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Biochemical Study of a New Inhibitor-Resistant β-Lactamase, SHV-84, Produced by a Clinical *Escherichia coli* Strain▽

Inhibitor-resistant TEM (IRT) β-lactamases derive from TEM-1 or TEM-2 enzymes by point mutations in the corresponding coding gene (http://www.lahey.org/studies). Inhibitor-resistant SHV (IRS) β-lactamases are less commonly identified than IRT enzymes, and mainly in *Klebsiella pneumoniae* strains (3, 4, 5, 8, 9). In this study, we performed a phenotypic, molecular, and biochemical characterization of a new IRS enzyme, SHV-84, produced by a clinical *Escherichia coli* strain.

E. coli INSRA4590 was isolated from a patient admitted in 1999 to Centro Hospitalar de Coimbra, Portugal. Antimicrobial susceptibility, isoelectric focusing for isoelectric point determination, identification of β-lactamase-encoding genes, and extraction and purification of a new β-lactamase were performed as previously described (1, 7). Antimicrobial susceptibility results were interpreted by using French Society of Microbiology criteria (2). *E. coli* INSRA4590 exhibited high-level resistance to the penicillins tested (except piperacillin) and to the combination of amoxicillin and clavulanic acid but was susceptible to the combination of piperacillin and tazobactam (Table 1); it was susceptible to all cephalosporins tested and to aztreonam.

The clinical strain expressed SHV-1 with a pI of 7.6 and the new SHV-84 β-lactamase with a pI of 7.4, which differed from SHV-1 by the amino acid substitution Lys234Arg. This mutation has been described in other IRS enzymes, such as SHV-56 (5) and SHV-72 (also encountered in Portugal) (8), in which it was associated with the substitution Leu35Gln and the substitutions Ile8Phe and Ala146Val, respectively. The recombinant SHV-encoding plasmid (pBK-SHV-84) and the corresponding transformant *E. coli* DH5α were obtained as previously described (8). The SHV-84-producing transformant DH5α-SHV-84 exhibited a β-lactam resistance phenotype similar to that of the clinical strain (Table 1), and the corresponding β-lactamase had a pI of 7.4.

The kinetic constants of the purified enzyme (≥99% pure; data not shown) and the concentrations of inhibitors required to inhibit enzyme activity by 50% (IC_{50}s) were determined as previously reported (6, 8) and compared with those of SHV-1. Unlike SHV-72, SHV-84 showed a lower affinity for penicillins (K_m, 64 to 101 μM) and decreased catalytic activity against these antibiotics (k_{cat}, 216 to 1,042 s^{-1}) than SHV-1 (K_m, 11 to 31 μM; k_{cat}, 220 to 1,937 s^{-1}) (Table 1). In SHV-72, mutations other than Lys234Arg might confer increased affinity of the enzyme for penicillins, as well as better catalytic activity against those antibiotics. SHV-84, like SHV-72, exhibited lower affinity for cephalothin (K_m, 169 to 141 μM) than SHV-1. No hydrolysis of extended-spectrum cephalosporins was detected. However, SHV-84 was less susceptible to clavulanic acid (IC_{50}, 2.21 μM) than SHV-1 (IC_{50}, 0.17 μM), as observed for SHV-72 (IC_{50}, 1.72 μM) (Table 1) (8). The IC_{50} of tazobactam for SHV-84 was 3.5-fold lower than that for SHV-1 (IC_{50}s, 0.03 and 0.15 μM, respectively).

In conclusion, this study underlines the importance of the Lys234Arg substitution in resistance to clavulanic acid in nature, since we demonstrate that this mutation alone is responsible for decreased susceptibility to β-lactamase inhibitors. These results corroborate those previously obtained by molecular dynamic simulation in a study using the model of the mutant SHV-72 enzyme, in which the authors suggested a change in the positioning of the Ser130 side chain induced by Arg234 (8).

Nucleotide sequence accession number. The new bla_{SHV} nucleotide sequence was submitted to the EMBL Nucleotide

TABLE 1. MICs for clinical, transformant, and recipient *E. coli* strains and kinetic parameters of SHV-84 and SHV-1 β-lactamases

Antimicrobial(s)	MIC (μg/ml) for *E. coli* strain:				Kinetic parameters of enzyme[a]					
					SHV-1[c]			SHV-84		
	DH5α	DH5α-SHV-1 (pBK-SHV-1)[b]	DH5α-SHV-84(pBK-SHV-84)[b]	INSRA4590 (SHV-84)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)	K_m (μM)	k_{cat} (s^{-1})	k_{cat}/K_m ($\mu M^{-1} \cdot s^{-1}$)
Penicillin G	ND[f]	ND	ND	ND	23 ± 0.42	1,937 ± 82	84.2 ± 2.0	64 ± 0.81	611 ± 43	9.5 ± 0.8
Amoxicillin	≤2	2,048	2,048	512	31 ± 1.29	1,044 ± 10	33.7 ± 1.1	101 ± 2.75	1,042 ± 116	10.4 ± 1.4
Amoxicillin + CLA[d]	≤2	8	>64	64	ND	ND	ND	ND	ND	ND
Ticarcillin	≤2	1,024	>4,096	>4,096	11 ± 3.40	220 ± 49	20.0 ± 1.7	83 ± 5.97	216 ± 20	2.5 ± 0.01
Piperacillin	1	64	128	64	24 ± 0.53	1,490 ± 96	62.1 ± 2.7	68 ± 2.90	406 ± 15	6.0 ± 0.03
Piperacillin + TAZ[e]	1	2	2	4	ND	ND	ND	ND	ND	ND
Cephalothin	1	16	8	8	40 ± 1.46	128 ± 33	3.2 ± 0.8	169 ± 0.31	4 ± 0.3	0.02 ± 0.002
Ceftazidime	0.06	0.5	0.25	0.25	142 ± 3.18	<0.1	NH[g]	NH	<0.1	NH
Cefotaxime	≤0.015	0.06	0.06	0.125	257 ± 20.65	<0.1	NH	NH	<0.1	NH
Aztreonam	≤0.015	0.125	0.03	0.03	ND	ND	ND	ND	ND	ND

[a] Values are means ± standard deviations.
[b] *E. coli* DH5α-SHV-1 and *E. coli* DH5α-SHV-84 were the transformants producing SHV-1 and SHV-84, respectively. The MICs for SHV-1 are from reference 8.
[c] The kinetic constants of SHV-1 are from reference 8.
[d] CLA, clavulanic acid at a fixed concentration of 2 μg/ml.
[e] TAZ, tazobactam at a fixed concentration of 4 μg/ml.
[f] ND, not determined.
[g] NH, not determinable because the hydrolysis rates were too low.

Sequence Database as bla_{SHV-84} with accession number AM087453.

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Vera Manageiro
Eugénia Ferreira*
National Reference Laboratory of Antimicrobial Resistance
Department of Infectious Diseases
National Institute of Health Dr. Ricardo Jorge
Av. Padre Cruz
1649-016 Lisbon, Portugal

Luís Albuquerque
Laboratory of Microbiology
Centro Hospitalar de Coimbra, EPE
Coimbra, Portugal

Richard Bonnet
Laboratoire de Bactériologie
UFR Médecine
University Clermont, EA3844
Clermont-Ferrand F-63001, France

Manuela Caniça*
National Reference Laboratory of Antimicrobial Resistance
Department of Infectious Diseases
National Institute of Health Dr. Ricardo Jorge
Av. Padre Cruz
1649-016 Lisbon, Portugal

*Phone and fax: 351 217519246
E-mail: manuela.canica@insa.min-saude.pt

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