

Clinical and Molecular Analysis of Extended-Spectrum β -Lactamase-Producing Enterobacteria in the Community Setting

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During a previous survey, five extended-spectrum β -lactamase (ESBL)-producing enterobacteria (ESBLE) (two *Enterobacter aerogenes* isolates expressing TEM-24b, two *Escherichia coli* isolates expressing TEM-21 or TEM-24b, and one *Klebsiella pneumoniae* isolate expressing SHV-4/TEM-15) responsible for urinary tract infections (UTIs) were found among 1,584 strains collected from community patients. The aim of the present study was to elucidate the route of emergence of these typically nosocomial organisms in the community. Thus, the files of the five patients were analyzed over at least a decade, and potentially related ESBLE from hospitals or clinics were examined. Their enzymes were characterized at a molecular level, and the strains were typed by amplified-primed PCR, enterobacterial repetitive intergenic consensus PCR, and restriction plasmid profile. All patients (C1 to C5) had risk factors for ESBLE acquisition, including past history of hospitalization (2.5 to 23 months before). Four (C1 and C3 to C5) had previously received antibiotics (concomitantly to 35 months earlier), two (C1 and C3) had indwelling urinary catheters and recurrent UTIs, and three (C2, C3, and C5) formerly experienced ESBLE-induced UTIs (2½ to 11½ months before). The same ESBLE and/or an identical or similar ESBL-encoding plasmid was identified in the hospital ward (C1 to C4) or in a clinic (C5) where the patients had previously resided. Patients C1 and C4, infected with different ESBLE carrying a closely related plasmid, were hospitalized in the same unit. Persistence of ESBLE over at least a 5-year period was demonstrated for patient C3. Thus, community-acquired UTIs in these patients likely resulted from nosocomially acquired ESBLE or an ESBL-encoding plasmid followed by a prolonged digestive carriage.

Extended-spectrum β -lactamase (ESBL)-producing enterobacteria (ESBLE) have emerged at the end of the 1980s within hospitals, causing outbreaks and/or hyperendemic situations in many centers. Several recent data have suggested that ESBLE are currently emerging within the community (6, 8, 9, 11, 12, 14, 16, 17, 19, 20, 22–24, 27, 28, 30, 31, 33). However, most of these studies simply noted the incidence of ESBLE-induced infections (9, 11, 12, 14, 16, 19, 27) or ESBLE digestive carriage (16, 22–24, 33). Others have described clinical cases in greater detail (6, 31) or identified the risk factors for ESBLE acquisition (8). Few studies have reported ESBL characterization (28) and analysis of strain relatedness (20, 30, 35). The latter investigations have generally been conducted in hospital laboratories and involved “community patients” without further information on their origin (35) or “nonhospitalized patients” admitted to the hospital for less than 48 h (20, 30), some of them being transferred from nursing homes (30), which are known to be a common source of ESBLE (26, 34).

A survey carried out by the Aquitaine Network of private laboratories in Southwestern France between January and May 1999 in the extrahospital setting indicated that 5 of 1,584

strains of enterobacteria (0.3%) from patients living at home were found to produce an ESBL (29). Although this was a significantly smaller number compared to the 34 out of 1,015 strains from residents of clinics and nursing homes (chi-square test, $P < 0.005$) (29), a similar and worrying incidence of ESBLE-induced infections in the community has previously been observed in France and in most other European countries (0.2 to 0.6%) (9, 11, 12, 14, 27), and an even higher frequency has been recorded in Poland (3.5%) (17). The ESBLEs of the five strains have been characterized by sequencing the encoding genes, and the ESBLE have been typed using both non-molecular and molecular methods (2). Nevertheless, as in previous studies, the mode of appearance of these typically nosocomial organisms in community patients remained to be elucidated.

The aim of this study was to assess the route of ESBLE acquisition in the five community patients. For this purpose, the medical files of the five patients were retrospectively analyzed as far as possible until the end of 2004. In addition, the five ESBLE were compared to those isolated in wards of the University Hospital of Bordeaux and in private health care centers of the region where the five patients had previously resided and to isolates recently recovered from the same patients in order to evaluate the carriage time period. Strain comparison included molecular characterization of ESBLEs and typing of ESBLE by amplified-primed PCR (AP-PCR), enter-

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obacterial repetitive intergenic consensus PCR (ERIC-PCR), and restriction plasmid profile.

MATERIALS AND METHODS

Patient data, bacterial strains, and culture conditions. Microbiological data were recorded from private and hospital laboratories of the Aquitaine region. Clinical data were obtained from the five community patients or their closest relatives, their private practitioners, and the medical staff of the clinics and/or the hospitals where they stayed. Patient charts were analyzed with respect to underlying diseases, hospitalization history, immunodepressor and antibiotic treatments, and infections due to other pathogens. Patients (C1 to C5) and private clinics (CLI-1 to CLI-3 and CLI-7) were numbered as indicated previously (2). Otherwise, five clinics (CLI-9 to CLI-12), where no ESBLE were found during our 1999 survey, were introduced in this study.

The ESBLE isolated from the five community patients (Kp7, Ec1, Ec5, and Ea1-a from patient C4 and Ea1-b from patient C5 belonging to the same clone, Ea1) (2) were compared to nine ESBLE recovered either from the same patients (Pm04 and Kp04 isolated in 2004 from C1 and C3, respectively) or from seven other patients (Kp97, Ea97, Kp98, Kp92, Kp93, and Ea99 from A, B, and D to H) in four separate sites of the University Hospital of Bordeaux (UH1 to UH4) and in clinic CLI-1 (Table 1). Identification to the species level was performed with the API 20E system (bioMérieux, France). *E. coli* strain ATCC 25922 was used as a control for MIC determination. Spontaneous nalidixic acid-resistant (Nal^r) and rifampin-resistant (Rif^r) mutants of *E. coli* K-12 (10) and *E. coli* DH5α were the recipient strains in conjugation and transformation experiments, respectively. Unrelated strains of *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter aerogenes* were introduced as controls for molecular typing. All bacterial strains were routinely cultured at 37°C in Mueller-Hinton (MH) medium (Diagnostics Pasteur, France).

Antibiotic susceptibility testing. The antibiotic susceptibility patterns of the ESBLE, their transconjugants, or recombinant clones were determined by a standard agar diffusion method in MH medium using 27 antibiotic disks (<http://www.sfm.asso.fr>). ESBLs were detected using the double-disk synergy test between clavulanic acid and ceftazidime, cefotaxime, or cefepime (18). MICs of eight β-lactams alone or in combination with clavulanic acid were determined by an agar dilution method (<http://www.sfm.asso.fr>).

Isoelectric focusing. Isoelectric focusing was performed in polyacrylamide gels containing ampholines with a pH range of 3.5 to 10.0 (Amersham Biosciences, France), as previously described (2). β-Lactamase activities were detected by an iodine-starch procedure in agar gel, with benzylpenicillin (75 μg/ml). The isoelectric points (pIs) of the studied β-lactamases were determined by comparison with reference β-lactamases of known pIs: TEM-1 (pI 5.4), TEM-15 (pI 6.0), TEM-3 (pI 6.3), TEM-21 (pI 6.4), TEM-24 (pI 6.5), SHV-1 (pI 7.6), and SHV-4 (pI 7.8) (2).

Conjugation experiments and plasmid analysis. Conjugation assays were carried out by the filter-mating procedure using the *E. coli* K-12 Nal^r Rif^r mutant as the recipient (10). Transconjugants were selected on MH agar containing nalidixic acid (100 μg/ml) and/or rifampin (100 μg/ml) plus ceftazidime (2 μg/ml). Plasmid DNA was extracted by an alkaline lysis method (5) and analyzed by electrophoresis on 0.8% (wt/vol) agarose gels with or without digestion by the EcoRI endonuclease (Promega, France).

PCR amplifications and sequence analysis. PCR experiments were performed with crude lysates obtained after boiling under standard conditions (94°C for 5 min and 35 subsequent cycles of 1 min at 94°C, 1 min at 50°C, and 1 min at 72°C and a final step at 72°C for 10 min). The pairs of primers specific to *bla*_{TEM} and *bla*_{SHV} amplifications have been described previously (2). The PCR products were purified using microcolumns of the Microspin Sephacryl S-400 purification system (Amersham Biosciences) and sequenced on both strands with sets of custom-made specific primers (Eurogentec, France), an automated fluorescent method based on dye terminator chemistry (AmpliTag DNA polymerase FS Dye Terminator Cycle Sequencing Ready Reaction kit; Applied Biosystems Division, Perkin-Elmer, France), and the ABI-Prism 310 sequencer (Applied Biosystems Division, Perkin-Elmer).

Cloning experiments. The entire nucleotide sequence coding for the SHV enzyme from Kp7 and Kp04 was cloned after PCR amplification using oligonucleotides carrying restriction sites at their ends, that is, the HindIII site in the forward primers and the EcoRI site in the reverse primers (2). Digested amplification products were then ligated into the pBK-CMV cloning vector (Stratagene-Europe/BIOCREST, The Netherlands) cut with the same enzymes. The ligation mixture was used to electrotransform *E. coli* DH5α cells which were subsequently plated onto medium containing ampicillin (100 μg/ml) and kanamycin (50 μg/ml).

TABLE 1. Characteristics of the studied strains of extended-spectrum β-lactamase-producing enterobacteria

Patient	Date (mo/day/yr)	Strain	Bacterial species	Patient origin (hospital site)	Specimen	Acquired β-lactamase(s) and antibiotic type ^a	Profile
							AP- and ERIC-PCR ^b Plasmid restriction
A	01/20/97	Kp97	<i>K. pneumoniae</i>	Neurology (UH1)	Sputum	TEM-3 KTN(A) CHL SSS TMP (TET) OFX	ND ^c ND
B	11/13/97	Ea97	<i>E. aerogenes</i>	Neurology (UH1)	Urine	TEM-24b KTN(A) CHL SSS TMP (TET) OFX	Ea-1 A-2
D	03/27/98	Kp98	<i>K. pneumoniae</i>	Neurology (UH1)	Blood	TEM-3 CHL SSS TMP NAL	ND ND
C1	06/03/99	Ec1	<i>E. coli</i>	Community	Urine	TEM-24b KTN(A) CHL SSS TMP	Ec1 A-1
C1	07/02/04	Pm04	<i>P. mirabilis</i>	Oncology (UH2)	Catheter	TEM-24b KTN(A) CHL SSS TMP TET OFX CS	ND ND
E	08/14/95	Kp95	<i>K. pneumoniae</i>	Gastroenterology (UH4)	Blood	TEM-21 GKTN(A) CHL SSS	ND B
C2	03/29/99	Ec5	<i>E. coli</i>	Community	Urine	TEM-21 TEM-1 GKTN(A) CHL SSS TET OFX	Ec5 B
F	08/05/92	Kp92	<i>K. pneumoniae</i>	Neurology (UH3)	Urine	SHV-4 KTN(A) (CHL) SSS TMP TET OFX	Kp7 C-2
G	01/08/93	Kp93	<i>K. pneumoniae</i>	Neurology (UH3)	Urine	SHV-4 KTN(A) (CHL) SSS TMP TET OFX	Kp7 C-2
C3	02/20/99	Kp7	<i>K. pneumoniae</i>	Community	Urine	SHV-4 TEM-15 KTN(A) (CHL) SSS TMP TET OFX	Kp7 C-1
C3	03/27/04	Kp04	<i>K. pneumoniae</i>	Clinic CLI-1	Sputum	SHV-4 TEM-15 KTN(A) (CHL) SSS TMP TET OFX	Kp7 C-1
C4	05/03/99	Ea1-a	<i>E. aerogenes</i>	Community	Urine	TEM-24b KTN(A) CHL SSS TMP (TET) OFX	Ea-1 A-1
H	03/31/99	Ea99	<i>E. aerogenes</i>	Clinic CLI-1	Urine	TEM-24b KTN(A) CHL SSS TMP (TET) OFX IPM	Ea-1 A-4
C5	04/16/99	Ea1-b	<i>E. aerogenes</i>	Community	Urine	TEM-24b KTN(A) CHL SSS TMP (TET) OFX IPM	Ea-1 A-2

^a K, G, Nt, T, and A, kanamycin, gentamicin, tobramycin, netilmicin, and amikacin, respectively; CHL, chloramphenicol; SSS, sulfonamides; TMP, trimethoprim; TET, tetracycline; OFX, ofloxacin; CS, colistin; IPM, imipenem. Parentheses indicate a low level of resistance. Resistances cotransferred with the extended-spectrum β-lactamase are indicated in boldface type.
^b A single number was assigned according to the concordant results obtained by five molecular typing methods (AP-PCR and ERIC-PCR with primers Ap12h, ERIC-2, ERIC-1R, 208, and 212).
^c ND, not determined.

Molecular typing. AP-PCR was performed using the primer AP12h, and ERIC-PCR was performed using ERIC2 and ERIC1R primers (2) and two additional primers, 208 and 272 (21). The amplification conditions were as follows: 94°C for 5 min and 45 subsequent cycles of 1 min at 94°C, 1 min at 37°C, and 1 min at 72°C, with a final step at 72°C for 10 min.

RESULTS

Clinical analysis. Patient C1 was a 55-year-old woman with type II diabetes and multiple sclerosis having led to paraplegia and the installation of a permanent urinary catheter since the early 1980s. For her disease, she was monitored in the neurology ward of UH1 (last hospitalization before the first ESBLE isolation in April 1997) and received multiple cycles of corticotherapy. This patient had recurrent urinary tract infections (UTIs) due to *E. coli* strain Ec1 elaborating the TEM-24b ESBL during our survey (6 March 1999). For these UTIs, patient C1 received multiple courses of antibiotics (last treatment, ceftriaxone in April 1996). In 2000, patient C1 developed breast cancer, which was followed up in the oncology center UH2 where, in February 2004, an ESBL-producing *Proteus mirabilis* strain (Pm04) was isolated on an indwelling central venous catheter.

Patient C2 was a 64-year-old woman with iron-deficient anemia related to a bulky hiatal hernia for two decades who experienced vascular brain damage in 1996 with sequellar hemiplegia. Thus, she was hospitalized in the neurology ward of UH3 (1996), the rehabilitation center CLI-2 (1996), and the gastroenterology wards of UH4 (1993 and August 1998) and of a clinic (CLI-9) (1986, 1990, 1995, and from December 1998 to January 1999). This incontinent patient carried diapers but had no indwelling urinary catheter. Upon CLI-9 discharge (14 January 1999), a urinalysis revealed the presence of an ESBL-producing *E. coli* strain (unavailable strain), but the patient returned home without an antibiotic prescription. The same *E. coli* (identical antibiotic type) Ec5 strain expressing a TEM-21 enzyme was found during our survey (29 March 1999).

Patient C3 was a 47-year-old man with type II diabetes, who in 1994 developed a paraplegia investigated first in a clinic (CLI-3) and then through seven hospitalizations and multiple consultations in the neurology ward of UH3 between 1995 and 1999, separated by stays in a rehabilitation center (CLI-2) from 1994 to September 1997. Micturitions were ensured by urinary tract self-catheterization since 1994. Subsequently, multiple episodes of UTIs occurred due to an ESBL-producing *K. pneumoniae* strain in January, August, and November 1998. The SHV-4/TEM-15-producing strain *K. pneumoniae* Kp7 was detected in our survey on 20 February 1999. Since 1994, patient C3, chronically treated with steroids, was given multiple courses of antibiotics including gentamicin concomitantly with ESBLE isolation. In February 2000, patient C3 underwent a cystectomy and the setting of a Bricker catheter in CLI-3. The ESBL-producing *K. pneumoniae* strain (same antibiotic type, unavailable isolates) was recovered from four blood cultures, three postoperative wound specimens, and two stool samples. In March 2000, a pulmonary carcinoma was discovered, and, until 2004, patient C3 underwent seven hospitalizations in various UH units and finally in CLI-1. Four urinalyses were positive for ESBL-producing *K. pneumoniae*, the last isolate being Kp04.

Patient C4 was a 68-year-old man with type II diabetes who

accumulated many clinical problems leading to several hospitalizations in various institutions: colic diverticulosis (1993, CLI-10), pancreatitis (1996, CLI-7), and four episodes of vascular brain damage, including two episodes in 1993 (neurology ward of UH3 and CLI-2) and June 1998 (neurology ward of UH1), where he received amoxicillin-clavulanate for a febrile peak, the last antimicrobial chemotherapy noted before ESBLE isolation. Since 1993, his micturitions required external manipulation. During our survey, a urine culture yielded the TEM-24b-producing *E. aerogenes* strain Ea1-a (3 May 1999).

Patient C5 was a 73-year-old asthmatic woman who was monitored for respiratory insufficiency in a day care center (CLI-11) since 1994 and was hospitalized in March 1998 in CLI-1 for severe acute bronchial superinfection and cardiac insufficiency, where she experienced a UTI due to an ESBL-producing *Serratia marcescens* strain. After recovery, she spent 3 weeks in a rest home (CLI-12), where she received amoxicillin for a bronchial superinfection, the last antibiotic treatment mentioned before ESBLE isolation. In the context of our survey, the imipenem-resistant TEM-24b-producing *E. aerogenes* strain Ea1-b was detected (4 April 1999).

Strain comparison. Patients C1 and C4, before isolation of Ec1 and Ea1-a, respectively, were hospitalized in the neurology ward of UH1, where three ESBLE were recovered between 1995 and 1999: Kp97, Ea97, and Kp98. Patient C2 was admitted 2 months prior to the isolation of Ec5 in CLI-9, where no ESBLE were found during our 1999 survey. However, she was previously hospitalized in the gastroenterology ward of UH4, where a single ESBLE, Kp95, was collected during the preceding 10-year period. Patient C3 received follow-up care in the neurology ward of UH3 before the isolation of Kp7. During a survey in UH3 at the beginning of the 1990s (4), two ESBL-producing *K. pneumoniae* strains were collected in this unit, Kp92 and Kp93. Patient C5, prior to the isolation of the imipenem-resistant strain Ea1-b, was hospitalized in CLI-1, where the imipenem-resistant strain Ea99 was recovered during our survey.

ESBL characterization. All strains included in this study produced an ESBL as detected by the disk synergy test and confirmed by β -lactam MIC determination (Table 2). Transconjugants (Tc) producing ESBLs were obtained for all strains except for Kp7 and Kp04 from patient C3. Variations in resistance profiles between parental strains and their transconjugants are likely related to the genetic background of each species. With regard to *E. coli*, higher MICs of amoxicillin-clavulanate in the clinical strains are probably due to a higher expression of the chromosomal AmpC enzyme. The β -lactamase contents of the clinical strains and their transconjugants were first analyzed by isoelectric focusing. Six strains (Ec1, Pm04, and the four *E. aerogenes* strains) harbored a single ESBL with a pI value of 6.5, and two strains each harbored an ESBL with a pI value of 6.4 (Ec5 and Kp95), 6.3 (Kp97 and Kp98), or 7.8 (Kp92 and Kp93). Both Kp7 and Kp04 exhibited two ESBL with pI values of 6.0 and 7.8, respectively. The clinical donor strains occasionally presented additional bands consistent with species-specific β -lactamases (chromosomal SHV-1 penicillinase with a pI value of 7.6 in *K. pneumoniae* or cephalosporinase with a pI value of >8.0 in *E. aerogenes*) or acquired TEM-1 enzyme (pI 5.4 in Ec5).

TABLE 2. MICs of β-lactam antibiotics alone or in combination with clavulanic acid for clinical strains, their transconjugants, or recombinant clones

Strain ^b	β-Lactamase content	MIC (mg/liter) ^a												
		AMX	AMX CLA	TIC	TIC CLA	CEF	FOX	CTX	CTX CLA	CAZ	CAZ CLA	ATM	ATM CLA	IPM
<i>E. coli</i> + Tc														
Ec1	TEM-24b	>512	32	>512	32	>512	8	2	0.1	512	2	64	0.05	0.1
TcEc1	TEM-24b	512	2	512	16	16	4	0.5	0.02	128	0.5	8	0.02	0.1
Ec5	TEM-21 + TEM-1	>512	32	>512	64	256	4	16	0.05	32	0.2	8	0.02	0.1
TcEc5	TEM-21	>512	8	>512	16	128	4	8	0.05	32	0.2	8	0.02	0.1
<i>P. mirabilis</i> + Tc														
Pm04	TEM-24b	256	1	128	0.5	32	4	0.5	0.02	16	0.05	≤0.1	≤0.01	0.1
TcPm04	TEM-24b	>512	4	>512	16	16	4	0.5	0.02	128	0.5	8	0.02	0.1
<i>K. pneumoniae</i> + Tc or Rc														
Kp92	SHV-4	>512	2	>512	32	512	32	8	0.1	128	0.5	256	0.05	0.1
TcKp92	SHV-4	>512	2	512	8	64	4	1	0.02	32	0.1	32	0.02	0.1
Kp93	SHV-4	>512	8	>512	32	512	32	8	0.1	128	1	128	0.05	0.1
TcKp93	SHV-4	>512	4	>512	8	128	4	2	0.02	16	0.1	16	0.02	0.2
Kp95	TEM-21	>512	4	>512	32	128	4	8	0.02	16	0.2	4	0.01	0.1
TcKp95	TEM-21	>512	4	>512	8	64	4	4	0.02	8	0.2	2	0.02	0.1
Kp97	TEM-3	>512	4	>512	32	256	16	8	0.1	64	1	16	0.05	0.05
TcKp97	TEM-3	>512	4	>512	4	64	4	2	0.02	8	0.2	4	0.02	0.1
Kp98	TEM-3	>512	4	>512	32	128	8	8	0.05	32	0.5	8	0.02	0.1
TcKp98	TEM-3	>512	8	>512	8	64	4	4	0.02	8	0.2	2	0.02	0.1
Kp7	SHV-4 + TEM-15	>512	2	>512	16	256	16	4	0.1	64	0.5	128	0.05	0.1
RcKp7	SHV-4	>512	2	>512	2	64	4	4	0.01	32	0.1	32	0.01	0.1
Kp04	SHV-4 + TEM-15	>512	4	>512	16	512	16	8	0.1	128	0.5	256	0.05	0.05
RcKp04	SHV-4	>512	4	>512	1	64	2	4	0.01	64	0.1	32	0.01	0.1
<i>E. aerogenes</i> + Tc														
Ea97	TEM-24b	>512	256	>512	64	>512	>512	8	4	512	16	64	8	0.1
TcEa97	TEM-24b	>512	2	>512	8	32	2	0.5	0.02	128	0.5	8	0.02	0.05
Ea99	TEM-24b	>512	512	>512	128	>512	>512	128	128	>512	256	256	128	4
TcEa99	TEM-24b	>512	4	>512	8	32	4	0.5	0.02	128	0.5	8	0.02	0.1
Ea1-a	TEM-24b	>512	256	>512	32	>512	>512	4	2	256	8	128	16	0.1
TcEa1-a	TEM-24b	>512	8	>512	8	16	8	0.5	0.02	128	0.5	8	0.02	0.1
Ea1-b	TEM-24b	>512	512	>512	256	>512	>512	32	32	512	32	64	16	4
TcEa1-b	TEM-24b	>512	4	>512	8	32	4	0.5	0.02	128	0.5	8	0.02	0.1
<i>E. coli</i> recipient strain														
K-12		4	2	2	2	4	1	≤0.1	0.02	0.2	0.2	≤0.1	0.02	0.1
DH5α		4	2	2	1	2	1	≤0.1	0.01	0.1	0.1	≤0.1	0.01	0.1

^a AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; TIC, ticarcillin; CEF, cephalothin; FOX, ceftoxitin; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; IPM, imipenem. Clavulanic acid (CLA) was used at a fixed concentration of 2 mg/liter.
^b Tc, transconjugant; Rc, recombinant clone.

According to the pIs of the ESBLs, PCR experiments were performed using crude DNA extracts from the clinical strains and their transconjugants (TcEc5) and primers specific for the TEM- or SHV-encoding genes, and then the amplicons were directly sequenced. The pI 7.8 ESBL-encoding gene of Kp7 and Kp04 has been amplified by PCR with *bla*_{SHV}-specific primers and cloned before sequencing. Sequence analysis demonstrated that *E. coli* Ec1, *P. mirabilis* Pm04, and all strains of *E. aerogenes*, i.e., Ea97, Ea99, Ea1-a, and Ea1-b, contained an identical *bla*_{TEM-24b} gene, differing from *bla*_{TEM-24} by the silent mutation T682C (15). Kp97 and Kp98 harbored the *bla*_{TEM-3} gene and were considered unrelated to the ESBL from the community. The transconjugants of *E. coli* Ec5 and *K. pneumoniae* Kp95 had the same nucleotide sequence of the *bla*_{TEM-21} gene (32). Finally, Kp92, Kp93, and the recombinant clones from Kp7 and Kp04 expressing the ESBL with a pI value of 7.8 exhibited an identical *bla*_{SHV-4} gene. The TEM amplicons from Kp7 and Kp04 were revealed to be a *bla*_{TEM-15} gene.

Epidemiological typing. The strains were first compared with regard to their antibiotic resistance phenotypes (Table 1). They exhibited marked differences, particularly in gentamicin, chloramphenicol, and cotrimoxazole resistances. Moreover,

they had different susceptibilities to ceftazidime and cefotaxime as confirmed by MIC determination (Table 2).

ESBLE belonging to the same species were then typed by AP-PCR and ERIC-PCR using five different primers. Results obtained were strictly concordant, making it possible to assign a single molecular type for each strain (Table 1). All TEM-24b-producing *E. aerogenes* strains exhibited the molecular type Ea1, corresponding to the epidemic clone disseminated among the French hospitals at the end of the 1990s (2, 7) (Fig. 1A). The TEM-24b-producing *E. coli* strain Ec1 and the TEM-21-expressing *E. coli* strain Ec5 gave clearly different profiles (Fig. 1B). The four *K. pneumoniae* strains elaborating the SHV-4 enzyme yielded a strictly identical pattern (molecular type Kp7) (Fig. 1C).

Finally, plasmid analysis was performed for all ESBL-producing the same ESBL. All transconjugants expressing the TEM-24b enzyme exhibited the same cotransferred resistances (Table 1), and their plasmids, digested by EcoRI, gave three related profiles, A-1 (TcEc1 and TcEa1-a), A-2 (TcPm04, TcKp97, and TcEa1-b), and A-4 (TcEa99) (Fig. 2A), consisting of five or six bands, four of which were identical. All transconjugants elaborating the TEM-21 ESBL possessed the same cotransferred resistances, and their plasmids, restricted by

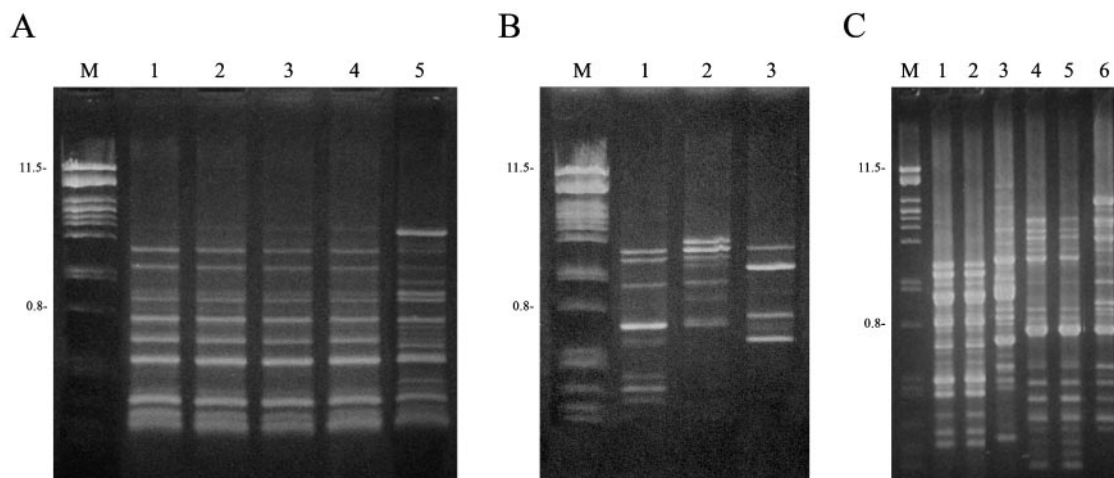


FIG. 1. AP-PCR and ERIC-PCR profiles of ESBLE. (A) AP-PCR profiles obtained with the primer ERIC-2 from TEM-24b-producing *E. aerogenes* strains Ea1-a (lane 1), Ea1-b (lane 2), Ea97 (lane 3), and Ea99 (lane 4) and from an unrelated strain of *E. aerogenes* (lane 5). (B) AP-PCR profiles from *E. coli* Ec1 (lane 1) and Ec5 (lane 2) obtained with the primer AP12h. (C) AP-PCR profiles obtained with primer 208 (lanes 1 to 3) and primer 272 (lanes 4 to 6) from Kp7 (lanes 1 and 4, respectively) and Kp04 (lanes 2 and 5) and from an unrelated strain of *K. pneumoniae* (lanes 3 and 6). M, size ladder (DNA of λ phage digested by PstI). Sizes are indicated in kilobases.

EcoRI, led to the identical profile B (Fig. 2B). The undigested plasmid profiles of the four clinical strains of SHV-4-producing *K. pneumoniae* comprised a single band for Kp92 and Kp93 and two additional bands for Kp7 and Kp04 (Fig. 3A). The EcoRI restriction plasmid profile C-1 of Kp7 and Kp04 shared many common bands with those of Kp92 and Kp93 (profile C-2) (Fig. 3B). The additional bands observed in the C-1 profile might be related to the presence of an additional plasmid(s), one possibly encoding the TEM-15 enzyme.

DISCUSSION

All five community patients (three females and two males, 47 to 73 years old) had predisposing factors for acquiring ESBLE (8, 30), including age over 60 years (C2, C4, and C5), diabetes (C1, C3, and C4), or neurological diseases (C1 and C3), as well as bed-ridden conditions (C1 and C3) (6). The comprehensive analysis of their medical files revealed that their underlying diseases were associated with a past history of hospitalization, recognized as the major cause of ESBLE infection (8). In addition, from molecular comparison with strains collected from hospital wards and private facilities where these patients previously resided, an epidemiological link between ESBLE of both community and hospitalized patients could be established, in contrast with results of a previous study (30). Although the source of each pathogen could not be definitely identified due to the absence of urinalysis during hospitalization (C1, C2, and C4) or the absence of storage of the isolated ESBLE (C3 and C5), our clinical and molecular analysis strongly argues for a nosocomial acquisition of ESBLE or ESBL-encoding plasmids. Thus, the neurology ward of UH1 was probably the source of the ESBLE or the ESBL-encoding plasmid of patients C1 and C4. Indeed, before ESBLE isolation, these patients were last hospitalized in this ward, and one TEM-24b-producing *E. aerogenes* strain belonging to the same clone as the ESBLE of patient C4 and containing the same encoding plasmid as the ESBL-producing *E.*

coli strain isolated from patient C1 was present in this unit before their hospitalization. The clone of TEM-24b, which for a decade now has led to an *E. aerogenes* epidemic in French hospitals, emerged in our region in the early 1990s (1) and has spread in hospitals and in private institutions such as clinic CLI-1 (2). Since then, due to the high transferability of the evolutive TEM-24b-encoding plasmid (25), TEM-24b-produc-

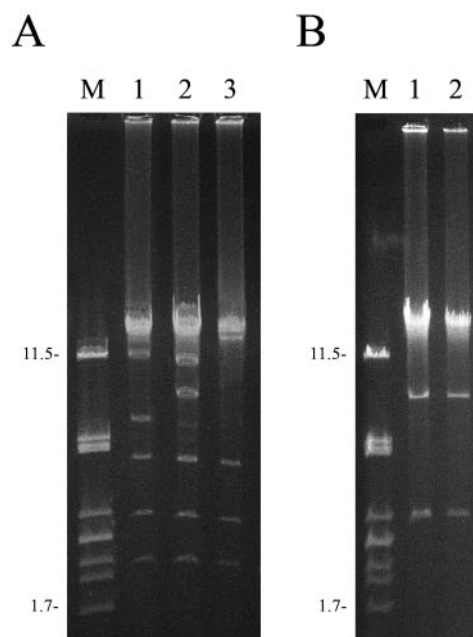


FIG. 2. EcoRI restriction profiles of TEM-24b (A)- and TEM-21 (B)-encoding plasmids. (A) Profile A-1 from the transconjugant of Ec1 (lane 1), profile A-2 from the transconjugant of Ea97 (lane 2), and profile A-4 from the transconjugant of Ea99 (lane 3). (B) Profile B from transconjugants of Ec5 and Kp95 (lanes 1 and 2, respectively). M, size ladder (DNA of λ phage digested by PstI).

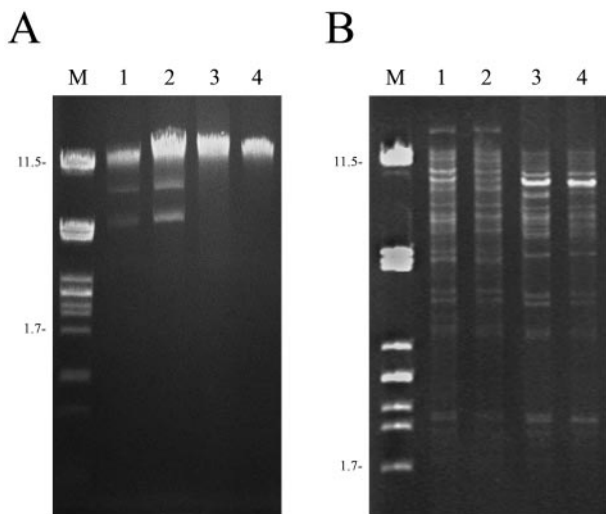


FIG. 3. Undigested plasmid profiles (A) and EcoRI restriction profiles (B) of SHV-4-encoding plasmids. (A) Undigested plasmid profiles from Kp7 (lane 1), Kp04 (lane 2), Kp92 (lane 3), and Kp93 (lane 4). (B) Profile C-1 from Kp7 and Kp04 (lanes 1 and 2, respectively) and profile C-2 from transconjugants of Kp92 and Kp93 (lanes 3 and 4, respectively). M, size ladder (DNA of λ phage digested by PstI).

ing strains belonging to other members of the family *Enterobacteriaceae* have increasingly been encountered (2). The TEM-24b-producing *E. aerogenes* of patient C5 most likely originated from the private clinic CLI-1. Indeed, (i) she was infected by an ESBLE during her stay, (ii) this institution had the highest incidence of ESBLE in our survey (8,7%; 11 TEM-24b-producing strains, including 9 *E. aerogenes* strains belonging to the epidemic clone, harboring plasmids with four related profiles, including A-2 as the ESBLE of patient C5), and (iii) the second imipenem-resistant ESBL-producing *E. aerogenes* of our survey was recovered from this clinic (2). Imipenem-resistant *E. aerogenes* strains are rare (< 5%), but such mutants may arise under treatment and then propagate even in patients who were never treated with this antibiotic and within the community as feared (30) and reported here for patient C5. Patient C2 might have been contaminated by the ESBLE or its epidemic ESBL-encoding plasmid in the gastroenterology ward of UH4, where she sojourned prior to ESBLE isolation. Actually, the single ESBLE isolate collected during the preceding 10 years in this ward carried the same TEM-21-encoding plasmid as the ESBLE of patient C2. The TEM-21 enzyme has rarely been identified in France but seems to be widespread in our region (2, 13, 32). Patient C3 almost certainly acquired the ESBLE during one of his multiple hospitalizations in the neurology ward of UH3. A survey in the early 1990s (4) showed that different *K. pneumoniae* strains carrying distinct SHV-4-encoding plasmids had disseminated in UH3. Two strains, confined to the neurology ward, were proven to be identical to the ESBLE of patient C3 and to exhibit a related plasmid profile.

Of note, the last hospitalizations of these patients occurred 2½ to 23 months prior to ESBLE isolation. Thus, our data demonstrate that an absence of hospitalization during the 3 (8, 12), 6 (14), or even 12 (30) preceding months does not guarantee that ESBLE are “community acquired,” as is sometimes stated (6, 19). Accordingly, 55.5% of the ESBLE-positive pa-

tients had not been hospitalized in the past 3 months in the study of Colodner et al. (8), and 45% had not been hospitalized in the preceding year in the study of Rodriguez-Baño et al. (30). In the study of Lescure et al. (19), four out of seven ESBLE carriers had not stayed in their own hospital within the five preceding years. However, private clinics, such as nursing homes (26, 34), can act as ESBLE reservoirs, as shown by the history of patient C5.

Antimicrobial therapy in the past 3 months (8, 14, 33), especially with β -lactams and quinolones (14), has been identified as the second risk factor for acquiring ESBLE (8). In this study, the association between antimicrobial therapy and ESBL acquisition was unclear. Indeed, four patients (C1 and C3 to C5) previously received antibiotics (concomitantly to 35 months earlier), but a single one (C3) received antibiotics during the previous 3 months to cure the ESBLE-induced UTI. In the study of Rodriguez-Baño et al. (30), 67% of the patients infected with ESBLE had antimicrobial treatment in the last 2 months, and 63% had more than two cycles of antimicrobial treatment in the preceding year. Nevertheless, the possibility that some antimicrobial therapies were not recorded for our patients cannot be eliminated.

Typically, enterobacteria, including ESBLE, act as uropathogens in the community as in the hospital (29, 30). Two patients (C1 and C3) had risk factors for UTIs and multiple episodes of UTIs, and three (C2, C3, C5) formerly presented UTIs due to ESBLE. The latter information, which is essential for case interpretation, rarely appears in this kind of study (8, 30).

The digestive tract is the reservoir of most uropathogens, and gastrointestinal colonization usually precedes UTIs due to ESBLE (31). A 2-year ESBLE carriage had been previously demonstrated in a hospitalized patient (3). In this study, patient C3 remained a digestive tract carrier of the same ESBLE for at least 5 years as demonstrated by (i) the intermittent isolation of the same ESBLE over this period, including two positive stool samples, and (ii) the identical antibody, SHV-4 production, molecular type profile, and restriction plasmid pattern of the two available ESBLE isolated from this patient in February 1999 and March 2004, respectively. Patient C1 may have continued to carry an evolutive TEM-24b-encoding plasmid over an almost 5-year period, although reinfection cannot be ruled out, since the two available ESBLE isolated from this patient in March 1999 and February 2004, respectively, expressed the same TEM-24b enzyme but belonged to different species and contained closely related but distinct plasmids. The other patients are suspected to have carried their ESBLE and/or the ESBL-encoding plasmid for at least 7 to 23 months, i.e., the time elapsed between the suggested contaminating hospital or clinic stay and the date of ESBLE isolation. Once established in the digestive tract, the ESBLE have no more reason to be eliminated from the commensal flora than other enterobacteria, particularly when patients receive antibiotics (16, 30, 31).

Thus, our findings contradict the data reported previously by Rodriguez-Baño et al. (30), who found no epidemiological relationship between patients infected with isolates producing the same type of ESBL and who concluded that a horizontal transfer of ESBLE during a previous hospital stay was improbable because they found no clonal relationship among the

strains obtained from their patients and those obtained from patients with nosocomially acquired ESBL. However, their investigation was restricted to ESBL-producing *E. coli*, the history of the patients was limited to the preceding year, plasmids were not analyzed, and hospital strains collected for years as strains from private institutions were not compared. In contrast, our conclusions are consistent with one recent study from England which showed that community and hospital patients were simultaneously infected with related ESBL and/or strains possessing the same ESBLs (35).

In conclusion, our data indicated that community-acquired UTIs due to ESBL in five patients likely resulted from a nosocomial acquisition of these organisms or their ESBL-encoding plasmids, followed by a prolonged digestive carriage. However, if control measures similar to those implemented in hospitals are not taken, the community reservoir will grow and ESBL will also become community acquired.

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