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Clonal and Horizontal Dissemination of *Klebsiella pneumoniae* Expressing SHV-5 Extended-Spectrum β-Lactamase in a Mexican Pediatric Hospital

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One hundred eighty-four clinical isolates of *Klebsiella pneumoniae* were recovered from August 1996 to October 1997 at the Pediatric Hospital of the Instituto Mexicano del Seguro Social in Mexico City, Mexico. Most of the isolates were collected from the neonatal intensive care unit and infant wards, which are located on the same floor of the hospital. Isolates were genotypically compared by pulsed-field gel electrophoresis with *Xba*I restriction of chromosomal DNA. Of 184 clinical isolates, 91 belonged to cluster A and comprised three subtypes (A1, A2, and A3), while 93 isolates, comprising two minor clones, B (10 isolates) and C (7 isolates), and 76 unique patterns, were considered unrelated isolates (URI). Susceptibility patterns were indistinguishable in both groups. Fifty extended-spectrum β-lactamase-producing isolates, including 34 from clone A and 16 from URI, were examined for further studies. Molecular and genetic analysis showed that 47 of 50 clinical isolates expressed the SHV-5 β-lactamase. This enzyme, in combination with TEM-1, was encoded in a ≥170-kb conjugative plasmid. Results indicate that dissemination of this resistance was due to clonal and horizontal spread.

Klebsiella spp. have been prominent among gram-negative bacilli causing nosocomial infections, as well as being an important source of transferable antibiotic resistance. During the 1970s there were frequent epidemics of gentamicin-resistant *Klebsiella pneumoniae* infections in hospitals (5). In recent years, following the overuse of expanded-spectrum cephalosporins, several outbreaks caused by extended-spectrum β-lactamases (ESBLs) have been reported (5, 18, 20, 23). ESBLs are enzymes with considerable hydrolyzing activity on a wide variety of β-lactam antibiotics, including oxyaminocephalosporins and aztreonam (12). Such enzymes have been shown to be derived from SHV- or TEM-type β-lactamases by one or more amino acid substitutions (11, 21). Since the first ESBL-expressing isolate was discovered, many types of ESBLs, exhibiting high degrees of diversity in their structures and activities, have been characterized and described. Several families reflecting evolutionary and/or functional similarities can be distinguished (19). Among the most prevalent types of ESBLs are members of the TEM and SHV families (3). To date about 17 amino acid positions in ESBL protein sequences have been reported to be heterogeneous (7). Isolation and sequencing of natural genes encoding ESBLs provide important data from an epidemiological point of view and contribute to our understanding of the structure and function of β-lactamases. The genes coding for ESBLs are usually carried by plasmids, which strongly facilitate their spread among strains of many species of gram-negative bacteria. Nevertheless, *K. pneumoniae* and *Escherichia coli* remain the most frequently reported ESBL producers (18, 20, 22, 23).

There is limited information in Mexico concerning molecular studies on the type of ESBL selected in vivo in multidrug-resistant enterobacteria (13, 24, 25, 26). In this work we used a molecular approach to determine the epidemiology of an outbreak produced by an endemic multidrug-resistant *K. pneumoniae* strain.

MATERIALS AND METHODS

Hospital setting. The study was carried out in a tertiary-care pediatric hospital in Mexico City with 194 beds and five areas of hospitalization: (i) a pediatric intensive-care unit (ICU) with 18 beds; (ii) an area for preschool children, with 40 beds; (iii) a neonatal ICU (NICU) with 24 beds; (iv) an area for infants, with 52 beds; and (v) an area for school-age children and adolescents, with 60 beds. All patients are referred from seven hospitals in Mexico City and from four Mexican states (Morelos, Guerrero, Querétaro, and Chiapas).

Bacterial strains. One hundred eighty-four clinical isolates of *K. pneumoniae* were isolated from August 1996 to November 1997. During this period an increase in the incidence of isolation was detected. The species of the organisms were verified by tests with the API 20E system (bioMerieux, Marcy L′Etoile, France). Genetic and molecular characterization studies for ESBL production were performed on 50 clinical isolates selected according to their pulsed-field gel electrophoresis (PFGE) patterns: 21 corresponding to clone A; 11 corresponding to subtype A1; 1 isolate each for subtype A2, subtype A3, clone B, and clone C; and 14 with unique patterns (UNP). All strains were isolated from blood or cerebrospinal fluid (CSF) cultures (see Table 2).

Antimicrobial agents. Pure salts of the following drugs were provided by the companies indicated: cefotaxime and cefpirome (Hoechst-Marion-Roussel, Romainville, France), ceftazidime (GlaxoWellcome, Mexico City, Mexico), aztreonam and cefepime (Bristol-Myers Squibb, Mexico City, Mexico), and clavulanic acid (SmithKline Beecham Pharmaceuticals, Mexico City, Mexico). Rifampin, tetracycline. and gentamicin were obtained from Sigma (St. Louis, Mo.).

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Susceptibility testing and ESBL test. MICs were determined by agar dilution on Mueller-Hinton agar according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS). Breakpoints for resistance (in micrograms per milliliter) were as follows: ampicillin, ≥32; carbenicillin, ≥64; chloramphenicol, ≥32; amikacin, ≥64; gentamicin, ≥16; ceftazidime, ≥32; cefotaxime, ≥64; ceftizoxime, ≥64; cefepime, ≥32; imipenem, ≥16; meropenem, ≥16; norfloxacin, ≥16. ESBL production was performed by the disk diffusion method with cefotaxime and ceftazidime alone and in combination with clavulanic acid, as recommended by the NCCLS (17).

Genome fingerprinting by PFGE. For PFGE typing, whole-cell DNA was obtained according to the method of Miranda et al. (16). DNA was digested with the *Xba*I restriction enzyme (Gibco BRL, Gaithersburg, Md.) and separated in a 1% agarose gel (Pulsed Field-Certified; Pronadisa, Madrid, Spain) with a Gene-Path System (Bio-Rad, Hercules, Calif.). The gel was stained with ethidium bromide and visualized with the Gel-Doc system (Bio-Rad). The images were interpreted with Multi-analyst software (Bio-Rad) according to the criteria of Tenover et al. (28).

IEF and bioassay. Isoelectric focusing (IEF) was conducted according to the method described by Matthew et al. (14) by using a Phast system minigel with a pH range of 3 to10 (Pharmacia, LKB). Extracts from TEM-1-, SHV-2-, and SHV-5-producing strains were used as standards for pIs of 5.4, 7.6, and 8.2, respectively. To determine the ESBLs encoded by the strains, a bioassay was performed as described by Silva-Sanchez and Aguilar-Zacarias (27).

Plasmid isolation and conjugation experiments. DNA was extracted from clinical isolates and transconjugants according to the method described by Kieser (10). DNA was visualized after vertical electrophoresis in 0.7% agarose gels stained with ethidium bromide. Plasmids R6K (40 kb), RP4 (5 kb), IR (93 kb), and pUD21 (170 kb) were used as molecular weight markers. Matings were performed as described by Miller (15), by using *E. coli* strain J53-2 (F^- *pro met* Rif^r). In all cases, transconjugants were selected on Luria agar supplemented with rifampin (200 μg/ml) in combination with cefotaxime (1 μg/ml) or ampicillin (100 μg/ml). For each successful mating experiment, 25 independent transconjugants were obtained from each selection medium and were tested on Luria plates supplemented with ampicillin (100 μg/ml), cefotaxime (1 μg/ml), kanamycin (25 μg/ml), tetracycline (25 μg/ml), chloramphenicol (10 μg/ml), and gentamicin (1 μg/ml).

TEM- or SHV-specific PCR and DNA sequencing. To amplify TEM-related genes from clinical isolates, oligonucleotide primers OT1 and OT2, described by Arlet and Philippon (1), were used for PCR. For SHV-specific PCR, primers SE5 and SB3 were used as described by Silva et al. (26). The PCR mixture for both amplifications (50 μl) contained 30 pmol of each primer, 300 ng of total DNA, 1× reaction buffer, 200 μM $MgSO_2$, 200 μM each deoxynucleoside triphosphate, and 2.5 U of *Taq* DNA polymerase. Enhancer buffer (Gibco BRL) at a final concentration of 3× was added to the reaction mixture to enhance the specificity of hybridization. The PCR amplification conditions for both reactions were as follows: initial denaturation (95°C for 5 min); 30 cycles of denaturation (94°C for 30s), annealing (58°C for 30s), and polymerization (72°C for 120s); and an additional polymerization step (72°C for 15 min) at the end of the program. The product of bla_{SHV} amplification was used to determine the nucleotide sequence with the fluorescence-based *Taq* FS Dye terminator cycle sequencing kit and the same primers. Sequence analysis was performed with Genetics Computer Group software and by BLASTx searching (of the EMBL, SwissProt, and PIR databases). Multiple alignment was performed with the Clustal W program (29).

Nucleotide sequence accession numbers. The nucleotide sequences of *shv* genes from strains X1529A, X1620A, X1623UNP, R1549UNP, and X1600UNP have been deposited in GenBank under accession no. AY386365 to AY386369, respectively.

RESULTS

Genomic typing of *K. pneumoniae* isolates. Between August 1996 and October 1997, 184 clinical isolates of *K. pneumoniae* were collected and typed by PFGE. Six different major PFGE types were identified. Most of the isolates corresponded to the major type (clone A), which included 91 clinical isolates (49.5%), suggesting the presence of one predominant clone of *K. pneumoniae*. The other 93 isolates (50.5%) comprised two minor groups, clone B (10 isolates) and clone C (7 isolates), and 76 UNP; all of these were referred as unrelated isolates (URI). Isolates from clone A could be further classified into subtypes A1, A2, and A3. A representative sample of the patterns is shown in Fig. 1. Most clinical isolates were obtained from patients in the NICU and the infant ward, both located on the same floor of the hospital. In order to perform further genetic and molecular characterizations, 50 strains of the 184 clinical isolates were selected, including 21 from clone A, 11 from subtype A1, 14 with UNP, and 4 isolates corresponding, respectively, to pattern A2, A3, B, or C. All 50 clinical isolates were identified as ESBL producers.

Antibiotic susceptibilities. MICs of various antibiotics were determined by agar dilution against the 184 *K. pneumoniae* clinical isolates. MICs at which 50 and 90% of the isolates were inhibited (MIC_{50} and MIC_{90}, respectively) are given in Table 1. All strains were resistant to β-lactam antibiotics and aminoglycosides and were susceptible to imipenem, meropenem, and norfloxacin. There was no difference in MICs between the clone A isolates and URI isolates.

Plasmid profiles and resistance transfer. All 50 clinical isolates harbored at least two plasmids, one of ≥170 kb and one of 40 to 90 kb. To identify the plasmid responsible for conferring cefotaxime resistance, all clinical isolates of *K. pneumoniae* were mated to a susceptible *E. coli* host. Transconjugants were selected on ampicillin or cefotaxime in independent selections and were designated with the letter X and the number of the parental strain. Successful matings were detected for 42 of the 50 clinical isolates; of these, 18 corresponded to clone A, 9 to subtype A1, 1 to subtype A3, 1 to clone B, and 13 to UNP. Ampicillin resistance transfer was successful in all 42 matings; however, when selection was done on cefotaxime, only 27 of 42 transconjugants (64.3%) were obtained. However, the transconjugants that grew only on ampicillin showed cefotaxime resistance in a later test. Frequencies of resistance transfer were 10^{-2} to 10^{-5} transconjugants per donor cell for ampicillin and 10^{-4} to 10^{-6} for cefotaxime. In general, resistance transfer frequencies were one- to twofold higher for ampicillin than for cefotaxime. All 42 transconjugants harbored only the largest plasmid (≥170 kb) and expressed resistance markers for other antibiotics such as tetracycline, kanamycin, gentamicin, and chloramphenicol. Eighteen transconjugants were susceptible only to tetracycline, 8 were susceptible only to chloramphenicol, 6 were susceptible only to tetracycline and chloramphenicol, and 10 were resistant to the six antibiotics tested.

IEF analysis and enzyme inhibition test. Crude sonicates of the 50 clinical isolates and 42 transconjugants were subjected to IEF in order to identify the β-lactamase profiles. All clinical isolates and transconjugants expressed a β-lactamase with a pI of 5.4 and two or three additional bands with pIs of 7.0, 7.3, 7.6, or 8.2 (Table 2). One predominant β-lactamase profile with pIs of 5.4, 7.3, and 8.2 was identified for 38 clinical isolates, of which 18 corresponded to clone A, 11 to subtype A1, 1 to subtype A3, 1 to clone B, and 7 to UNP. Three other, minor β-lactamase profiles were identified for clinical isolates: the first with pIs of 5.4, 7.0, and 8.2, the second with pIs of 5.4 and 8.2, and the last with pIs of 5.4 and 7.6. In all cases the last enzyme, with a pI of 8.2 or 7.6, was determined to have the capacity to hydrolyze cefotaxime in the bioassay, indicating correspondence to an ESBL. When β-lactamase profiles were determined for transconjugants, a major pattern of enzymes with pIs of 5.4 and 8.2 was identified. This pattern was ex-

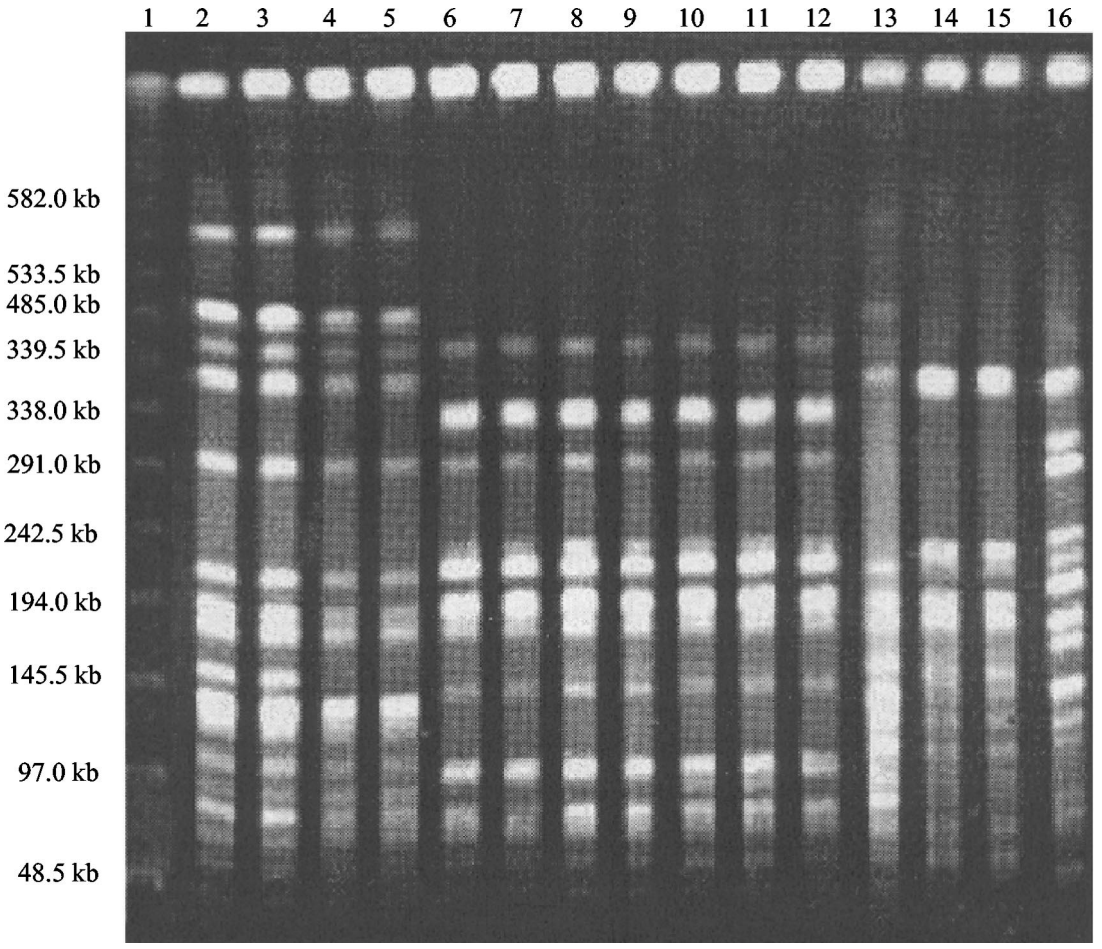

FIG. 1. Representative agarose gel PFGE of *Xba*I-digested genomic DNA of ESBL-producing *K. pneumoniae* isolates. Lane 1, molecular size marker of lambda ladder; lanes 2 to 5 and 13, clone B; lanes 6 to 12, clone A; lanes 14 and 15, clone C; lane 16, UNP.

pressed in 40 of 42 transconjugants, of which 17 corresponded to clone A, 9 to subtype A1, 1 to subtype A3, 1 to clone B, and 12 to UNP. Two transconjugants, 1 from clone A and 1 with a UNP, showed a β-lactamase pattern with pIs of 5.4 and 7.6.

TABLE 1. Comparison of antimicrobial susceptibilities between *K. pneumoniae* clone A isolates and URI

Antibiotic	MIC (μg/ml)[a] for:			
	Clone A		URI[b]	
	50%	90%	50%	90%
Ampicillin	>64	>64	>64	>64
Carbenicillin	>256	>256	>256	>256
Chloramphenicol	>16	>16	>16	>16
Amikacin	>64	>64	16	>64
Gentamicin	>64	>64	64	>64
Ceftazidime	>64	>64	64	>64
Cefotaxime	<16	>64	<16	>64
Ceftizoxime	<8	64	>8	>64
Cefepime	<4	16	<4	32
Imipenem	<4	<4	<4	<4
Meropenem	<4	<4	<4	<4
Norfloxacin	<2	2	<2	2

[a] 50% and 90%, MIC_{50} and MIC_{90}.
[b] Include clinical isolates of clone B, clone C, and UNP.

PCR amplification of bla_{TEM} and bla_{SHV} genes. According to the pIs of the β-lactamases obtained, the enzyme with a pI of 5.4 should correspond to the TEM-1 β-lactamase, and enzymes with a pI of 7.6 or 8.2 should correspond to SHV-derived β-lactamases. In order to corroborate this hypothesis, PCR assays were performed with specific oligonucleotides. Total-DNA preparations of five different clinical isolates and the respective transconjugants, representing the four β-lactamase profiles, were tested for the presence of bla_{TEM} and bla_{SHV} genes. In the first case, an amplification product of the expected size (503 bp) was obtained for all clinical isolates and transconjugants tested, indicating that the β-lactamase with a pI of 5.4 corresponded to TEM-1 (Table 3). When PCR amplification was performed for the detection of bla_{SHV} genes, a product of approximately 900 bp was observed in all cases.

Sequencing of ESBL-encoding genes. The products of PCR amplification of the bla_{SHV} genes from *E. coli* transconjugants X1600UNP, X1620A, and X1623UNP and from clinical isolate R1549A, all of which expressed an enzyme with a pI of 8.2, were used for sequencing. The results were compared with the sequence of the bla_{SHV-1} gene and indicated that all contained the Gly238Ser and Glu240Lys substitutions, which correspond to the SHV-5 β-lactamase. Meanwhile, the DNA sequence of the PCR product from *E. coli* transconjugant X1529A, produc-

TABLE 2. Epidemiological and molecular characteristics of *K. pneumoniae* ESBL-producing clinical isolates

Strain no.	Date of isolation (day/mo/yr)	Ward	Sample	PFGE pattern	β-Lactamase pattern (pI)[a]	Conjugation	Resistance pattern of transconjugant[b]
1533A	16/8/1996	School-age	Blood	A	**5.4**, 7.3, (**8.2**)	+	a
1545A	12/9/1996	School-age	Blood	A	**5.4**, 7.3, (**8.2**)	+	b
1607A	12/11/1996	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	d
1517A	18/2/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	b
1589A	10/4/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	b
1591A	8/5/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	b
1594A	26/5/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	c
1596A	8/7/1997	NICU	Blood	A	5.4, 7.3, (8.2)	−	
1599A	15/7/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	a
1598A	15/7/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	c
1610A	21/7/1997	Infant	Blood	A	**5.4**, 7.3, (**8.2**)	+	c
1602A	8/8/1997	NICU	Blood	A	5.4, 7.3, (8.2)	−	
1604A	20/8/1997	Infant	Blood	A	**5.4**, 7.3, (**8.2**)	+	d
1606A	25/8/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	c
1611A	8/9/1997	NICU	Blood	A	5.4, 7.3, (8.2)	−	
1620A	29/9/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	a
1625A	30/9/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	d
1516A	31/1/1997	NICU	Blood	A	**5.4**, 7.3, (**8.2**)	+	d
1525A1	15/8/1996	NICU	Blood	A1	5.4, 7.3, (8.2)	−	
1547A1	12/9/1996	NICU	Blood	A1	**5.4**, 7.3, (**8.2**)	+	b
1574A1	11/11/1996	NICU	Blood	A1	5.4, 7.3, (8.2)	−	
1510A1	27/12/1996	School-age	Blood	A1	**5.4**, 7.3, (**8.2**)	+	d
1513A1	3/1/1997	NICU	Blood	A1	**5.4**, 7.3, (**8.2**)	+	b
1583A1	21/1/1997	NICU	Blood	A1	**5.4**, 7.3, (**8.2**)	+	b
1582A1	27/1/1997	NICU	Blood	A1	**5.4**, 7.3, (**8.2**)	+	b
1584A1	30/1/1997	Infant	Blood	A1	**5.4**, 7.3, (**8.2**)	+	b
1515A1	31/1/1997	NICU	CSF	A1	**5.4**, 7.3, (**8.2**)	+	b
1585A1	19/3/1997	NICU	CSF	A1	**5.4**, 7.3, (**8.2**)	+	b
1523A1	2/4/1997	NICU	CSF	A1	**5.4**, 7.3, (**8.2**)	+	c
1511A3	2/1/1997	NICU	Blood	A3	**5.4**, 7.3, (**8.2**)	+	b
1528UNP	23/8/1996	Infant	Blood	UNP	**5.4**, 7.3, (**8.2**)	+	a
1549UNP	17/9/1996	Infant	Blood	UNP	**5.4**, 7.3, (**8.2**)	+	b
1608UNP	19/11/1996	NICU	Blood	UNP	**5.4**, 7.3, (**8.2**)	+	d
1578B	25/11/1996	Infant	Blood	B	**5.4**, 7.3, (**8.2**)	+	b
1603UNP	11/6/1997	NICU	Blood	UNP	5.4, 7.3, (8.2)	−	
1621UNP	26/9/1997	NICU	Blood	UNP	**5.4**, 7.3, (**8.2**)	+	c
1628UNP	3/10/1997	NICU	Blood	UNP	**5.4**, 7.3, (**8.2**)	+	c
1627UNP	6/10/1997	School-age	Blood	UNP	**5.4**, 7.3, (**8.2**)	+	b
1521A	17/3/1997	NICU	Blood	A	**5.4**, 7.0, (**8.2**)	+	b
1560UNP	25/9/1996	Infant	Blood	UNP	**5.4**, 7.0, (**8.2**)	+	a
1563UNP	25/9/1996	Infant	Blood	UNP	**5.4**, 7.0, (**8.2**)	+	a
1600UNP	4/8/1997	School-age	Blood	UNP	**5.4**, 7.0, (**8.2**)	+	b
1509A	20/12/1996	NICU	Blood	A	**5.4**, (**8.2**)	+	a
1542UNP	8/8/1996	NICU	Blood	UNP	**5.4**, (**8.2**)	+	a
1568C	2/10/1996	Infant	CSF	C	5.4, (8.2)	−	
1601UNP	4/8/1997	Outpatient	CSF	UNP	**5.4**, (**8.2**)	+	c
1623UNP	1/10/1997	Infant	Blood	UNP	**5.4**, (**8.2**)	+	b
1529A	26/8/1996	NICU	Blood	A	**5.4**, (**7.6**)	+	a
1520UNP	4/3/1997	Emergency	Blood	UNP	**5.4**, (**7.6**)	+	a
1562A2	30/9/1996	Infant	CSF	A2	5.4, (7.6)	−	

[a] A pI in parentheses indicates that the enzyme is an (ESBL), as detected by bioassay. Boldfaced data are values observed for transconjugants.
[b] a, resistant to ampicillin, cefotaxime, kanamycin, tetracycline, chloramphenicol, and gentamicin; b, resistant to all antibiotics except tetracycline; c, resistant to all antibiotics except chloramphenicol; d, resistant to all antibiotics except tetracycline and chloramphenicol.

ing the enzyme with a pI of 7.6, encoded only the Gly238Ser amino acid substitution, corresponding to the SHV-2 gene (Table 3).

DISCUSSION

A total of 184 clinical isolates of *K. pneumoniae* were recovered between August 1996 and October 1997 at the Pediatric Hospital of the Instituto Mexicano del Seguro Social in Mexico City. Most of the isolates were recovered from the NICU and the infant ward, which are located on the same floor. According to the genotyping, a single clonal spread was detected by PFGE in 50% of the isolates while the other 50% comprised URI, which included two minor clones (B and C) and UNP. Both groups harbor a multidrug resistance plasmid. Therefore, this outbreak was due to the spread of an epidemic strain of multidrug-resistant *K. pneumoniae* (2, 5, 8, 26). At the same time, it was due to the dissemination of a multidrug resistance plasmid to other clinical isolates, as has occurred in other hospitals (13, 23).

TABLE 3. Comparison of amino acid sequences of SHV derivatives and detection of TEM β-lactamase in *K. pneumoniae* clinical isolates and transconjugants

Strain no.[a]	β-Lactamase pattern (pI)[b]	TEM[c]	ESBL	Amino acid at position:		Source or reference	GenBank accession no.
				238	240		
	(7.6)	−	SHV-1	G	E	7	AF148850
	(7.6)	−	SHV-2	S	E	7	AF148851
	(8.2)	−	SHV-5	S	K	7	X55640
X1529A	5.4, (7.6)	+	SHV-2	S	E	This work	AY386365
X1620A	5.4, (8.2)	+	SHV-5	S	K	This work	AY386366
X1623UNP	5.4, (8.2)	+	SHV-5	S	K	This work	AY386367
R1549UNP	5.4, 7.3, (8.2)	+	SHV-5	S	K	This work	AY386368
X1600UNP	5.4, 7.0, (8.2)	+	SHV-5	S	K	This work	AY386369

[a] R, clinical isolate; X, transconjugant.
[b] A pI in parentheses indicates an ESBL, as detected by a bioassay.
[c] Presence (+) or absence (−) as determined by PCR.

Recent studies of hospital-associated infections in the United States have reported that SHV-4 and SHV-5 are becoming the predominant types of ESBLs found in nosocomial isolates of *K. pneumoniae*. In Germany, SHV-2 and SHV-5 seem to be predominant; and in France, SHV-3, SHV-4, and TEM-3 are more common. SHV-2 is widespread internationally (9).

In order to characterize cefotaxime resistance at the molecular level, 50 clinical isolates were randomly selected; they included type A, with three subtypes, and URI, which included two minor clones (B and C) and UNP. In all clinical isolates, TEM-1 and SHV-5 (and in a minor proportion, SHV-2) enzymes were encoded in a self-transferable ≥170-kb plasmid. In addition to these two enzymes, a third, non-ESBL β-lactamase with a pI of 7.0 or 7.3 was expressed. This β-lactamase was not transferred by conjugation to the *E. coli* recipient strain, suggesting that this enzyme is not carried on the same plasmid. The prevalence of ESBL producers was almost the same throughout the study period, suggesting that SHV-type β-lactamase-producing strains were already endemic in the hospital.

The identification of SHV-2 and SHV-5 makes it possible to speculate on the evolutionary sequence of SHV-type ESBLs, i.e., from SHV-2 to SHV-5, according to the mutation process. Because only one amino acid substitution (Gly240Lys) is required, it may be assumed that in this hospital the mutation process in *K. pneumoniae* clinical isolates was sequential from SHV-2 to SHV-5, thereby disseminating to other strains and/or patients.

When the mating experiments were developed in both genotyping groups (A and URI), the results were very similar, 82 and 88%, respectively. Two antibiotics were used independently for selection, ampicillin and cefotaxime, and frequencies of transfer of resistance to ampicillin were always one- to twofold higher than those for cefotaxime. Considering that the ≥170-kb plasmid encodes two β-lactamases, TEM-1 and SHV-2 or SHV-5, these results suggest that the higher frequency for ampicillin resistance transfer may have been due to the fact that TEM-1 expresses preferentially to the ESBL enzyme, conferring resistance to ampicillin in *E. coli* transconjugants. The cefotaxime phenotype depends on the expression of the SHV-derived enzyme. Also, variations in non-β-lactam resistance markers associated with the ≥170-kb plasmid suggest the ability of the plasmid to accept or lose these genes. These could be included in other mobile genetic elements such as transposons or integrons (4). Also, the fact that a minor number of strains were unable to transfer resistance could be due to loss of conjugation by means of a possible mutation along the *tra* operon, which showed diversity in the clinical isolates. In these isolates, the high frequency of conjugation increases the possibility of dissemination of multidrug resistance among other genera in vivo.

Other studies investigating β-lactamases and ESBL types in different clinical isolates from Mexico, Poland, and Taiwan (6, 13, 26) have documented the SHV-5 enzyme in association with the TEM-1 β-lactamase. In the future, our laboratory will undertake investigations using molecular biology techniques to determine whether the plasmids expressing these enzymes are related at the molecular level.

On the basis of susceptibilities to antibiotics, genotyping, β-lactamase production, and conjugation experiments, it is possible that spread of an endemic strain and horizontal gene transfer were responsible for the high frequency of detection of *K. pneumoniae* ESBL producers in this setting. Most of these strains were obtained from the NICU and the infant ward, indicating a localized dissemination within the hospital and pointing to a potential source of spread of an SHV-5 ESBL-encoding plasmid in the hospital.

In conclusion, this study highlights the need to establish an antimicrobial resistance surveillance network for *K. pneumoniae* to monitor the trends and new types of resistance mechanisms in this hospital. Also, the factors responsible for the selection and dissemination of this plasmid encoding the SHV-derived enzyme and clone A need to be identified, controlled, and, where possible, prevented so as to avoid major outbreaks.

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