)	V_{max}/K_m	K_m (μM)	V_{max}/K_m
Ampicillin	42	100	13	100	11	100
Mezlocillin	45	73	8	163	7	116
Cephaloridine	201	4	30	28	31	11
Cephalothin	90	1	5	53	3	51
Ceftazidime	–[a]		72	1	23	4
Cefotaxime	–[a]		4	10	7	7
Aztreonam	>1,000[b]		3[b]		0.02[b]	
Carumonam	400[b]		207[b]		22[b]	
Tigemonam	71[b]		0.15[b]		0.008[b]	

[a] Poor affinity.
[b] Only K_i were calculated.

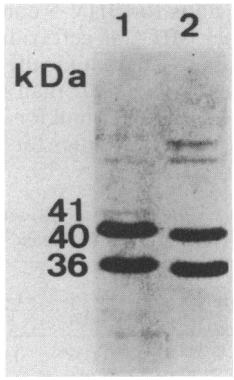

FIG. 2. Outer membrane proteins of *Klebsiella* species. Lanes: 1, *K. pneumoniae* 2222; 2, *K. pneumoniae* 160. Molecular masses are indicated at left.

TABLE 4. Quantities of inhibitor necessary to decrease the β-lactam susceptibilities of different strains harboring plasmid-mediated β-lactamases to a chosen MIC[a]

Strain	β-Lactamase(s)	AMX MIC (μg/ml)	Concn (μg/ml) of β-lactamase inhibitor needed to achieve an AMX MIC of 8 μg/ml			PIP MIC (μg/ml)	Concn (μg/ml) of β-lactamase inhibitor needed to achieve a PIP MIC of 16 μg/ml		
			CLA	SUL	TAZ		CLA	SUL	TAZ
K. pneumoniae 160	SHV-1[b], TEM-1, SHV-5[c]	>2,048	8	64	>512	256	0.25	8	8
K. pneumoniae 2222	SHV-1[b]	32	<0.015	0.5	0.06	16	<0.015	<0.015	<0.015
K. pneumoniae 2222	SHV-1[b], SHV-5[c]	>2,048	0.25	8	4	128	<0.015	2	1
E. coli BM694		2	<0.015	<0.015	<0.015	1	<0.015	<0.015	<0.015
E. coli BM694	SHV-1[c]	>2,048	4	512	8	512	0.5	256	64
E. coli BM694	SHV-2[c]	2,048	0.5	4	2	128	0.015	4	1
E. coli BM694	SHV-5[c]	>2,048	0.25	4	2	128	0.015	0.25	0.12

[a] Chosen MICs were arbitrarily chosen on the basis of levels easily achieved in serum. Abbreviations: AMX, amoxicillin; PIP, piperacillin; AZT, aztreonam; CTZ, ceftazidime; CTX, cefotaxime; CLA, clavulanic acid; SUL, sulbactam; TAZ, tazobactam.
[b] Chromosome-mediated β-lactamase.
[c] Plasmid-mediated β-lactamase.

arbitrary MICs of the different antibiotics. Similar results were obtained with SHV-2, although the I_{50} concentration of clavulanic acid was slightly higher than that observed with SHV-5 (Table 5).

Identification of the plasmid encoding SHV-5. A plasmid of ca. 150 kb (pAFF-1) was shown to harbor the SHV-5 β-lactamase gene by hybridization with a probe containing the SHV-3 gene (Fig. 3). A 3.6-kb *Bam*HI fragment from pAFF2 was cloned into pACYC184 (pAFF3). pAFF3 expressed the SHV-5 enzyme (data not shown) and also hybridized with the SHV-3 probe (Fig. 3). No hybridization with these plasmids with an intragenic probe of the TEM gene (15) was observed (data not shown).

DISCUSSION

SHV-5 is a novel broad-spectrum β-lactamase which has a pI of 8.2 and was, like many other broad-spectrum β-lactamases (12, 15, 17, 23, 26), discovered in *K. pneumoniae*. Similarly to SHV-2 and many of the other new broad-spectrum β-lactamases described to date, it increases resistance to aminopenicillins, ureidopenicillins, and many broad-spectrum cephalosporins but does not affect resistance to β-lactam antibiotics with a C6 or C7 α-methoxy substituent. SHV-5 is unusual because it causes higher resistance to ceftazidime and, in particular, to monobactams than do other broad-spectrum β-lactamases. This is related to the higher rate of hydrolysis, the higher efficiency of hydrolysis (V_{max}/K_m) and, concomitantly, the lower K_is of the different compounds for SHV-5 compared with SHV-2. SHV-5 caused higher MICs of ceftazidime than of cefotaxime, whereas (apparently paradoxically) the hydrolytic efficiency for cefotaxime was higher than that for ceftazidime. This is similar to the situation found for TEM-7 (6) and might be due to differences in the affinities for some essential PBPs (8) or rates of diffusion across the outer membrane (31) or both, minimizing the hydrolytic effect of SHV-5 on cefotaxime.

A peculiarity of the clinical isolate *K. pneumoniae* 160 is its decreased susceptibility to compounds containing an α-methoxy substituent at C6 or C7. This might be explained by a defect in some of its outer membrane proteins which are probably porins (9, 24). Reduced outer membrane permeability of *K. pneumoniae* 160 could also explain the higher MICs of other broad-spectrum cephalosporins compared with those for the transconjugant of *K. pneumoniae* 2222. As was already described for other members of the family *Enterobacteriaceae* with altered membrane permeability caused by reduced amounts of porins (5), imipenem was not affected. Similarly, the susceptibility to SM 7338 remained unaltered.

The susceptibility to antibiotics hydrolyzable by β-lactamases was restored in transconjugants of SHV-5 with lower concentrations of inhibitors than in the clinical isolate *K. pneumoniae* 160. In the latter case, the presence of TEM-1 could necessitate higher concentrations of inhibitors, in particular sulbactam, which is less active than clavulanic

TABLE 5. K_i and I_{50} values obtained with different plasmid-mediated β-lactamases

β-Lactamase	K_i (μM) with[a]:			I_{50} (μM) with:		
	CLA	SUL	TAZ	CLA	SUL	TAZ
SHV-1	0.19	1.7	0.057	0.057	7.5	0.15
SHV-2	0.12	0.19	0.06	0.018	0.39	0.063
SHV-5	0.10	0.18	0.036	0.0045	0.40	0.022

[a] CLA, Clavulanic acid; SUL, sulbactam; TAZ, tazobactam.

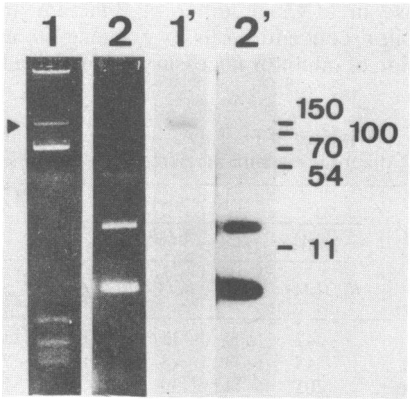

FIG. 3. Hybridization of plasmid pAFF1 coding for SHV-5 and of a cloned 3.8-kb fragment of pAFF2 in pACYC184 (pAFF3 [ca. 8 kb]) with a probe containing the SHV-3 gene. Lanes (indicating plasmids in *K. pneumoniae* 160): 1, pAFF1 (▶); 2, pAFF3 (open and covalently closed circular forms); 1' and 2', corresponding hybridizations. Plasmids used as molecular weight standards (indicated at right) were pIP55 (150 kb), pIP112 (100 kb), pIP135 (70 kb), RP4 (54 kb), and ColE1 (11 kb).

TABLE 4—*Continued*

AZT MIC (μg/ml)	Concn (μg/ml) of β-lactamase inhibitor needed to achieve an AZT MIC of 1 μg/ml			CTZ MIC (μg/ml)	Concn (μg/ml) of β-lactamase inhibitor needed to achieve a CTZ MIC of 1 μg/ml			CTX MIC (μg/ml)	Concn (μg/ml) of β-lactamase inhibitor needed to achieve a CTX MIC of 1 μg/ml		
	CLA	SUL	TAZ		CLA	SUL	TAZ		CLA	SUL	TAZ
256	0.5	8	8	128	1	16	8	16	0.06	8	2
0.12	<0.015	<0.015	<0.015	0.12	<0.015	<0.015	<0.015	0.06	<0.015	<0.015	<0.015
64	0.03	2	1	64	0.12	2	2	8	0.03	2	1
0.06	<0.015	<0.015	<0.015	0.12	<0.015	<0.015	<0.015	0.06	<0.015	<0.015	<0.015
0.12	<0.015	<0.015	<0.015	0.5	<0.015	<0.015	<0.015	0.12	<0.015	<0.015	<0.015
4	0.03	4	2	8	0.5	8	2	4	<0.015	4	2
128	0.06	2	1	64	0.25	4	1	8	0.03	2	2

acid or tazobactam against TEM-1 when combined with amoxicillin (7, 11). However, higher quantities of inhibitors were also necessary to decrease the MICs of aztreonam, ceftazidime, and cefotaxime, which are not susceptible to the TEM enzyme. Since very similar quantities of SHV-5 enzyme were produced by *K. pneumoniae* 160 and the transconjugant, as demonstrated by similar rates of hydrolysis of cefotaxime (data not shown), this suggests that a decrease in permeability might also interfere with inhibitor efficacy. As demonstrated previously for SHV-2 (15), the lower K_i and I_{50} values of the different inhibitors, in particular sulbactam, for SHV-5 compared with SHV-1 could explain the lower quantities of inhibitors needed to restore the arbitrary MICs for the transconjugants producing these plasmid-mediated β-lactamases.

The substrate profile, with hydrolysis of ampicillin higher than that of penicillin and hydrolysis of cefotaxime lower than those reported for some TEM derivatives, suggests that SHV-5 is an SHV derivative (3, 12, 15). A strong argument in favor of this hypothesis is the hybridization between the 150-kb plasmid, as well as that of a subcloned 3.6-kb fragment expressing the SHV-5 β-lactamase, with a probe of a gene expressing a distinct SHV-related enzyme. Furthermore, recent partial nucleotide sequence analysis has revealed complete homology of the deduced amino acid sequence of SHV-5 with stretches of SHV-1 (1) (data not shown). Thus, this novel β-lactamase was denominated SHV-5 since it appears to be the fifth SHV-type enzyme (3, 12, 16). If nucleotide sequencing of the SHV-5 gene confirms this hypothesis, one could expect one or several amino acid substitutions in positions similar to those found in other broad-spectrum β-lactamases (1, 6, 27), i.e., in the proximity of the boxes which are evolutionarily conserved in this family of enzymes (13).

On a therapeutic level, combinations of β-lactam antibiotics with β-lactamase inhibitors should theoretically be as useful against SHV-5-producing strains as against strains producing TEM-type or other SHV-type broad-spectrum β-lactamases. However, β-lactamase inhibitors and β-lactam antibiotics containing a C6 or C7 α-methoxy substituent, such as cefoxitin or moxalactam, which are not normally hydrolyzed by these enzymes, should be given with caution because of the possibility that some β-lactamase-producing strains may also have a decreased membrane permeability which could affect the efficacy of these compounds. Alternatively, although the I_{50}s of the inhibitors for SHV-5 were lower than those for SHV-1, the efficacy of the inhibitors might also be reduced against strains overproducing SHV-5.

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