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Molecular Epidemiology of *Klebsiella pneumoniae* Strains That Produce SHV-4 β-Lactamase and Which Were Isolated in 14 French Hospitals

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Preliminary results suggested that the diffusion in France of the SHV-4 extended-spectrum β-lactamase was probably due to the spread of one single epidemic strain of *Klebsiella pneumoniae*. In this study, we tested various phenotypic and genotypic markers to compare *K. pneumoniae* strains producing this enzyme isolated in 14 French hospitals between 1987 and 1989. All of the strains were of the same capsule serotype, K25. Twelve of them were of the same biotype: weak urease activity and no sucrose fermentation. Among the six plasmid profiles observed, one accounted for eight strains. Large plasmids of 170 kb encoding SHV-4 β-lactamase were present in all strains of *K. pneumoniae* and could be transferred by conjugation with high frequency to *Escherichia coli* J53-2 or HB101 from all except one strain. Plasmid *Eco*RI restriction patterns suggested that these plasmids were closely related and similar to pUD18 encoding SHV-3 β-lactamase, originally described in France and differing from SHV-4 by one amino acid substitution. Ribotyping with *Eco*RI and *Hin*dIII and genomic fingerprinting with *Xba*I by pulsed-field gel electrophoresis were concordant and suggested that 12 of the isolates recovered from the 14 hospitals were probably the same strain. Dissemination in France of the SHV-4 extended-spectrum β-lactamase was thus essentially due to the diffusion of a single *K. pneumoniae* clone.

Acquired resistance to third-generation cephalosporins and aztreonam is a recent clinical problem. This now-widespread resistance results from the production of enzymes derived from TEM- or SHV-type β-lactamases by one or more amino acid substitutions (15, 30). These extended-spectrum β-lactamases have been identified most often among strains of *Klebsiella pneumoniae* responsible for outbreaks of nosocomial infections particularly in intensive care units but also in oncology units and chronic care facilities (3, 4, 6, 7, 15, 17, 18, 24, 25, 31). Outbreaks due to *K. pneumoniae* producing TEM-derived enzymes are usually limited to only one ward or hospital. However, TEM-3 β-lactamase is widespread in France as a result of the dissemination of a plasmid carrying the corresponding gene (29). Among the SHV-type-derived enzymes, SHV-2 and SHV-5 are the most common internationally (14, 15, 18, 30), and yet outbreaks due to *K. pneumoniae* or *Salmonella enterica* producing these enzymes have been limited to one or two hospitals (4, 14, 18). SHV-3 and SHV-4 seem to be a particular problem in France. *K. pneumoniae* strains producing SHV-3 β-lactamase were first described in a Parisian hospital where they were responsible for an outbreak of nosocomial infections (17). The first strain of *K. pneumoniae* producing SHV-4 β-lactamase was isolated from one patient coming from the same hospital. In the following months, multiresistant *K. pneumoniae* strains producing the same extended-spectrum β-lactamase, belonging to capsule serotype K25, were isolated in clusters in five hospitals in the Paris area (8). We subsequently observed in our hospital an outbreak of nosocomial infections caused by *K. pneumoniae* strains producing this β-lactamase (3). Capsule serotyping, biotyping, antibiotic resistance phenotyping, plasmid profiles, and plasmid restriction patterns suggested that a single strain (K25) was responsible for this intrahospital outbreak (3). Similar observations were reported by de Champs et al. (10) for *K. pneumoniae* producing CAZ-5 β-lactamase (an enzyme very similar to SHV-4) in another French hospital. The diffusion of TEM-3 β-lactamase is mainly the result of the dissemination of plasmids (7, 10, 19, 23, 29, 34). In contrast, the interhospital diffusion of the SHV-4 β-lactamase seems to be due to the propagation of a single bacterial strain. To analyze this epidemic phenomenon, we used various molecular methods to compare *K. pneumoniae* isolates producing SHV-4 β-lactamase recovered from 14 French hospitals.

MATERIALS AND METHODS

Bacterial strains and plasmids. Fourteen clinical isolates of *K. pneumoniae*, isolated in France during 1987 and 1988, producing SHV-4 β-lactamase were examined (Table 1): 12 were isolated in 12 hospitals in Paris, France, and the Parisian suburbs, and two came from other areas (Tours and Nice, France). Each strain was the first SHV-4 β-lactamase-producing *K. pneumoniae* strain isolated in each hospital. Isolates were identified by using the API 20E system (BioMérieux SA, Marcy l'Etoile, France). Capsular serotyping (32) was done at the Institut Pasteur (Paris, France). *Escherichia coli* HB101 resistant to streptomycin and *E. coli* K-12 J 53-2 resistant to rifampin were used as recipients for conjugation experiments. p453 (SHV-1), pCFF04 (TEM-3), and pUD18 (SHV-3) were used for plasmid analysis.

Antimicrobial susceptibility. Susceptibility to antimicrobial agents was tested by the disk diffusion method on Mueller-Hinton agar (Sanofi Diagnostics Pasteur S.A., Marnes-la-Coquette, France) as described in the recommendations of the Comité de l'Antibiogramme de la Société Française de Microbiologie (1). Extended-spectrum β-lactamase was detected by

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using the double-disk synergy test (21). MICs of various β-lactam antibiotics, including cefotaxime, ceftazidime, and aztreonam, in the presence of clavulanic acid (2 μg/ml) were determined by using serial twofold dilutions of antibiotics in Mueller-Hinton agar and inocula of about 10^5 CFU per spot.

Conjugation. Matings were performed by mixing equal volumes (1 ml) of exponentially growing cultures of *K. pneumoniae* and *E. coli* as described elsewhere (3). Transconjugants were selected on plates supplemented with rifampin (100 μg/ml) or streptomycin (500 μg/ml) and ceftazidime (2 μg/ml) or kanamycin (25 μg/ml). The frequency of transfer was expressed relative to the number of donor cells.

β-Lactamase analysis. Analytical isoelectric focusing was performed on polyacrylamide gels (17) with crude cell sonic extracts. β-Lactamase activity was detected by the iodometric method with ceftriaxone (20 mg/100 ml of gel) (17) and then by the classic chromogenic nitrocefin test. SHV-2 (pBP60-1; pI 7.6), SHV-3 (pUD18; pI 7.0), and SHV-4 (pUD21; pI 7.8) were used as pI markers.

Preparation and hybridization of plasmid DNA. Plasmid DNA was extracted from *K. pneumoniae* by the method of Wheatcroft and Williams (40). Plasmid DNA was extracted from *E. coli* transconjugants by the method of Takahashi and Nagano (37) and digested with *Eco*RI as described in the recommendations of the supplier (Boehringer Mannheim France S.A., Meylan). Digested and nondigested DNA samples were analyzed by electrophoresis on 0.7% agarose gels in Tris acetate buffer (Seakem GTG; FMC Bioproducts, Rockland, Maine). DNA was transferred by the Southern method (36) onto a nylon membrane (GeneScreen Plus; New England Nuclear Products, Boston, Mass.) and probed for hybridization under stringent conditions (65°C; 1 M NaCl, 1% sodium dodecyl sulfate, [SDS] 10% dextran sulfate) with an intragenic DNA probe specific for the SHV-type β-lactamase prepared by PCR as described previously (2).

Ribotyping. Total DNA of *K. pneumoniae* was prepared as described by Grimont and Grimont (11). After digestion with *Eco*RI and *Hin*dIII (Boehringer Mannheim), the digested DNA was electrophoresed at 25 V for 18 h in an 0.7% agarose gel (Seakem GTG; FMC Bioproducts) in Tris acetate buffer. The DNA was transferred by the method of Southern onto a nylon membrane and probed for hybridization under stringent conditions as described above. The probe used was cDNA from ribosomal 16S and 23S from *E. coli* (Boehringer Mannheim) prepared with a cDNA Synthesis Kit (Boehringer Mannheim) and labeled with [α-^{32}P]dCTP by random priming (Boehringer Mannheim). Phage lambda DNA digested with *Hin*dIII and labeled by the same method was used as a molecular weight marker.

Genome fingerprinting by pulsed-field gel electrophoresis (PFGE). Chromosomal DNA was prepared as described previously (35). Overnight cultures were harvested and resuspended in Pett IV solution (1 M NaCl, 10 mM Tris [pH 7.6]). The suspensions were mixed with an equal volume of 1.6% Incert agarose (FMC Bioproducts) and allowed to solidify in 100-μl molds. Plugs were incubated in 1 ml of lysis buffer (6 mM Tris [pH 7.6], 1 M NaCl, 0.1 M EDTA, 0.5% Brij 58, 0.4% deoxycholate, 0.5% Sarkosyl; Sigma Chimie, Saint Quentin Fallavier, France) with 1 mg of lysozyme per ml and 20 μg of DNase-free RNase (Boehringer Mannheim) per ml. After 18 h at 37°C, the lysis buffer was removed and the plugs were incubated in 1 ml of ESP buffer (0.5 M EDTA [pH 9], 1% Sarkosyl) supplemented with 50 μg of proteinase K (Boehringer Mannheim) per ml for 18 h at 50°C. The plugs were then washed four times with 1 ml of TE buffer (10 mM Tris [pH 8], 1 mM EDTA [pH 8]) and digested with 40 U of *Sma*I or *Xba*I for 6 h as described in the recommendations of the supplier (Boehringer Mannheim). Fragments of DNA were separated by PFGE in a 1% agarose gel (Agarose Gold; FMC Bioproducts) in TBE buffer (0.0445 M Tris [pH 8], 0.0445 M boric acid, 0.001 M EDTA [pH 8]) at 12°C by using the Pulsaphor system (Pharmacia France S.A., Saint-Quentin en Yvelines, France). The electrophoresis conditions were 200 V for 16 h with pulse times ranging from 10 to 25 s. The DNA size marker was a lambda ladder (Bio-Rad S.A., Ivry sur Seine, France). After electrophoresis, gels were stained with ethidium bromide, washed, and photographed under UV light.

RESULTS

Phenotypic analysis and antimicrobial susceptibility. All 14 *K. pneumoniae* strains belonged to the same capsule serotype K25. All strains except two (strains 5 and 7) were classified into the same particular biotype by the API 20E system: they had a weak urease activity and did not ferment sucrose.

All of the clinical isolates of *K. pneumoniae* were resistant to amoxicillin, ticarcillin, and piperacillin (MICs, ≥128 μg/ml) and to ceftazidime, aztreonam, and cefotaxime (90% MICs, 128, 128, and 16 μg/ml, respectively). In the presence of 2 μg of clavulanic acid per ml, the susceptibility of all strains to ceftazidime, cefotaxime, and aztreonam was restored (90% MICs, 2, 0.5, and 0.25 μg/ml, respectively). All strains were susceptible to moxalactam (MICs, ≤0.5 μg/ml) and imipenem (MICs, ≤0.25 μg/ml). Antimicrobial susceptibility tests by the disk diffusion method indicated strong synergy between amoxicillin or clavulanic acid and either cefotaxime, ceftazidime, or aztreonam for all of the *K. pneumoniae* isolates. The resistance patterns given by the disk diffusion test could be classified into six groups. One group was predominant and included nine strains (with resistance phenotype 1 [RP1]): decreased susceptibility to cefoxitin, resistance to aminoglycosides (except gentamicin), tetracycline, trimethoprim, and fluoroquinolones, and decreased susceptibility to chloramphenicol. RP2 (strain 3) differed from the RP1 only by susceptibility to all aminoglycosides. RP3 (strain 5) differed from RP1 by susceptibility to cefoxitin and quinolones and by a high level of resistance to chloramphenicol. RP4 (strain 7) differed from RP3 by susceptibility to chloramphenicol. RP5 (strain 9) differed from RP1 only by susceptibility to tetracycline. RP6 (strain 14) differed from RP1 only by susceptibility to trimethoprim.

Conjugation experiments. All *K. pneumoniae* strains except one (strain 14) transferred oxyimino β-lactam antibiotic resistance to *E. coli* K-12 with high frequency (10^{-4}) in mating experiments for all. For 11 strains, resistance markers cotransferred were aminoglycosides (except gentamicin), tetracycline, and trimethoprim (Table 1). For two strains, one of these markers was not cotransferred. The results of transfer of kanamycin resistance were similar except for one strain (no transconjugants for strain 3) and for frequency (10^{-2} to 10^{-3}).

Isoelectric focusing. By using the nitrocefin procedure, two major bands of β-lactamase activity with pIs of 7.6 (corresponding to chromosomal β-lactamase of *K. pneumoniae*) and 7.8 (with higher activity) were detected in all *K. pneumoniae* strains (data not shown). An additional band of activity was detected in one strain (pI 5.4) (Table 1). By using the iodometric technique, with ceftriaxone as the substrate, only one band of activity with a pI of 7.8 was detected in *K. pneumoniae* strains and in *E. coli* transconjugants selected on ceftazidime. This band corresponded to the SHV-4 extended-spectrum β-lactamase. Biochemical identification of some of isolates was previously reported (3, 8).

Plasmid analysis. Six different plasmid profiles were ob-

TABLE 1. Origin, characteristics,[a] and molecular characterization of *K. pneumoniae* strains producing SHV-4 β-lactamase

Strain	Date of isolation (mo/yr)	City	Hospital	Resistance phenotype[b]	β-lactamase pIs	Plasmid profile[b]	Resistances cotransferred with SHV-4 β-lactamase[c]	Ribotype		Genomic *Xba*I restriction pattern[d]
								*Eco*RI[b]	*Hin*dIII[d]	
1	6/87	Paris	Broca[e]	RP1	7.7, 7.8	P1	K, T, N, A, Tc, Tp	E1	H1a	X1a
2	9/87	Créteil	H. Mondor	RP1	7.7, 7.8	P1	K, T, N, A, Tc, Tp	E2	H1a	X1a
3	9/87	Paris	C. Bernard	RP2	7.7, 7.8	P1	Tc, Tp	E1	H1a	X1a
4	9/87	Paris	Saint-Louis	RP1	7.7, 7.8	P2	K, T, N, A, Tc, Tp	E1	H1a	X1b
5	9/87	Paris	Pitié-Salpétrière	RP3	5.4, 7.7, 7.8	P3	K, T, N, A, Tc, Tp	E3	H2	X2
6	10/88	Paris	Lariboisière	RP1	7.7, 7.8	P1	K, T, N, A, Tc, Tp	E1	H1b	X1c
7	12/88	Paris	Necker	RP4	7.7, 7.8	P4	K, T, N, A, Tc, Tp	E4	H3	X3
8	11/87	Garches	R. Poincaré	RP1	7.7, 7.8	P1	K, T, N, A, Tc, Tp	E1	H1a	X1d
9	2/89	Ivry/seine	C. Foix	RP5	7.7, 7.8	P1	K, T, N, A, Tp	E1	H1a	X1e
10	12/87	Corbeil	Corbeil	RP1	7.7, 7.8	P5	K, T, N, A, Tc, Tp	E1	H1a	X1a
11	3/89	Tours	Bretonneau	RP1	7.7, 7.8	P1	K, T, N, A, Tc, Tp	E1	H1b	X1a
12	2/89	Nice	Pasteur	RP1	7.7, 7.8	P6	K, T, N, A, Tc, Tp	E1	H1a	X1f
13	12/87	Villejuif	P. Brousse[f]	RP1	7.7, 7.8	P2	K, T, N, A, Tc, Tp	E1	H1a	X1a
14	12/87	Villejuif	G. Roussy[g]	RP6	7.7, 7.8	P1	No transconjugant	E1	H1b	X1g

[a] All strains were of capsular serotype K25. By using the API 20E system, all strains except two (strains 5 and 7) were determined to have a weak urease activity and to not ferment sucrose.
[b] See Results for details.
[c] K, kanamycin; T, tobramycin; N, netilmicin; A, amikacin; Tc, tetracycline; Tp, trimethoprim.
[d] See Results and figures for details.
[e] Origin of patient, Hospital Pitié-Salpétrière.
[f] Origin of patient, Greece.
[g] Origin of patient, Hospital of Compiègne.

served among the *K. pneumoniae* strains (Fig. 1; Table 1). One of these, P1, was common to eight strains and corresponded to the presence of a single large plasmid. In the other strains, in addition to a large plasmid, plasmids with lower molecular weights were detected (Fig. 1). P2 (strains 4 and 13) and P5 (strain 10) corresponded to the presence of an additional very small plasmid; P3 and P4 were characterized by the presence of additional plasmids of about 70 kb (strain 5) and 150 kb (strain 7), and P6 (strain 12) was characterized by the presence of an additional plasmid of about 40 kb. In *E. coli* transconjugants, acquisition of resistance to oxyimino-β-lactam antibiotics was correlated with the transfer of the large plasmids. After digestion with *Eco*RI of these plasmids (in *E. coli* transconjugants and in *K. pneumoniae* strain 14), the restriction profiles observed were similar and comparable to that of pUD18 (encoding the SHV-3 β-lactamase) (Fig. 2A). The sizes of these plasmids were all estimated to be between 160 to 180 kb. Some differences, especially additional DNA fragments, were noted: one of about 17 kb in the transconjugant of *K. pneumoniae* 2 and in *K. pneumoniae* 14, one of about 10 kb in the transconjugant of *K. pneumoniae* 10, and one of about 2.5 kb in the transconjugants of *K. pneumoniae* 8, 9, and 12. Hybridization with the intragenic DNA probe mapped the gene encoding the SHV-4 β-lactamase to a DNA fragment of about 13 kb, common to all plasmids and to pUD18 (Fig. 2B).

Ribotyping. Four different rDNA gene restriction patterns

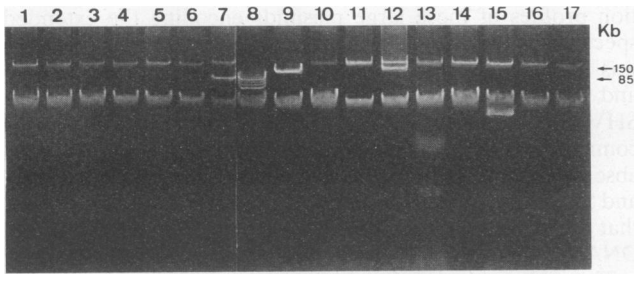

FIG. 1. Plasmid profiles of *K. pneumoniae* producing SHV-4 β-lactamase. Lanes: 1, strain 1; 2, strain 2; 3, strain 3; 4, strain 4; 5, strain 6; 6, strain 8; 7, strain 7; 8, p453 (SHV-1); 9, pCFF04 (TEM-3); 10, pUD18 (SHV-3); 11, strain 9; 12, strain 5; 13, strain 10; 14, strain 11; 15, strain 12; 16, strain 13; 17, strain 14. Molecular mass markers are indicated on the right.

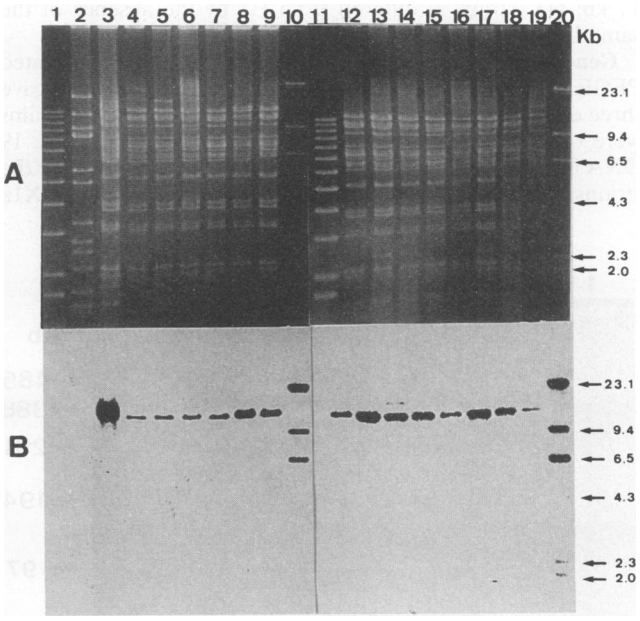

FIG. 2. (A) *Eco*RI restriction pattern of plasmids from the *E. coli* transconjugants and from the *K. pneumoniae* 14. Lanes: 1 and 11, 1-kb DNA ladder; 2, pCFF04 digested by *Eco*RI; 3, pUD18 digested by *Eco*RI; 4 to 9 and 12 to 18, digested plasmid DNA from transconjugants from *K. pneumoniae* 1 to 13; 19, digested plasmid DNA from *K. pneumoniae* 14; 10 and 20, phage λ digested by *Hin*dIII. (B) Corresponding autoradiography after hybridization with the SHV probe and phage λ.

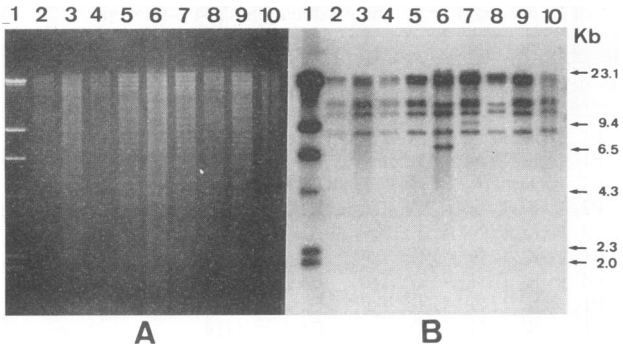

FIG. 3. Restriction endonuclease analysis of total DNA of *K. pneumoniae* producing SHV-4 β-lactamase obtained after digestion with *Hin*dIII (A) and corresponding rDNA restriction fragment length polymorphism patterns (B). Lanes: 1, phage λ digested by *Hin*dIII; 2 to 10, *K. pneumoniae* 1 to 9, respectively.

were obtained by digestion with *Eco*RI of DNA from the 14 strains (Table 1). Eleven strains belonged to the ribotype E1 (12 bands between 17 and 1.5 kb). Ribotypes E2, E3, and E4 each had one isolate (strains 2, 5, and 7). E2 differs from E1 by an additional band of 9.4 kb and by the absence of a band of 7 kb. E3 differs from E1 by the absence of two bands of 17 and 16 kb and by an additional band of 8 kb. E4 differs from E1 by the absence of three bands of 17, 16, and 9 kb, and by an additional band of 14 kb. Restriction with *Hin*dIII produced three ribotypes: 12 strains were of the ribotype H1 with minor variations (an additional band of 10 kb with low intensity) in three of them; strains 5 and 7 presented different *Hin*dIII ribotypes (Fig. 3; Table 1); H2 (strain 5) was different from H1 by an additional band of 7 kb and by the absence of a band of 17 kb; H3 (strain 7) differed from H1 by the absence of the same band of 17 kb.

Genomic fingerprinting by PFGE. Only *Xba*I generated PFGE profiles that were easy to analyze. The 14 strains gave three different types of profiles (Fig. 4; Table 1). Twelve strains were of profile X1 with only minor variations among the 19 DNA fragments observed; the profile X1a was observed for the strains 1, 2, 3, 10, 11, and 13. Other profiles differing from X1a by the presence or absence of one or two DNA fragments or by different weights were observed for strains 4 (X1b), 6 (X1c), 8 (X1d), 9 (X1e), 12 (X1f), and 14 (X1g). Strains 5 and 7 had completely different PFGE profiles.

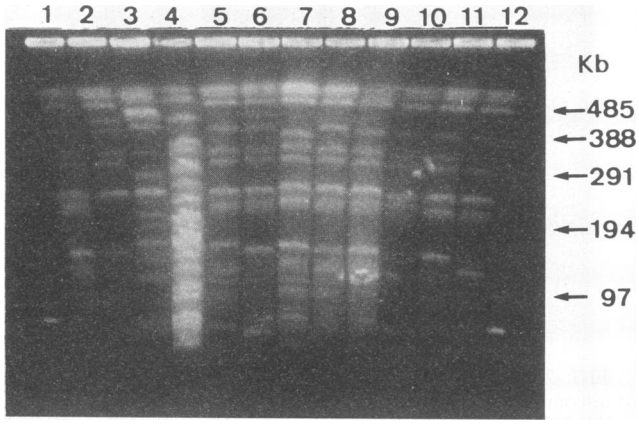

FIG. 4. PFGE of total DNA digested by *Xba*I of *K. pneumoniae* producing SHV-4 β-lactamase. Lanes: 1, *K. pneumoniae* 1; 2, *K. pneumoniae* 4; 3, *K. pneumoniae* 5; 4, *K. pneumoniae* 7; 5, *K. pneumoniae* 6; 6, *K. pneumoniae* 8; 7 to 12, *K. pneumoniae* 9 to 14, respectively.

DISCUSSION

Transferable resistance to oxyimino-β-lactam antibiotics in members of the family *Enterobacteriaceae* is a worldwide problem. The resistance mechanism mainly involves the production of enzymes derived from the TEM or SHV type of β-lactamase (15, 30). These extended-spectrum β-lactamases have been found mostly in *K. pneumoniae* strains responsible for outbreaks of nosocomial infections particularly in intensive care units but also in oncology units and nursing homes (3, 7, 25, 31). More recently, outpatients have presented with a producing strain (33). Some of these enzymes have been described all over the world (SHV-2 and SHV-5), whereas others have been reported only in one or a few countries, for example, TEM-10 and TEM-12 in the United States (15, 25) and TEM-3, SHV-3, and SHV-4 in France (8, 15, 17, 29, 30, 34).

The first five strains of *K. pneumoniae* (K25) producing the SHV-4 extended-spectrum β-lactamase were isolated in a cluster in the Paris area (8). Other *K. pneumoniae* strains producing this enzyme were subsequently isolated mostly in the Paris area but also from other areas in France, and it was interesting to compare these novel strains to the five first described (8).

All of the strains had the same capsule serotype, K25, as previously observed. This serotype is uncommon in Europe (≤1.8%) (32). Twelve of the 14 strains analyzed in this study were classified into the same particular biotype by the API 20E system (strains 5 and 7 excepted). The great majority of *K. pneumoniae* strains producing the CAZ-5 β-lactamase (an enzyme very similar to SHV-4) were also of this biotype (10). This biotype is associated with decreased susceptibility to cefoxitin and chloramphenicol and resistance to fluoroquinolones, suggesting that decreased permeability may be one of the mechanisms of resistance to these antimicrobial agents (13, 27). Strains 5 and 7 did not show patterns of resistance compatible with this mechanism, and strain 5 produced an additional β-lactamase with a pI of 5.4 (probably TEM-1).

Eight of the 14 strains had the same plasmid profile, and the other six strains displayed five different profiles. A very large plasmid (160 to 180 kb) encoding the SHV-4 β-lactamase was present in all *K. pneumoniae* strains. This plasmid was easily transferred to *E. coli* from each of the 14 strains except one. Strains 5 and 7 were again different in that they were the only strains to carry an additional large plasmid. The *Eco*RI restriction profiles of these large plasmids encoding the extended-spectrum β-lactamase varied slightly between strains. There was no correlation between these variations and the number and the nature of resistance markers cotransferred with the SHV-4 β-lactamase. An SHV probe hybridized with a single, common DNA fragment of about 13 kb, on the basis of the absence of *Eco*RI restriction site in the genes encoding SHV-3 and SHV-5 β-lactamases (5, 26). This plasmid thus differs from that carrying the CAZ-5 β-lactamase, where more than one DNA fragment hybridizes with the SHV probe (10).

These plasmids encoding SHV-4 β-lactamase were similar to pUD18 (encoding SHV-3), which is consistent with the SHV-4 β-lactamase being derived from SHV-3. The first strain of *K. pneumoniae* producing SHV-4 β-lactamase was recovered from a patient previously colonized by a strain of *K. pneumoniae* producing SHV-3 and treated with ceftazidime for an

infection due to *Pseudomonas aeruginosa* (16a). SHV-3 and SHV-4 differ from each other by only one amino acid substitution at position 213 of the mature protein (26, 28), and both differ from other SHV type β-lactamases by the presence of a leucine at position 180 (26, 28), which affects the pI but not the enzymatic activity. Furthermore, pUD18 and pUD21, encoding SHV-3 and SHV-4, respectively, carry the same resistance markers and belong to the same incompatibility group (FI) (16).

This *K. pneumoniae* strains in this group have the same capsule serotype but different plasmid profiles. Genomic fingerprinting should be able to determine whether they are in fact the same. Ribotyping has been shown by Bingen et al. (6) to be a good genotypic marker for *K. pneumoniae* strains. Although genomic fingerprinting by PFGE has been reported only once for *K. pneumoniae* (22), it is a powerful technique for epidemiologic investigation: we had previously observed that 12 unrelated strains of *K. pneumoniae* producing extended-spectrum β-lactamases had very different DNA profiles by using PFGE (data not shown). In the present study, ribotyping and genomic fingerprinting by PFGE gave consistent results. Of the 14 strains studied, only two were very different from the others in the two analyses. These two strains (5 and 7), isolated in 1988, 1 year after the isolation of the first *K. pneumoniae* strain producing the SHV-4 β-lactamase, were recovered in Paris. Surprisingly, strains isolated later, both in Paris and in other areas, had genomic fingerprinting profiles very similar to the first strains isolated. The minor variations observed in PFGE analysis between the different X1 profiles allowed the observation of a chromosomal evolution.

In conclusion, the dissemination of the SHV-4 β-lactamase in France has been mainly the result of the dissemination of a single strain, in spite of the high frequency of transfer in vitro of the plasmids harboring this β-lactamase. During one outbreak with SHV-4 in our hospital, only one strain of *E. coli* producing SHV-4 β-lactamase was recovered from a patient simultaneously infected by a *K. pneumoniae* isolate producing this enzyme. This contrasts with the TEM-3 β-lactamase (another enzyme essentially described in France), whose diffusion in France was principally the result of a plasmid dissemination (7, 19, 29, 34). Surprisingly, this clonal dissemination was not the characteristic of a particularly virulent strain (39); it could be explained by the transfer of patients between hospitals, especially between the Parisian hospitals which belong to the same administrative authority (Assistance Publique-Hôpitaux de Paris). Moreover, we have confirmed that SHV-4 β-lactamase was probably derived from SHV-3. Finally, phenotyping on the basis of capsular serotype seems to be insufficient to characterize *K. pneumoniae* strains. Plasmid profiles and plasmid fingerprinting are good methods to analyze outbreaks of short duration (3, 38), but genomic fingerprinting is indispensable for the comparison of strains isolated during outbreaks lasting a longer time (6, 38).

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