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Evidence of In Vivo Transfer of a Plasmid Encoding the Extended-Spectrum β-Lactamase TEM-24 and Other Resistance Factors among Different Members of the Family *Enterobacteriaceae*

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The epidemiological study of several multidrug-resistant *Enterobacteriaceae* isolated from five patients demonstrated in vivo dissemination of a 100-kb plasmid encoding the extended-spectrum β-lactamase TEM-24 from a clonal strain of *Enterobacter aerogenes* to different strains of *Klebsiella pneumoniae, Escherichia coli, Proteus vulgaris, Proteus mirabilis*, and *Serratia marcescens*.

In France, plasmid-mediated extended-spectrum beta-lactamases (ESBLs) have been mostly described from strains of *Klebsiella pneumoniae* (1, 2, 3, 6, 7, 15), but more recently infections caused by strains of *Enterobacter* spp. producing the TEM-24 ESBL have increased (5, 14, 24). The same phenomenon was observed in our University Hospital (2,000 beds, in Dijon, France). In 1996, 1997, and 1998 we isolated, respectively, 16, 37, and 50 *Enterobacter aerogenes* strains producing TEM-24 among totals of 78, 70, and 70 nonrepetitive ESBL-producing strains. All these strains were analyzed by pulsed-field gel electrophoresis (PFGE). During our continuous survey we found that five patients were cocolonized or coinfected with different multidrug-resistant species of enterobacteria. Following the use of imipenem, two strains of *Proteus mirabilis* and *E. aerogenes* resistant to this molecule were recovered from one patient. We report here the epidemiological study and the β-lactamase characterization of all the strains isolated from the five patients.

The origins of the strains are given in Table 1. The detection of ESBL production was performed by the double-disk synergy test (19) but with a quarter of the disk containing third-generation cephalosporin for *Proteus* sp. (9).

Analyses of chromosomal DNAs by PFGE were performed as described previously (15) but with a pulse range from 40 to 5 s for 20 h at 180 V for strains of *E. aerogenes, K. pneumoniae, Serratia marcescens*, and *Escherichia coli*. For *P. mirabilis* and *Proteus vulgaris*, we used a pulse range from 25 to 5 s for 20 h at 180 V (Fig. 1 and 2). A single profile was found for the strains of *E. aerogenes*, similar to that of the epidemic strain described in 1996 (24). For the other enterobacteria, strains from the same species (ESBL or not ESBL producing) isolated from the same patient shared concordant PFGE patterns, suggesting their clonal origin. Nevertheless, the strains of the same species isolated from the five patients were not related. This result excluded the possibility that resistant strains of *K. pneumoniae, E. coli*, or *P. vulgaris* were disseminated between the patients or that there was a common source of contamination.

A large plasmid of about 100 kb was isolated by the method of Birnboim and Doly from the ESBL-producing strains (4). The restriction patterns obtained after digestion of the plasmid by *Eco*RI were very similar. The plasmid was easily transferred

TABLE 1. Origins of the strains

Patient	Ward	Date of isolation	Source of isolate	Organism[a]	pI(s) of β-lactamase
1	Intensive care unit	20 April 1998	Sputum	EA (+)	6.5, 8.3
		22 May 1998	Stool	EA (+)	6.5, 8.3
				EC (+)	6.5
		17 June 1998	Sputum	EA (+)	6.5, 8.3
				KP (+)	6.5, 7.7
				PV (+)	6.5, >8.3
		17 June 1998	Stool	EA (+)	6.5, 8.3
2	Urology surgery unit	24 October 1998	Stool	EA (+)	6.5, 8.3
		31 October 1998	Surgical wound	EA (+)	6.5, 8.3
				KP (+)	6.5, 7.7
				PV (+)	6.5, >8.3
3	Dermatology unit	24 August 1998	Urine	EA (+)	6.5, 8.3
		18 November 1998	Stool	EA (+)	6.5, 8.3
				EC (+)	6.5
				KP (+)	6.5, 7.7
4	Neurosurgery unit	29 September 1999	Sputum	EC (−)	
		3 October 1999	Stool	EA (+)	6.5, 8.3
		20 November 1999	Sputum	EA (+)	6.5, 8.3
				EC (+)	6.5
				EC (−)	
		3 December 1999	Sputum	EA (+)	6.5, 8.3
		28 February 2000	Sputum	PM* (−)	5.4
		29 March 2000	Surgical wound	PM* (+)	5.4, 6.5
				EA* (+)	6.5, 8.3
5	Rehabilitation unit	24 February 2000	Sputum	EA (+)	6.5, 8.3
		6 March 2000	Sputum	EA (+)	6.5, 8.3
				SM (+)	6.5, >8.3
				SM (−)	>8.3

[a] EA, *E. aerogenes*; EC, *E. coli*; KP, *K. pneumoniae*; PV, *P. vulgaris*; PM, *P. mirabilis*; SM, *S. marcescens*; (+), ESBL-producing strain; (−), non-ESBL-producing strain; *, strain resistant to imipenem.

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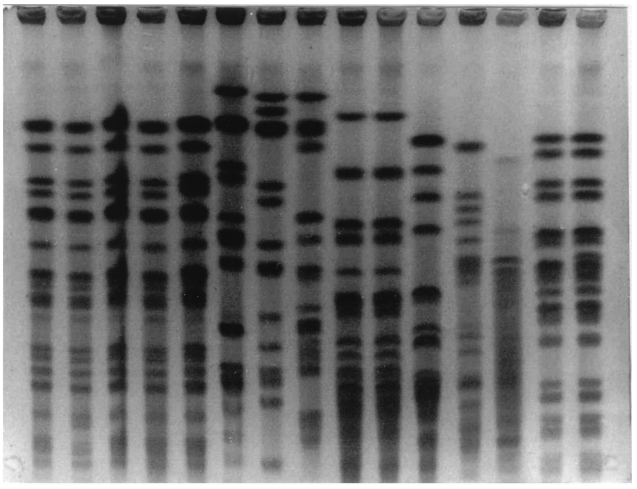

FIG. 1. PFGE of total DNAs from *E. aerogenes* (lanes A to E), *K. pneumoniae* (lanes F to H), *S. marcescens* (lanes I to K), and *E. coli* (lanes L to O) cut by *Xba*I. Lane A, patient 1 isolate; lane B, patient 3 isolate; lane C, patient 2 isolate; lane D, patient 4 isolate; lane E, patient 5 isolate; lane F, patient 1 isolate; lane G, patient 3 isolate; lane H, patient 2 isolate; lanes I and J, patient 5 isolate; lane K, unrelated strain; lane L, patient 1 isolate; lane M, patient 3 isolate; lanes N and O, patient 4 isolate.

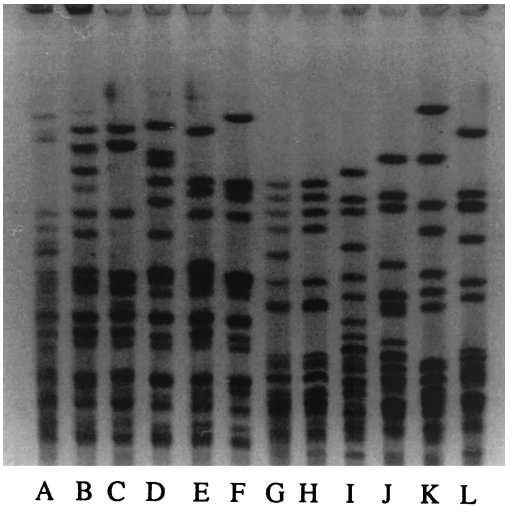

FIG. 2. PFGE of total DNAs from *P. vulgaris* (lanes A to F) and *P. mirabilis* (lanes G to L) cut by *Sma*I. Lane A, patient 1 isolate; lane B, patient 2 isolate; lanes C to F, unrelated strains; lanes G and H, patient 4 isolate; lanes I to L, unrelated strains.

from *E. aerogenes, K. pneumoniae, P. vulgaris, P. mirabilis*, and *S. marcescens* to *E. coli* K-12 C600, which is resistant to sodium azide (selection with 256 μg of sodium azide per ml and 8 μg of netilmicin per ml) or from *E. coli* to *K. pneumoniae* 10031, which is resistant to rifampin (selection with 100 μg of rifampin per ml and 8 μg of netilmicin per ml). Resistance to β-lactams was cotransferred with resistance to aminoglycosides (amikacin, kanamycin, netilmicin, tobramycin), sulfonamides, and chloramphenicol. The MICs of β-lactams (Table 2) were determined in Mueller-Hinton broth by a microdilution method for the clinical strains and their transconjugants. The levels of resistance were very similar among the transconjugants. For the *P. mirabilis* strains, extended-spectrum cephalosporin MICs were very low. This may explain why it was difficult to detect ESBL production in this species. *P. mirabilis* and *E. aerogenes* strains isolated from patient 4 in February and in March were resistant to imipenem (respectively, MICs of 8 and 16 to 32 μg/ml). Isoelectric focusing was performed as previously reported (24). The β-lactamase activity was located in the gels by an iodine starch procedure (20). A β-lactamase with a pI of 6.5 was detected in all the ESBL-producing strains as well as in their transconjugants. PCR was performed on plasmids

TABLE 2. MICs of beta-lactam antibiotics for the clinical strains presented in Table 1

Patient	Organism[a]	MIC (μg/ml)[b]						
		TIC	PIP	CAZ	CTX	ATM	FEP	CEF
1	EA	>2,048 (>2,048)	256 (16)	512 (128)	32 (1)	64 (16)	2 (0.5)	>1,024 (32)
	EC	>2,048 (>2,048)	256 (16)	256 (64)	4 (0.5)	64 (8)	2 (0.25)	256 (32)
	KP	>2,048 (>2,048)	32 (16)	128 (128)	1 (1)	16 (16)	0.5 (0.5)	64 (32)
	PV	256 (>2,048)	8 (32)	16 (128)	1 (1)	0.5 (16)	1 (0.5)	512 (32)
2	EA	>2,048 (>2,048)	256 (16)	512 (128)	32 (1)	64 (16)	2 (0.5)	>1,024 (32)
	KP	>2,048 (>2,048)	64 (32)	256 (128)	1 (1)	32 (16)	0.5 (0.5)	64 (32)
	PV	128 (>2,048)	8 (32)	16 (128)	0.5 (1)	0.25 (16)	1 (0.5)	256 (32)
3	EA	>2,048 (>2,048)	512 (16)	512 (128)	32 (0.5)	64 (16)	1 (0.5)	>1,024 (32)
	EC	>2,048 (>2,048)	64 (16)	256 (64)	2 (0.5)	32 (8)	1 (0.25)	128 (32)
	KP	>2,048 (>2,048)	64 (32)	128 (128)	2 (1)	32 (16)	1 (0.5)	128 (64)
4	EA	>2,048 (>2,048)	256 (16)	512 (128)	32 (1)	64 (16)	2 (0.25)	>1,024 (32)
	EC	>2,048 (>2,048)	256 (16)	128 (32)	2 (0.5)	32 (8)	2 (0.25)	32 (16)
	PM	512 (>2,048)	128 (16)	8 (64)	1 (1)	0.25 (4)	0.5 (0.25)	16 (16)
5	EA	>2,048 (>2,048)	256 (16)	512 (128)	32 (1)	64 (16)	2 (0.5)	>1,024 (32)
	SM	>2,048 (>2,048)	64 (16)	256 (128)	1 (1)	32 (8)	1 (0.5)	256 (128)

[a] EA, *E. aerogenes*; EC, *E. coli*; KP, *K. pneumoniae*; PV, *P. vulgaris*; PM, *P. mirabilis*; SM, *S. marcescens.*

[b] TIC, ticarcillin; PIP, piperacillin; CAZ, ceftazidime; CTX, cefotaxime; ATM, aztreonam; FEP, cefepime; CEF, cephalothin. Values in parentheses are MICs for the transconjugants.

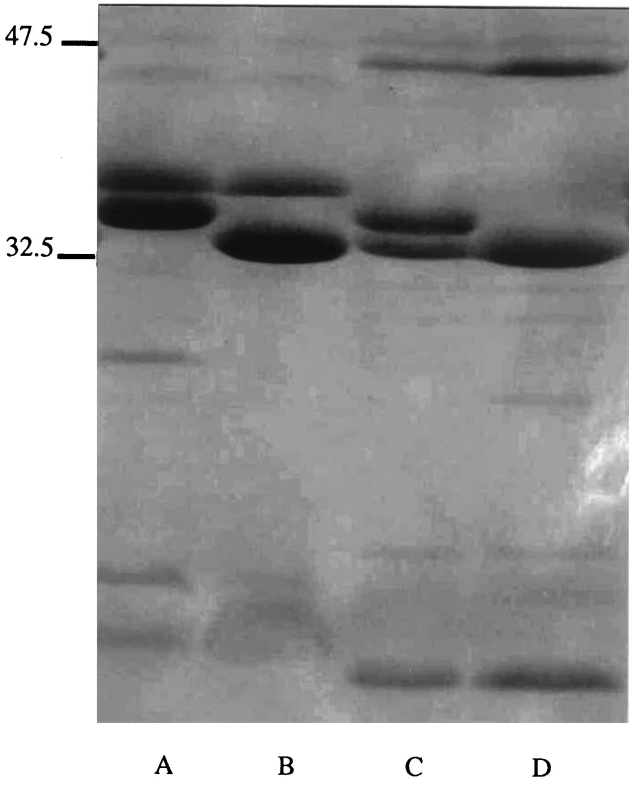

FIG. 3. Outer membrane protein profiles of *P. mirabilis* and *E. aerogenes*. Lane A, *P. mirabilis* ATCC 29906; lane B, *P. mirabilis* imipenem-resistant strain from patient 4; lane C, *E. aerogenes* imipenem-susceptible strain from patient 4; lane D, *E. aerogenes* imipenem-resistant strain from patient 4. Molecular mass standards in kilodaltons are given on the left.

extracted from the transconjugants and from the non-ESBL-producing *P. mirabilis* strain which was resistant to imipenem with primers J (forward, 5′-CTTATTCCCTTTTTTGCGGC-3′) and E (reverse, 5′-GGTCTGACAGTTACCAATGC-3′) (8) at positions 236 and 1079 of the TEM family gene β-lactamase according to Sutcliffe numbering (25). The sequence of the gene encoding the β-lactamase with a pI of 5.4 produced by the *P. mirabilis* strain was identical to that of TEM-1b (16, 25), and the sequence of the gene encoding the β-lactamase with a pI of 6.5 was identical to that of the extended-broad-spectrum β-lactamase TEM-24b (8, 17).

These results demonstrate clearly that there has been an in vivo transfer of the plasmid encoding ESBL TEM-24 from *E. aerogenes* to *K. pneumoniae, E. coli, P. mirabilis, S. marcescens*, and *P. vulgaris*. In all cases, the ESBL was first detected in the strain of *E. aerogenes* and only later in other species in a site colonized by *E. aerogenes*. For patients 1, 2, and 3 we unfortunately did not keep the non-ESBL-producing species of *Enterobacteriaceae* isolated before the *E. aerogenes* strain. The transfer probably occurred in the wound of patient 2 because the resistant strains of *K. pneumoniae* and *P. vulgaris* were never found in the stools. For the two other patients, we isolated the non-ESBL-producing strain (*E. coli* and *P. mirabilis* for patient 4, *S. marcescens* for patient 5) from the same site as we did the identical TEM-24-producing strain. The transfer in vivo of plasmid has already been described (12, 21, 22, 24), but each report concerned only one patient. This study, the first one describing five patients, proves that the spread of plasmid is no longer exceptional and can concern species for which the ESBL TEM-24 had not yet been described, like *P. vulgaris*.

The analysis of the outer membrane proteins of the strains of *P. mirabilis* and *E. aerogenes* resistant to imipenem and isolated from patient 4 was carried out as previously reported (Fig. 3) (18, 23). A band of 40 kDa was not detected in the strain of *E. aerogenes* that is resistant to imipenem as already reported (10, 11, 13). In the *P. mirabilis* strain resistant to imipenem all the major bands were present. The resistance was probably due to some modifications in the penicillin-binding proteins, which we already described for this species (23). This report is the first description of the selection of two different species of *Enterobacteriaceae* resistant to imipenem in a single patient following treatment with imipenem. If such strains were to be more often isolated, there would soon be no medical therapies available.

In conclusion, the *E. aerogenes* strain producing TEM-24 isolated in our hospital represents a serious danger: it spreads very easily and is at the origin of plasmid dissemination among *Enterobacteriaceae*.

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