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Survey and Molecular Genetics of SHV β-Lactamases in *Enterobacteriaceae* in Switzerland: Two Novel Enzymes, SHV-11 and SHV-12

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Sixty isolates of *Enterobacteriaceae* resistant to β-lactam antibiotics were collected over a period of 2 years in Switzerland and screened by hybridization for the carriage of SHV genes. Thirty-four positive strains were found, and their SHV genes were amplified and sequenced. SHV extended-spectrum β-lactamases (ESBLs) were found: 13 strains contained SHV-2a, 12 harbored SHV-2, and SHV-5 was found twice. Four strains were shown to contain SHV-1. In addition, we report two new SHV variants, termed SHV-11 (non-ESBL) and SHV-12 (ESBL). In spite of the carriage of SHV ESBLs, many strains showed only low resistance to one or more third-generation cephalosporins. In addition, 26 did not transfer the *bla*$_{SHV}$ gene in mating experiments.

Extended-spectrum β-lactamases (ESBLs), such as the plasmid-mediated class A TEM and SHV type enzymes, have developed by stepwise mutations in their structural genes, resulting in either single or multiple amino acid changes in the encoded enzymes. These enzymes are often the cause for resistance to newer cephalosporins and monobactams in members of the family *Enterobacteriaceae* (for reviews see references 13, 24, 43, and 55). Since their discovery in Germany in 1983 (30) SHV ESBL-producing isolates have been found in various countries in Europe, such as Austria (48), France (40), Italy (33), and Greece (58), as well as in the United States (10, 26) and Australia (37). SHV ESBLs have been termed SHV-2 through SHV-7, including an SHV-2 variant, SHV-2a (46). Very recently, a new enzyme was described, termed SHV-9 (47). Until now, no significant information on the prevalence of ESBL-producing strains in Switzerland has been available. A survey of SHV ESBL-producing clinical isolates from major hospitals in Switzerland was carried out between July 1993 and July 1995 in order to (i) assess the prevalences of various types of SHV derivatives, (ii) detect the possible existence of new SHV enzymes, and (iii) investigate the SHV-producing isolates for phenotypical and genotypical characteristics.

MATERIALS AND METHODS

Bacterial strains. Clinical isolates were collected between 1993 and 1995 from clinical microbiology laboratories in Aarau, Basel, Bern, Geneva, La Chaux-de-Fonds, Lausanne, Lugano, Lucerne, Sion, St. Gallen, and Zürich, and, in one case, from a general practitioner's surgery. All strains were initially isolated and identified in the respective microbiology laboratories, and isolates with reduced susceptibility or resistance to oxyimino cephalosporins were forwarded to our laboratory for further investigation. The exact criteria were as follows: strains belonging to the genera *Escherichia*, *Klebsiella*, and *Salmonella* were selected if they produced inhibition zone diameters of <21 mm around discs of cefotaxime (30 μg), <19 mm around ceftriaxone (30 μg), or <16 mm around ceftazidime (30 μg) when tested according to the methods of the National Committee for Clinical Laboratory Standards (NCCLS) (39). All incoming strains were systematically screened for the presence of a *bla*$_{SHV}$ gene by hybridization with an intragenic gene probe (see DNA techniques).

Escherichia coli HK225 (*E. coli* K-12 [29] resistant to rifampin and streptomycin [Rif^r Str^r]) was used as a receptor in conjugation experiments.

Antibiotics. Ampicillin, amoxicillin, and clavulanic acid were supplied by SmithKline Beecham Pharmaceuticals, Surrey, England; cefepime was from Bristol-Myers Squibb, New York, N.Y.; cefotaxime was provided by Roussel, Paris, France; cephalothin was from Eli Lilly, Indianapolis, Ind.; ceftazidime, cefuroxime, and nitrocefin were from Glaxo, Greenford, England; ceftriaxone was obtained from Hoffmann-La Roche, Basel, Switzerland; imipenem was obtained from Merck Sharpe & Dohme, Glattbrugg, Switzerland; rifampin was supplied by Ciba-GEIGY, Basel, Switzerland.

Susceptibility testing. MICs were determined by the microdilution method according to the guidelines of the NCCLS (38).

The condition used for testing the β-lactam-β-lactamase inhibitor combination, amoxicillin-clavulanic acid (Augmentin), was a 2-to-1 ratio of amoxicillin to clavulanic acid.

DNA techniques. Plasmid DNA was prepared from transconjugant *E. coli* strains with a plasmid kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total DNA was extracted following standard protocols (52). Restriction enzymes and buffers were purchased from Boehringer Mannheim, Rotkreuz, Switzerland, and total DNA or plasmid DNA was digested according to the manufacturer's guidelines. DNA was electrophoresed on 0.7% agarose gels and visualized with ethidium bromide under UV light.

DNA was blotted onto nylon membranes (GeneScreen *Plus*; New England Nuclear Research Products, Boston, Mass.) after agarose gel electrophoresis, according to the method of Southern (56). DNA hybridization was performed under high-stringency conditions. For the screening of strains for the presence of an SHV gene, an intragenic 0.55-kb *Nhe*I-*Not*I probe for the β-lactamase gene of SHV-2a was digoxigenin labeled by using digoxigenin-11-dUTP and a random-primed labeling kit (Boehringer). Hybridization experiments with plasmid or total DNA of transconjugants were performed with a 1,018-bp PCR amplimer of an SHV-2a gene, into which digoxigenin-11-dUTP was incorporated during amplification. Chemiluminescent detection was performed with CDP-*Star* reagent according to the manufacturer's instructions (Boehringer). Ribotyping was performed by hybridizing *Bst*EII-digested total DNA with linear plasmid pKK3535 (11), which was labeled with [α-^{32}P]dCTP (Amersham, Little Chalfont, England) and a random-primed labeling kit (Boehringer).

bla$_{SHV}$ genes were amplified by PCR as described previously (42). The PCR conditions employed were as follows: initial denaturation at 94°C for 5 min, then 30 cycles consisting of 95°C for 30 s, 68°C for 30 s, and 72°C for 50 s each.

DNA sequencing of the PCR products was performed on both strands with a Sequenase kit (United States Biochemical, Cleveland, Ohio) with [α-^{35}S]dATP label (Amersham) according to the manufacturer's instructions, except for annealing, which was performed by heating the samples to 94°C for 3 min, with subsequent cooling on dry ice.

Mating experiments. Conjugational transfer of β-lactam resistance was performed by a modified filter mating method (53), with clinical isolates as donors and *E. coli* HK225 as the recipient. After conjugation, bacterial suspensions were plated onto Luria-Bertani agar plates containing 50 μg of ampicillin and 200 μg of rifampin per ml. The resulting transconjugants were purified and identified with API 20E strips (bioMerieux, Marcy l'Etoile, France).

Nucleotide sequence accession numbers. Sequences containing new or silent point mutations are filed in the EmblBank database under the accession numbers X98098 (KPAA-1), X98099 (KPZU-13), X98100 (KPZU-8), X98101 (KPZU-12), X98102 (KPZU-10), X98103 (KPGE-2), X98104 (KPLA-4), and X98105 (KPLA-1).

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TABLE 1. Properties of bacterial strains[a]

Strain[b]	Species or transconjugant	SHV	Ribotype[c]	MIC (μg/ml) of:							
				CF	COX	CAZ	CTX	CRO	PM	IPM	AUG[d]
KPAA-1	*K. pneumoniae*	SHV-1		2	1	0.125	0.03	0.015	0.03	0.06	1
KPBE-2	*K. pneumoniae*	SHV-2		≥256	8	1	4	8	0.5	0.06	4
KPGE-1	*K. pneumoniae*	SHV-2	A	≥256	16	64	32	32	0.5	0.125	16
KPGE-2	*K. pneumoniae*	SHV-5	A	≥256	16	64	16	32	0.5	0.125	16
KPLA-1	*K. pneumoniae*	SHV-12		≥256	128	≥256	128	256	32	0.25	16
KPLA-2	*K. pneumoniae*	SHV-2		≥256	64	8	32	32	4	0.125	8
KPLA-3	*K. pneumoniae*	SHV-2a		256	16	4	16	16	0.5	0.125	8
KPLA-4	*K. pneumoniae*	SHV-5		≥256	64	128	32	64	2	0.125	4
KPLA-5	*K. pneumoniae*	SHV-2a	B	≥256	8	1	16	32	1	0.5	32
KPLA-6	*K. pneumoniae*	SHV-2a	B	≥256	8	1	8	8	0.25	0.25	16
KPLA-7	*K. pneumoniae*	SHV-2a		≥256	64	8	32	32	4	0.25	16
KPLA-8	*K. pneumoniae*	SHV-2a	C	≥256	64	16	128	16	0.5	0.25	16
KPLA-9	*K. pneumoniae*	SHV-2a		≥256	64	16	128	64	16	0.5	16
KPLA-10	*K. pneumoniae*	SHV-2a	C	≥256	32	1	8	8	4	0.5	32
KPZU-1	*K. pneumoniae*	SHV-2		≥256	256	64	256	256	256	0.06	16
KPZU-4	*K. pneumoniae*	SHV-2a		≥256	256	32	128	128	256	0.25	16
KPZU-6	*K. pneumoniae*	SHV-2a		≥256	256	64	128	256	16	0.25	16
KPZU-7	*K. pneumoniae*	SHV-2	D	≥256	8	2	8	8	1	0.125	4
KPZU-8	*K. pneumoniae*	SHV-1	D	32	2	0.5	0.03	0.03	0.125	0.06	32
KPZU-9	*K. pneumoniae*	SHV-1	D	32	1	0.5	0.03	0.06	0.06	0.06	8
KPZU-10	*K. pneumoniae*	SHV-2a	D	≥256	32	8	32	32	4	0.125	8
KPZU-11	*K. pneumoniae*	SHV-2	D	≥256	256	64	128	256	128	0.25	16
KPZU-12	*K. pneumoniae*	SHV-11		2	2	0.125	0.025	0.008	0.008	0.5	1
KPZU-13	*K. pneumoniae*	SHV-1		≥256	16	128	8	16	0.5	0.25	4
ECAA-1	*E. coli*	SHV-2		256	≥256	64	256	128	32	0.125	32
ECLA-1	*E. coli*	SHV-2		≥256	≥256	1	≥256	≥256	128	0.25	8
ECLA-2	*E. coli*	SHV-2		64	2	0.125	1	2	0.06	0.125	2
ECLA-4	*E. coli*	SHV-2a		256	4	0.125	8	16	0.25	0.25	8
ECZP-1	*E. coli*	SHV-12		≥256	32	64	32	32	2	0.06	8
ECZK-1	*E. coli*	SHV-2		≥256	≥256	32	≥256	≥256	128	0.06	8
ECZU-1	*E. coli*	SHV-2		≥256	8	2	0.5	0.5	0.015	0.125	32
ENLA-1	*E. cloacae*	SHV-2		≥256	128	1	16	16	1	0.5	64
SWLA-1	*S. wien*[e]	SHV-2a	E	≥256	64	8	64	64	8	0.06	16
SWLA-2	*S. wien*	SHV-2a	E	≥256	128	32	128	128	64	0.125	16
KL-1	Conj. KPLA-1	SHV-12		≥256	128	64	128	128	8	0.03	4
KL-2	Conj. KPLA-2	SHV-2		≥256	64	4	64	64	8	0.03	4
KL-7	Conj. KPLA-7	SHV-2a		≥256	64	8	16	16	4	0.03	8
KZ-6	Conj. KPZU-6	SHV-2a		≥256	≥256	32	256	≥256	128	0.125	4
KZ-7	Conj. KPZU-7	No SHV		256	64	2	4	8	1	0.015	4
KZ-10	Conj. KPZU-10	No SHV		64	2	0.25	0.03	0.06	0.03	0.03	4
EL-1	Conj. ECLA-1	No SHV		≥256	≥256	1	32	64	2	0.06	8
EL-4	Conj. ECLA-4	SHV-2a		≥256	128	16	128	128	64	0.03	8
EP-1	Conj. ECZP-1	SHV-12		≥256	64	32	32	16	1	0.06	4
EK-1	Conj. ECZK-1	SHV-2		≥256	≥256	64	≥256	≥256	256	0.06	8
EN-1	Conj. ENLA-1	SHV-2		≥256	128	16	64	64	16	0.03	8
HK225[f]	*E. coli*			16		0.5	0.5	0.25	ND	0.25	4

[a] Abbreviations: CF, cephalothin, CAZ, ceftazidime; COX, cefuroxime; CRO, ceftriaxone; CTX, cefotaxime; PM, cefepime; IPM, imipenem; AUG, amoxicillin-clavulanic acid combination; Conj., transconjugant; ND, not determined.

[b] In strain designations, the last two letters of a four-letter designation indicate the origin as follows: AA stands for Aarau, BE for Bern, GE for Geneva, LA for Lausanne, ZU for Zürich, ZP for general practitioner in Zürich, and ZK for the Children's Hospital of Zürich.

[c] Strains with no classification belong to individual ribotypes. Transconjugants were not ribotyped.

[d] The amoxicillin-clavulanic acid combination was a 2:1 ratio of amoxicillin to clavulanic acid.

[e] *S. wien*, *S. enterica* serotype *wien.*

[f] *E. coli* recipient used for transfer experiments.

RESULTS

Detection of SHV ESBL-producing clinical isolates. Of 60 strains with reduced susceptibility to expanded-spectrum cephalosporins which had been collected, 34 (24 *Klebsiella pneumoniae*, 7 *Escherichia coli*, 1 *Enterobacter cloacae*, and 2 *Salmonella enterica* serotype *wien*) hybridized with the intragenic bla_{SHV} probe (data not shown). The bla_{SHV} genes were amplified by PCR, and the presence of $bla_{SHV\text{-}ESBL}$ was established in 29 strains by restriction enzyme digests of the resulting 1,018-bp amplimers as described elsewhere (42). These results were confirmed by the nucleotide sequencing of all 34 amplimers (Table 1). Five strains contained SHV non-ESBL enzymes.

Nucleotide sequences. The sequencing of the 34 amplimers revealed that 13 strains contained SHV-2a, 12 strains contained SHV-2, 2 isolates harbored SHV-5, and 4 strains carried SHV-1, a non-extended-spectrum SHV. In addition, two new SHV variants, termed SHV-11 and SHV-12, were detected in one and two isolates, respectively (see Table 2). These variants

TABLE 2. Silent point mutations in the open reading frames of amplified SHV genes compared to known sequences

Species or strain (reference)	Enzyme	Codon for amino acid[a]:						
		His-112	Leu-137	Leu-138	Arg-215	Arg-222	Glu-240/Lys-240	Gly-245
K. pneumoniae (3)	LEN-1	CA**C**	CT**G**	CT**G**	CG**G**	CG**C**	GA**A**	GG**C**
K. pneumoniae (35)[b]	SHV-1	CA**C**	CT**G**	CT**A**	CG**G**	CG**C**	GA**G**	GG**G**
KPAA-1	SHV-1			CT**G**			GA**A**	GG**G**
KPZU-8	SHV-1			CT**A**			GA**G**	GG**G**
KPZU-9	SHV-1			CT**A**			GA**G**	GG**G**
KPZU-13	SHV-1			CT**G**			GA**G**	GG**C**
KPZU-12	SHV-11	CA**T**[c]		CT**G**			GA**G**	GG**G**
S. typhimurium (17)	SHV-2[d]	CA**C**	CT**G**	CT**G**	CG**G**	CG**C**	GA**G**	GG**G**
K. ozaenae[e] (45)	SHV-2	CA**C**	CT**G**	CT**A**	CG**G**	CG**C**	GA**G**	GG**G**
KPBE-2	SHV-2			CT**A**			GA**G**	GG**G**
KPGE-1	SHV-2			CT**G**			GA**G**	GG**G**
KPLA-2	SHV-2			CT**A**			GA**G**	GG**G**
KPZU-1	SHV-2			CT**A**			GA**G**	GG**G**
KPZU-7	SHV-2			CT**A**			GA**G**	GG**G**
KPZU-11	SHV-2			CT**A**			GA**G**	GG**G**
ECAA-1	SHV-2			CT**A**			GA**G**	GG**G**
ECLA-1	SHV-2			CT**A**			GA**G**	GG**G**
ECLA-2	SHV-2			CT**A**			GA**G**	GG**G**
ECZK-1	SHV-2			CT**G**			GA**G**	GG**G**
ECZU-1	SHV-2			CT**A**			GA**G**	GG**G**
ENLA-1	SHV-2			CT**A**			GA**G**	GG**G**
K. pneumoniae (41)	SHV-2a	CA**C**	CT**G**	CT**G**	CG**G**	CG**C**	GA**G**	GG**G**
K. pneumoniae (46)	SHV-2a	CA**C**	CT**G**	CT**G**	CG**G**	CG**C**	GA**G**	GG**G**
KPLA-3	SHV-2a			CT**G**			GA**G**	GG**G**
KPLA-5	SHV-2a			CT**G**			GA**G**	GG**G**
KPLA-6	SHV-2a			CT**G**			GA**G**	GG**G**
KPLA-7	SHV-2a			CT**G**			GA**G**	GG**G**
KPLA-8	SHV-2a			CT**G**			GA**G**	GG**G**
KPLA-9	SHV-2a			CT**G**			GA**G**	GG**G**
KPLA-10	SHV-2a			CT**A**		CG**T**[c]	GA**G**	GG**G**
KPZU-4	SHV-2a			CT**G**			GA**G**	GG**G**
KPZU-6	SHV-2a			CT**G**			GA**G**	GG**G**
KPZU-10	SHV-2a			CT**G**			GA**G**	GG**G**
ECLA-4	SHV-2a			CT**G**			GA**G**	GG**G**
SWLA-1	SHV-2a			CT**G**			GA**G**	GG**G**
SWLA-2	SHV-2a			CT**G**			GA**G**	GG**G**
K. pneumoniae (40)	SHV-3	CA**C**	CT**G**	CT**A**	CG**G**	CG**C**	GA**A**	GG**G**
K. ozaenae (20)	SHV-5	CA**C**	CT**G**	CT**A**	CG**G**	CG**C**	AA**G**	GG**G**
KPGE-2	SHV-5			CT**G**	CG**A**[c]		AA**A**	GG**G**
KPLA-1	SHV-12			CT**G**			AA**G**	GG**G**
KPLA-4	SHV-5		CT**A**[c]	CT**A**			AA**A**	GG**G**
ECZP-1	SHV-12			CT**G**			AA**G**	GG**G**
E. coli (10)	SHV-7	CA**C**	CT**G**	CT**G**	CG**G**	CG**C**	AA**A**	GG**G**

[a] Amino acid numbering is according to the consensus numbering of Ambler et al. (1).
[b] Discrepancies between different SHV-1 sequences as outlined by Bradford et al. (10) are ignored.
[c] Newly discovered silent point mutations.
[d] Now determined to be SHV-2a.
[e] *K. ozaenae*, *Klebsiella ozaenae.*

differ from SHV-1 and SHV-5, respectively, by a leucine-to-glutamine substitution at position 35 of the SHV protein (numbering in accordance with Ambler et al. [1]), analogous to SHV-2a (46). The amino acid changes of SHV-11 and SHV-12 are listed in Table 3.

The nucleotide sequences of the SHV genes of all four strains carrying SHV-1 as well as that of the SHV-11 gene showed the same differences from the only other registered DNA sequence of SHV-1 (35), as do all other bla_{SHV} sequences so far reported. The dissimilarities in the sequences reported by Mercier and Levesque (35) concern the amino acid exchanges lysine to asparagine at position 192 (lysine 192→asparagine) and leucine 193→valine, followed by an additional glycine.

Furthermore, the interchange of alanine 140 and threonine 141 in the amino acid sequence of SHV-1 reported by Barthélémy et al. (6) was not observed in any of our SHV-1 genes.

Several of the sequenced SHV genes contained additional silent point mutations within the open reading frames, compared to known sequences. Some of them are reported here for the first time. They are listed in Table 2.

The most frequent silent point mutation was found in the coding triplet for leucine 138, which is, in the majority of the bla_{SHV-2} genes (10 of 12), encoded by CT**A**, whereas in 12 of 13 bla_{SHV-2a} genes it is encoded by CT**G.**

Molecular epidemiology. Ribotyping showed that 13 strains could be assigned to five different groups (designated ribotypes A to E). Ribotypes A, B, C, and E comprised two isolates each, whereas ribotype D included five strains (see Table 2). All other strains were found to have unique ribotyping patterns. *K.*

TABLE 3. Amino acid changes of SHV-11 (non-ESBL) and SHV-12 (ESBL) and comparison to SHV-1, SHV-2a, and SHV-5 at selected positions

β-Lactamase	Amino acid at position[a]:			Source or reference
	35	238	240	
SHV-1	Leu	Gly	Glu	35
SHV-11	**Gln**	Gly	Glu	This study
SHV-2a	Gln	Ser	Glu	46
SHV-5	Leu	Ser	Lys	20
SHV-12	**Gln**	Ser	Lys	This study

[a] Amino acid numbers are in accordance with Ambler et al. (1). Changes are indicated in boldface.

pneumoniae KPGE-1 and KPGE-2, belonging to ribotype A, were urine isolates from two different patients in the same unit of the same hospital. Although indistinguishable in ribotype, the two strains harbored different SHV derivatives, i.e., SHV-2 and SHV-5, respectively. *K. pneumoniae* KPLA-5 and KPLA-6 (ribotype B) were isolated on the same day from the stool of the same patient. Both isolates were carriers of SHV-2a. No detailed information was available for *K. pneumoniae* KPLA-8 and KPLA-10, both harboring SHV-2a and belonging to ribotype C, except that they originated from different patients in the same hospital. The two *S. enterica* serotype *wien* strains, SWLA-1 and SWLA-2, ribotype E, were isolated from two patients originally from Libya and hospitalized in the same clinic. Both strains also produced SHV-2a. Ribotype D comprised 5 *K. pneumoniae* strains (KPZU-7 to KPZU-11), which were isolated over a period of 2 weeks initially from the blood and subsequently from the cerebrospinal fluid of a patient suffering from a skull injury. Three of these strains were shown to carry SHV-1, one strain harbored SHV-2, and one harbored SHV-2a (Table 2). Conjugative transfer of resistance determinants from clinical isolates to *E. coli* was successful in 11 of 34 cases. Large plasmids, which were estimated to be approximately 80 kb (data not shown), were isolated from all transconjugants except EK-1 and KZ-6, which contained smaller plasmids of approximately 30 and 5 kb, respectively. Plasmid isolation was unsuccessful from KZ-7 and KZ-10. The plasmids and the total DNA of KZ-6, KZ-7, and KZ-10 were subjected to restriction endonuclease digestions with *Bam*HI. However, no prevailing plasmid fingerprint was detected, with only KL-2, EL-4, and EN-1 sharing strong comigrating bands, together with KZ-6, in which weak plasmid bands became visible only after restriction endonuclease treatment of the total DNA. All four strains had a restriction fragment of 3.6 kb and one of 1 kb in common (Fig. 1). Subsequent hybridization with a bla_{SHV}-specific probe revealed that these strains shared the location of the bla_{SHV} gene on the 3.6-kb fragment. Several strains harbored two or more bla_{SHV} genes, as shown in Fig. 1.

As can be seen by the absence of hybridizing bands in Fig. 1, the transfer of resistance markers into HK225, yielding the transconjugants EL-1, KZ-7, and KZ-10, did not involve the expected bla_{SHV} genes.

Phenotypical properties. MICs were determined to obtain a resistance pattern for each individual wild-type and transconjugant strain. The results are summarized in Table 1. The clinical isolates harboring extended-spectrum SHVs were resistant to most β-lactam antibiotics but retained susceptibility to imipenem. Several strains also exhibited resistance to the amoxicillin-clavulanic acid combination. The MICs of all third-generation cephalosporins tested were somewhat low for several strains despite the carriage of extended-spectrum SHV genes by the strains. Others, for which the MICs of cefotaxime and ceftriaxone were high, remained susceptible to ceftazidime. This can be observed in strains KPLA-2, KPLA-7, KPZU-10, SWLA-1, and, most obviously, in ECLA-1, for which the MICs of cefotaxime and ceftriaxone are both ≥256 μg/ml but that of ceftazidime is only 1 μg/ml. These properties are shared by the respective transconjugants KL-2, KL-7, KZ-10, and EL-1 (Table 1).

DISCUSSION

Sixty clinical isolates of *Enterobacteriaceae* that were considered to have either reduced susceptibility or resistance to β-lactam antibiotics in various clinical microbiology laboratories in Switzerland were subjected to further investigation. In general, the disk diffusion method is employed for susceptibility testing in these laboratories. However, it has been shown that such tests are not sufficiently sensitive for the detection of SHV ESBL-producing isolates (2, 12, 25, 42). Bearing this in mind, one must assume that a number of ESBL producers pass through diagnostic microbiology laboratories undetected. This may account for the relatively low number of SHV-producing *Enterobacteriaceae* found during the two years of the survey. We identified strains carrying $bla_{SHV\text{-}ESBL}$ genes by the PCR-*Nhe*I method described previously (42) and confirmed the results by nucleotide sequencing.

The results give an insight into the prevalence of various types of SHV derivatives in Switzerland, with SHV-2 and SHV-2a clearly prevailing. SHV-5 is frequent in Germany (7), Italy (33), and England (16), and SHV-4 is frequent in France (15). In addition, two new SHV variants, SHV-11 and SHV-12, are described here for the first time. They carry a leucine-to-glutamine substitution at position 35. This point mutation, although far from the active site and known not to alter the isoelectric point of SHV-2a compared to that of SHV-2 (46), has been shown to have an influence on the resistance levels of

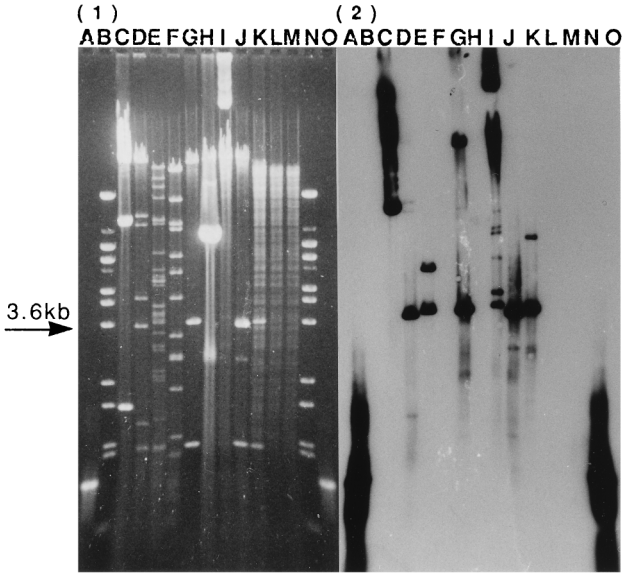

FIG. 1. (1) Ethidium bromide-stained agarose gel showing *Bam*HI-restriction digestion of plasmids isolated from transconjugants. Lanes: A and O, 1,018-bp PCR amplimer of SHV-2a; B and N, bacteriophage λ*Bst*EII molecular size marker; C, KL-1; D, KL-2; E, KL-7; F, EL-1; G, EL-4; H, EK-1; I, EP-1; J, EN-1; K, KZ-6; L, KZ-7; M, KZ-10. (2) Southern transfer of the gel hybridized with the 1,018-bp gene probe consisting of $bla_{SHV\text{-}2a}$. Lanes are as defined for panel 1.

the host strain (41). Such variants, therefore, have to be considered independent SHV derivatives.

Interestingly, this particular point mutation is shared by the chromosomally encoded β-lactamase LEN-1 (3), which otherwise has nearly 90% amino acid identity with SHV-2. LEN-1 also has leucine 138 encoded by CT**G**, as do the majority of the SHV-2a genes sequenced in this survey. In contrast, leucine 138 in most SHV-2 enzymes presented here is encoded by CT**A**, as in the nucleotide sequence of SHV-1 reported by Mercier and Levesque (35). These findings suggest a possible separate evolutionary development of SHV-2a and SHV-2. The fact that a number of silent mutations but no new amino acid exchanges were detected among the sequenced genes implies that the evolutionary range of variation of the SHV enzyme may be limited, possibly due to decreasing stability of the enzyme (50, 54) associated with reduced kinetic efficiency (12).

Ribotyping gave no evidence for a clonal spread in any of the hospitals. The relationships between isolates could only be established in a few instances and did not involve more than five strains, as was the case with the *K. pneumoniae* isolates belonging to ribotype D. Moreover, the occurrence of various types of SHV derivatives even among the isolates of the same ribotype makes dissemination of resistance genes among the isolates improbable. This interpretation is supported by the fact that only 11 of the 34 clinical isolates transferred their resistance markers in mating experiments. Furthermore, 3 of the 11 transconjugants did not harbor an SHV gene, implying localization of that gene on a nonconjugative plasmid (see also reference 9) or on the chromosome within the respective donor. These three transconjugants, EL-1, KZ-7, and KZ-10, possibly contain other *bla* genes, probably on additional plasmids, which were transferred during conjugation rather than the bla_{SHV} genes. This phenomenon of preferential transfer in the case of the carriage of multiple-resistance plasmids by a donor has been reported before (10, 31, 34, 51) and has been attributed to selective conditions during mating (10). Some MICs for the three transconjugants (EL-1, KZ-7, and KZ-10) differ greatly from those for their donors, confirming that the bla_{SHV} genes are not solely responsible for the entire resistance phenotypes of the respective donors. Moreover, other MICs shown in Table 1 indicate that some of the clinical isolates possess more than one β-lactamase because, although they carry the same SHV type enzyme, they show differences in susceptibility (e.g., KPLA-8 and KPLA-9 for cefepime and ECAA-1 and ECLA-1 for ceftazidime).

So far, SHV ESBLs have been found predominantly in *Klebsiella* spp. and *E. coli*. Few authors detected them in various serotypes of *S. enterica* (8, 18, 22, 23), and all reported the presence of SHV-2. Several different TEM ESBLs have been found in this genus (4, 5, 36, 49, 57). In this paper, two isolates carrying SHV-2a of *S. enterica* serotype *wien*, originally from Libyan patients, are reported. A careful sequence comparison revealed that a known *S. typhimurium* isolate (17) also carried an SHV-2a which had, however, been correctly termed SHV-2 in 1990 since SHV-2a was not differentiated from SHV-2 until 1991 (46). This finding, together with the fact that all SHV-carrying *Salmonella* isolates were found in countries around the Mediterranean Sea, leads to the speculation that of the isolates reported previously, more may actually harbor SHV-2a than SHV-2.

ESBLs of the TEM and particularly of the SHV type are very rarely found in other *Enterobacteriaceae* genera, such as *Enterobacter*, *Serratia*, and *Citrobacter*, etc., in which chromosomal AmpC cephalosporinases predominate (24, 32). Nevertheless, *Serratia marcescens* isolated in Greece has been reported to produce an SHV-5-like enzyme (identified by isoelectric focusing) (19) and *S. marcescens* as well as *E. cloacae* have been found to carry SHV-4 (44). In addition, Venezia et al. (59) presented evidence for the production of SHV-5 by *Klebsiella oxytoca*, *E. cloacae*, and *Citrobacter freundii* in the United States. A single isolate of *Enterobacter gergoviae* from China was found to carry SHV-2 by DNA sequencing (14). In this context, it is important to note that ENLA-1 is the first strain of *E. cloacae* reported to carry SHV-2.

Identical enzymes harbored by different isolates are known to vary in the levels of resistance to different antimicrobial compounds that they convey to their hosts (32, 41). Consequently, the MICs presented here vary considerably (Table 1). The ceftazidime MICs for several strains analyzed in this study were modest. However, these strains exhibited elevated resistance to other third-generation cephalosporins, and consequently many had failed to be detected as ESBL producers when screened with a commercial ceftazidime/clavulanic acid synergy test (42). In addition, other strains that were shown to harbor a $bla_{SHV\text{-}ESBL}$ gene remained apparently susceptible not only to ceftazidime but to all cephalosporins tested except cephalothin. Such strains, although they show reduced susceptibility to newer cephalosporins, remain susceptible according to the breakpoints established by the NCCLS. Interpretive problems that result from these circumstances are inevitable and especially misleading in the case of ceftazidime, which is recommended for the detection of ESBL producers (21, 28). Moreover, treatment failures due to undetected ESBL-producing pathogens have already been reported (27). No clear connection can be made between β-lactamase production and the in vitro resistance level of the host strain. Other factors must therefore be taken into consideration, e.g., permeability changes or the influence of the specific host environment.

Obviously, the mere presence of a $bla_{SHV\text{-}ESBL}$ gene in a host strain does not guarantee clear-cut high resistance to all extended-spectrum β-lactams in vitro. Nevertheless, such organisms should be regarded as potentially resistant to all extended-spectrum cephalosporins, regardless of the actual susceptibilities detected, as proposed on the basis of clinical and animal model data (20, 32, 45).

One must conclude that more clinical isolates than anticipated harbor $bla_{SHV\text{-}ESBL}$ genes. In many cases they appear not to be transmissible, and their respective enzymes are often produced at low levels. A clonal spread or dissemination of a single resistance plasmid, therefore, would occur only sporadically, under special conditions, thus leading to nosocomial outbreaks.

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