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Translocation of Antibiotic Resistance Determinants Including an Extended-Spectrum β-Lactamase between Conjugative Plasmids of *Klebsiella pneumoniae* and *Escherichia coli*

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The extended-spectrum β-lactamase CAZ-7, derived from TEMs, was produced by two different strains of the family *Enterobacteriaceae*, *Klebsiella pneumoniae* and *Escherichia coli*, isolated from the same patient. Both isolates were resistant to amikacin. In addition, the *K. pneumoniae* strain was TEM-1 producing and resistant to gentamicin. An *E. coli* HB101 transconjugant obtained from *K. pneumoniae*, selected on ceftazidime, showed that CAZ-7 and amikacin resistance were encoded by an 85-kb Inc7 or M plasmid, while an *E. coli* HB101 transconjugant obtained from *E. coli* under the same conditions showed that CAZ-7 and amikacin resistance were encoded by a >150-kb Inc6 or C plasmid. Two other *E. coli* HB101 transconjugants obtained from *K. pneumoniae*, selected on gentamicin or chloramphenicol, showed that TEM-1 and gentamicin resistance could be encoded either by a >150-kb Inc6 or C plasmid or by an 85-kb Inc7 or M plasmid. It was hypothesized that the genes for β-lactam and aminoglycoside resistances were located on translocatable sequences. *Eco*RI digestion and hybridizations obtained with bla_{tem}, *aacA4*, and IS*15* probes demonstrated that the CAZ-7 gene, amikacin resistance gene, and IS*15* element were clustered on an ~20-kb fragment common to 85- and >150-kb plasmids. *E. coli* HB101 transconjugants from *K. pneumoniae* and *E. coli* isolates were used to obtain translocations of CAZ-7 and amikacin resistance and of TEM-1 and gentamicin resistance between the 85- and >150-kb plasmids. This study shows a typical example of in vivo gene dissemination involving transposable elements which translocate multiresistance genes, including an extended-spectrum β-lactamase.

Since 1984, members of the family *Enterobacteriaceae* that produce extended-spectrum β-lactamases (ESbla) derived from TEM (CTX-1/TEM-3, CAZ-1/TEM-5, CAZ-2, CAZ-3, CAZ-6, CAZ-7) or SHV (CAZ-4, CAZ-5) have been isolated from colonized or infected patients in our hospitals (4, 5, 20, 22, 24). Previous studies on these enzymes, especially CTX-1, indicated a combination of plasmid and strain dissemination (6, 19, 23).

During the outbreaks, TEM-derived enzymes were encoded by closely related 85-kb (Inc7 or M) plasmids (5, 6, 19).

Among TEM-derived ceftazidimases, the CAZ-7 enzyme has the same pI as CTX-1, but CAZ-7 has a greater hydrolytic activity against ceftazidime (Caz) and aztreonam than against cefotaxime.

In March 1988, a CAZ-7 enzyme (5) encoded by an 85-kb plasmid was produced by a *Klebsiella pneumoniae* strain isolated in a urine sample from a patient in an intensive care unit. In this sample, another *K. pneumoniae* strain producing only a TEM-1 enzyme encoded by a >150-kb plasmid was also isolated. Three months later, we detected a CAZ-7 enzyme encoded by a >150-kb plasmid produced by an *Escherichia coli* strain isolated from urine of the same patient.

In this study, β-lactamase production and other resistance markers of these three strains are analyzed. Plasmid content analysis, restriction patterns, and DNA hybridizations were performed to localize the β-lactamases (CAZ-7 or TEM-1) and the aminoglycoside resistance genes in the plasmids.

Reciprocal transfers of the 85- and >150-kb plasmids encoding β-lactamases CAZ-7 or TEM-1 associated with aminoglycoside resistances were carried out to elucidate the mechanism underlying the in vivo transfer of the multiple resistance genes between these two plasmids.

MATERIALS AND METHODS

Bacterial strains and plasmids (Table 1). Three strains were isolated from the same patient.

K. pneumoniae CF1314 was isolated from urine on 8 March 1988. By production of CAZ-7 (5) and TEM-1, it had a multiple resistance to β-lactams and was also resistant to all aminoglycosides, chloramphenicol (Cm), sulfonamides, tetracyclines (Tc), and trimethoprim. Genes encoding CAZ-7, amikacin (Ak), sulfonamide, and tetracycline resistance were located on an 85-kb plasmid.

K. pneumoniae CF1004 was isolated from the same sample with a multiple resistance to β-lactams by production of TEM-1, to all aminoglycosides except amikacin, to chloramphenicol, sulfonamides, tetracyclines, and trimethoprim. Genes encoding TEM-1 and resistance to gentamicin, chloramphenicol, and trimethoprim were located on a >150-kb plasmid.

E. coli CF1302 with a multiple resistance to β-lactams by production of CAZ-7, to all aminoglycosides except gentamicin, to chloramphenicol, to sulfonamides, and to trimethoprim, was isolated 3 months later (7 June 1988). Genes encoding all these antibiotic resistances were located on a >150-kb plasmid.

The plasmid-mediated β-lactamases CAZ-7 and TEM-1 were transferred by conjugation (14) into *E. coli* HB101

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TABLE 1. Resistance phenotype, β-lactamase, and plasmid contents of three strains isolated from the same patient and of their *E. coli* HB101 transconjugants

Strain	Antibiotics used for selection[a]	pI	Resistance phenotype (β-lactamases and associated markers[b])	Plasmid content (kb) (large plasmids)
K. pneumoniae CF1314		7.7		
		6.3	TEM-1 CAZ-7 Km Tm Gm Ak Nt Cm Su Tc Tp	150 110 85
		5.4		
E. coli TR1	Caz	6.3	CAZ-7 Km Tm Ak Nt Su Tc	85
E. coli TR2	Cm	5.4	TEM-1 Km Tm Gm Nt Cm Su Tp	150
E. coli TR3	Gm	5.4	TEM-1 Km Tm Gm Nt Tc	85
K. pneumoniae CF1004		7.7	TEM-1 Km Tm Gm Nt Cm Su Tc Tp	150 110
		5.4		
E. coli TR4	Gm	5.4	TEM-1 Km Tm Gm Nt Cm Su Tp	150
E. coli CF1302		6.3	CAZ-7 Km Tm Ak Nt Cm Su Tp	150 85
E. coli TR5	Caz	6.3	CAZ-7 Km Tm Ak Nt Cm Su Tp	150

[a] Antibiotics used for selection: ceftazidime (4 μg/ml), chloramphenicol (64 μg/ml), and gentamicin (4 μg/ml).
[b] Km, kanamycin; Tm, tobramycin; Nt, netilmicin; Su, sulfonamides; Tp, trimethoprim. The boxed resistance markers correspond to the translocated sequences.

recA13 (17). Two mutants, *E. coli* HB101 Rif^r and *E. coli* HB101 Nal^r, were selected from *E. coli* HB101 on medium with rifampin (300 μg/ml) or nalidixic acid (50 μg/ml) and were used as recipients in mating experiments.

Antibiotic concentrations for isolation were as follows: ceftazidime, 4 μg/ml; gentamicin, 4 μg/ml; chloramphenicol, 64 μg/ml; tetracyclines, 10 μg/ml; nalidixic acid, 50 μg/ml; and rifampin, 300 μg/ml.

Reciprocal transfer of plasmids encoding β-lactamases (TEM-1 or CAZ-7) associated with aminoglycoside-modifying enzymes (Gm^r or Ak^r) were performed by using the three *E. coli* HB101 Rif^r or Nal^r transconjugants TR1, TR2, and TR3 obtained from *K. pneumoniae* CF1314 and the transconjugant TR5 from *E. coli* CF1302 as donors and *E. coli* HB101 Rif^r or Nal^r as a recipient strain.

Analytical isoelectric focusing. Extracts prepared by ultrasonic disintegration were applied on filter paper strips to commercially obtained polyacrylamide gels containing ampholines with a pH range of 3.5 to 10 (LKB Products). Electrofocusing was carried out by the procedure recommended by the manufacturer, with the LKB 2117 Multiphor apparatus. β-Lactamases of known pI (TEM-1, pI 5.4; CAZ-7, pI 6.3; SHV-1, pI 7.7; CTX-1, pI 6.3) were focused in parallel with the extracts. The enzyme activities were located in the gels with chromogenic cephalosporin CM 32150.

Determination of β-lactamase kinetic constants (K_m, V_{max}). The K_m and V_{max} values of the β-lactamases were obtained by the computerized microacidometric method described by Labia et al. (10). The K_m and V_{max} values of the β-lactamase CAZ-7 were determined by using partially (close to 50-fold) purified (by preparative gel electrofocusing) extracts obtained from *E. coli* transconjugants. The relative V_{max} rates of hydrolysis for cefotaxime, ceftazidime, and aztreonam were compared with the rate for benzylpenicillin, which was taken as 100%.

Tests for incompatibility. Tests for incompatibility were performed as previously described (3), with *E. coli* BM21 harboring plasmid pIP135 (Tra$^+$; Inc 7 or M; Gm^r Sm^r Sp^r Su^r Tc^r; 79.3 kb) (12) and *E. coli* BM2961 harboring plasmid pIP1434 (Inc6 or C, TEM-1, AAC(6')-IV, Su^r; 148 kb) (2).

Plasmid DNA isolation. Plasmid DNA was extracted from clinical isolates and transconjugants by the alkaline lysis method (17) by a protocol which is a modification of the procedure initially described by Birnboim and Doly (1). DNA electrophoresis was performed in 0.7% agarose. Gels were stained with ethidium bromide and photographed with Polaroid film with a UV light source.

Restriction endonuclease analysis. Plasmid DNA obtained from crude preparations was digested with restriction endonuclease *Eco*RI according to the manufacturer's recommendations (Bethesda Research Laboratories, Inc.). After digestion, the samples were applied to 1% agarose gels, electrophoresed, stained, and photographed as described above.

Hybridization. Specific DNA probes were obtained by purification of a 560-bp fragment of pBR322 after cleavage with *Pst*I and *Ssp*I (TEM probe), a 344-bp fragment of pAZ505 after cleavage with *Dde*I-*Ava*I [AAC(6')IV probe) (27), and a 670-bp fragment of pIP1088 after cleavage with *Sal*I (IS*15* probe) (11). DNA was labeled with ^{32}P by nick translation (17), and hybridization was carried out under stringent conditions (16) after transfer to nitrocellulose by the method of Southern (25).

RESULTS

Table 1 shows the five transconjugants obtained. Three types of transconjugants were obtained from *K. pneumoniae* CF1314:TR1 producing the ESbla CAZ-7 encoded by an 85-kb plasmid and TR2 and TR3 producing a TEM-1 enzyme encoded either by a >150-kb plasmid (in TR2) or by an 85-kb plasmid (in TR3). Transconjugant TR4 was obtained from *K. pneumoniae* CF1004. It was identical to TR2. Transconjugant TR5 was obtained from *E. coli* CF1302. It produced ESbla CAZ-7 encoded by a >150-kb plasmid. Incompatibility testing revealed that the 85-kb plasmids belonged to incompatibility group 7 or M and that the >150-kb plasmids belonged to group 6 or C. The 85- and >150-kb plasmids were both present in the CAZ-7-producing isolates, *K. pneumoniae* CF1314 and *E. coli* CF1302.

Whatever the donor strain (*K. pneumoniae* or *E. coli*), the

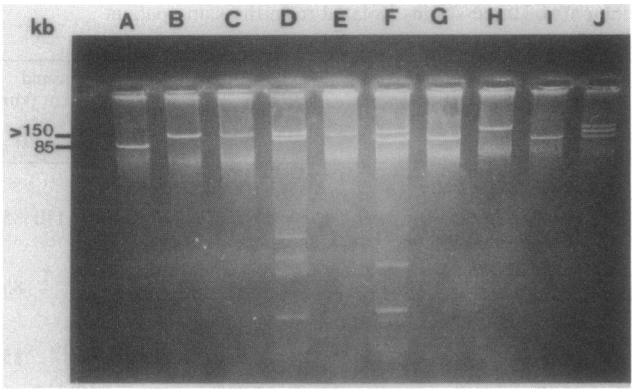

FIG. 1. Agarose gel electrophoresis of plasmid DNA from wild-type strains and their transconjugants (in *E. coli* HB101). Lanes: A, pCFF04 (85 kb); B, pCFF14 (>150 kb); C and D, *E. coli* transconjugant TR4 (TEM-1) and *K. pneumoniae* CF1004 (TEM-1), respectively; E and F, *E. coli* transconjugant TR5 (CAZ-7) and *E. coli* CF1302 (CAZ-7), respectively; G, H, I, and J, *E. coli* transconjugants TR3 (TEM-1) TR2 (TEM-1), and TR1 (CAZ-7) and *K. pneumoniae* CF1314 (TEM-1 plus CAZ-7), respectively. (Sizes are indicated on the left.)

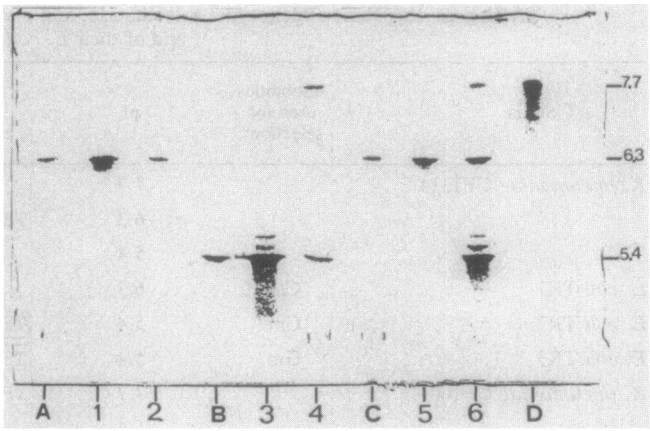

FIG. 2. Analytical isoelectric focusing (pH range, 3.5 to 10) of β-lactamases from wild-type strains and their *E. coli* transconjugants. Lanes A, B, C, and D, β-lactamases of known pI: CTX-1, pI 6.3; TEM-1, pI 5.4; CAZ-7, pI 6.3; and SHV-1, pI 7.7, respectively. Lanes 1 and 2, *E. coli* CF1302 and its transconjugant, TR5 (CAZ-7), respectively. Lanes 4 and 3, *K. pneumoniae* CF1004 and its transconjugant, TR4 (TEM-1), respectively. Lanes 6 and 5, *K. pneumoniae* CF1314 and its transconjugant, TR1 (CAZ-7). The enzymes were located in the polyacrylamide gel by using a cephalosporin chromogen (CM 32150) procedure.

transfer frequency of the 85-kb plasmid was about 10^{-4} and the transfer frequency of the >150-kb plasmid was about 10^{-5}.

Transfer of ceftazidime resistance. Conjugative transfer occurred from two different donors, *K. pneumoniae* CF1314 and *E. coli* CF1302.

The *E. coli* HB101 transconjugant TR1 was obtained from *K. pneumoniae* CF1314 after selection on ceftazidime. It harbored resistance to ceftazidime and to aminoglycosides except gentamicin and was also resistant to sulfonamides and tetracyclines. The resistance pattern to β-lactams was due to synthesis of the ESbla CAZ-7 with pI = 6.3. TR1 harbored a single plasmid of approximately 85 kb (Fig. 1).

The transconjugant TR5 was obtained from *E. coli* CF1302 under the same conditions as TR1. It harbored a single >150-kb plasmid encoding the same resistance to ceftazidime, aminoglycosides, and sulfonamides as TR1 but differed from the latter in its resistance to chloramphenicol and its susceptibility to tetracyclines.

A comparison of isoelectric points (Fig. 2) and kinetic constants (Table 2) showed that the ESbla produced by the transconjugants TR1 and TR5 was CAZ-7.

Genes encoding ceftazidime resistance via CAZ-7 and amikacin resistance were located either on the 85- or the >150-kb plasmid.

Transfer of ampicillin resistance (Table 1 and Fig. 1 and 2). Transfer of ampicillin resistance by conjugation to *E. coli* HB101 was obtained from *K. pneumoniae* CF1314 (CAZ-7 and TEM-1 producing) and from *K. pneumoniae* CF1004 (TEM-1 producing). Both strains were isolated from the first urine sample.

From *K. pneumoniae* CF1314 two types of transconjugants, TR2 and TR3, were obtained after selection on chloramphenicol and gentamicin, respectively, both producing a TEM-1 enzyme associated with gentamicin resistance. Comparison of the plasmid contents of TR2 and TR3 showed that TR2 harbored a >150-kb plasmid encoding TEM-1, Gm^r, and Cm^r and that TR3 harbored an 85-kb plasmid encoding TEM-1, Gm^r, and Tc^r.

From *K. pneumoniae* CF1004 the transconjugant TR4 was obtained after selection on gentamicin. It harbored a single plasmid of >150 kb encoding the same resistances as TR2, and so the two wild-type *K. pneumoniae* strains harbored the same >150-kb R plasmid.

Transfer experiments and analysis of plasmid contents showed that the >150-kb plasmid always encoded resistance to chloramphenicol, while tetracycline resistance was always encoded by the 85-kb plasmid.

Comparison of the 150-kb (Inc6 or C) and 85-kb (Inc7 or M) plasmids by digestion and hybridization. (i) Plasmids (150 kb [Inc6 or C]) in transconjugants TR5 (CAZ-7), TR4, and TR2 (TEM-1) (Fig. 3A). Comparison of the *Eco*RI DNA fragments showed similar restriction patterns for these plasmids. The restriction patterns of the two plasmids encoding TEM-1 in TR2 and TR4 were indistinguishable, while a fragment of ~6.5 kb was absent in the CAZ-7-encoding plasmid of TR5.

Comparison of these plasmids with pIP1434 (148 kb, Inc6 or C) showed that three fragments (11, 9, and 3 kb) were missing in plasmids of TR2, TR4, and TR5, which in contrast contained an additional fragment of about 20 kb.

(ii) Plasmids (85 kb [Inc 7 or M]) in transconjugants TR1 (CAZ-7) and TR3 (TEM-1) (Fig. 3B). Comparison of the *Eco*RI DNA fragments showed similar restriction patterns of these plasmids. Fragments A (10.7 kb) and C (2.5 kb) of pIP135 (10) were absent in CAZ-7- and TEM-1-encoding

TABLE 2. Comparative activity with ceftazidime, cefotaxime, and aztreonam of the β-lactamases (pI = 6.3) encoded by the two plasmids of 85 kb (TR1) and 150 kb (TR5)

Substrate	CAZ-7 (TR1), pI 6.3[a]		CAZ-7 (TR5), pI 6.3[a]	
	V_{max}	K_m (μM)	V_{max}	K_m (μM)
Benzylpenicillin	100	7.8	100	7
Cefotaxime	9.8	43.8	10.5	51.4
Ceftazidime	98	125.4	86	85.3
Aztreonam	28	31.2	21	38

[a] Values are relative to those for benzylpenicillin (taken as 100%).

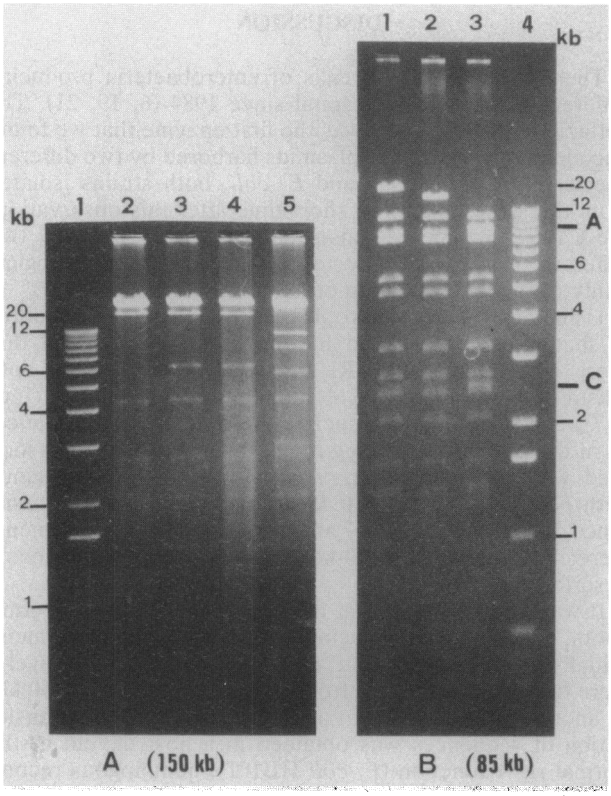

FIG. 3. Comparison of the *Eco*RI fragment patterns of the 150-kb plasmids (A) and the 85-kb plasmids (B). (A) Lanes: 1, ladder DNA (Bethesda Research Laboratories); 2, transconjugant TR5 (CAZ-7); 3, transconjugant TR4 (TEM-1); 4, transconjugant TR2 (TEM-1); 5, pIP1434 (Inc6 or C). (B) Lanes: 1, transconjugant TR3 (TEM-1); 2, transconjugant TR1 (CAZ-7); 3, pIP135 (Inc7 or M); 4, ladder DNA (Bethesda Research Laboratories).

plasmids in TR1 and TR3, respectively, which in contrast contained an additional fragment of ~20 kb (slightly larger in TR3).

Localization of the resistance determinants and IS*15* (Fig. 4). The presence of bla_{tem} (CAZ-7 or TEM-1), *aacA4* (amikacin resistance) and IS*15* was localized by Southern hybridization in the transconjugants TR1 to TR5. The same *Eco*RI ~20-kb fragments of plasmids hybridized with a specific probe for bla_{tem} and IS*15*. By using intragenic probe for *aacA4*, hybridization was also obtained on the same fragment in TR5 (>150 kb) and TR1 (85 kb) plasmids encoding amikacin resistance (associated with ESbla CAZ-7).

In vitro translocation of markers between the 85- and >150-kb plasmids (Fig. 5). We tried to reproduce in vitro the phenomenon observed in vivo: the ESbla CAZ-7, first encoded by an 85-kb plasmid in *K. pneumoniae* CF1314, was afterward encoded by a >150-kb plasmid in *E. coli* CF1302 from the same patient. A transfer by conjugation of plasmids encoding β-lactamase TEM-1 or CAZ-7 associated with gentamicin or amikacin resistance, respectively, was performed between two *E. coli* HB101 transconjugants obtained from the wild-type strains CF1314 and CF1302.

E. coli HB101 Rif^r transconjugant TR1, harboring an 85-kb plasmid (CAZ-7 Ak^r Tc^r), was mated with *E. coli* HB101 Nal^r transconjugant TR2 harboring a >150-kb plasmid (TEM-1 Gm^r Cm^r) (Fig. 5). The same transconjugants harboring the two plasmids were obtained by selecting on medium with nalidixic acid plus Caz when TR1 was donor and on medium with rifampin plus Gm when TR2 was donor. However, it was easier to obtain such transconjugants by using TR1 as donor since the transfer frequency of the 85-kb plasmid was higher ($\sim 10^{-4}$) than that of the >150-kb plasmid ($\sim 10^{-5}$).

For the last cross, the transconjugants harboring the 85- and >150-kb plasmids were used as donors and *E. coli* HB101 Rif^r or Nal^r were used as recipient strains. In this

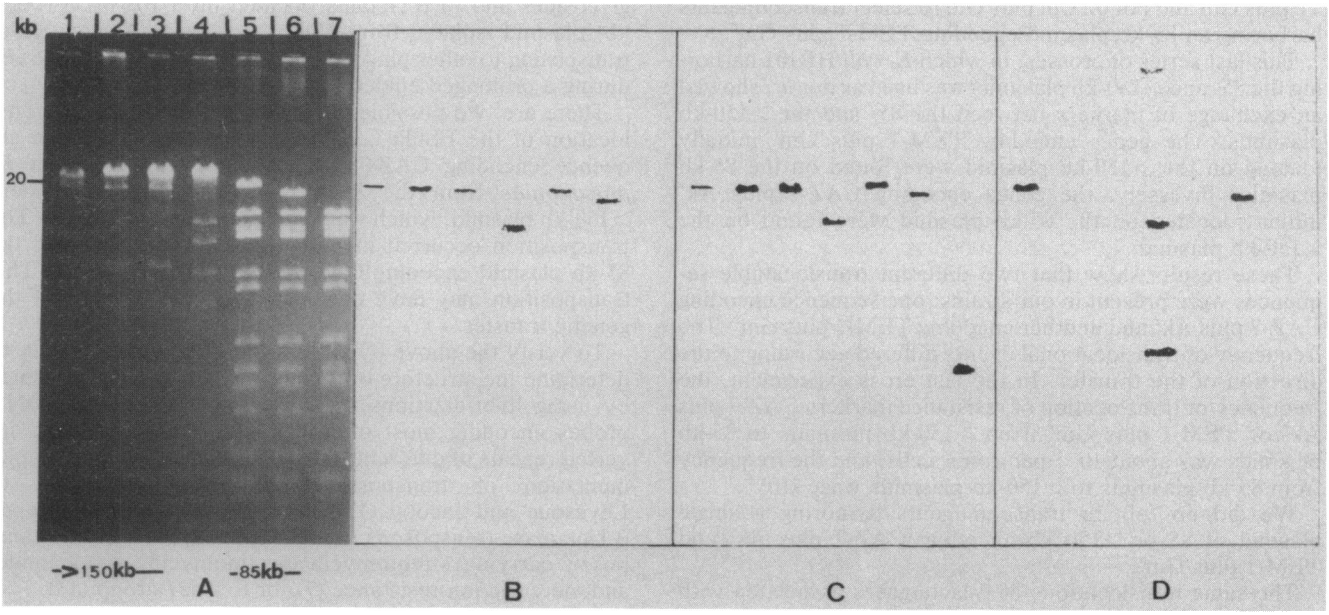

FIG. 4. (A) Agarose (0.7%) electrophoresis of *Eco*RI-digested plasmids (150 and 85 kb). Lanes 1 to 4, >150-kb plasmids. Lanes: 1, transconjugant TR5 (CAZ-7 plus Ak^r); 2, transconjugant TR4 (TEM-1 plus Gm^r); 3, transconjugant TR2 (TEM-1 plus Gm^r); 4, pIP1434 (TEM-1 plus AAC(6′)IV). Lanes 5 to 7, 85-kb plasmids. Lanes: 5, transconjugant TR3 (TEM-1 plus Gm^r); 6, transconjugant TR1 (CAZ-7 plus Ak^r); 7, pIP135. (B to D) Autoradiograms obtained after Southern transfer of the DNA from the agarose gel in panel A, followed by hybridization with the ^{32}P-labeled TEM probe (B), IS*15* probe (C), and AAC(6′)IV probe (D).

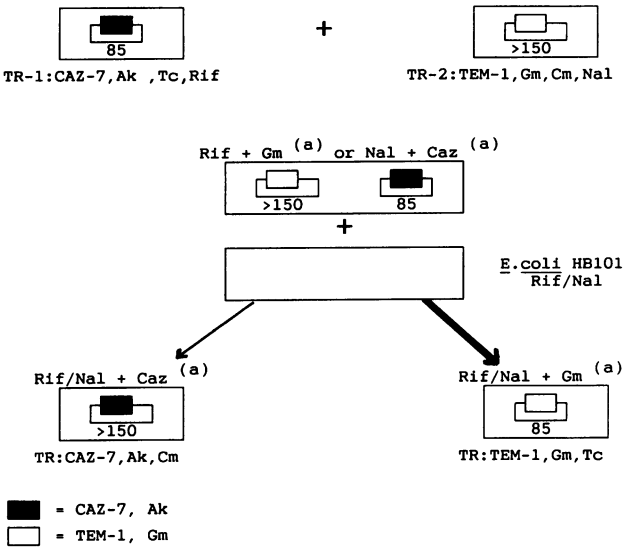

FIG. 5. Schematic representation of reciprocal transfer of plasmids encoding β-lactamase TEM-1 or CAZ-7 associated with, respectively, gentamicin or amikacin resistance between *E. coli* HB101 transconjugants TR1 (CAZ-7 Ak^r; 85 kb) and TR2 (TEM-1, Gm^r; >150 kb). a, antibiotics used for selection. Rif, rifampin; Nal, nalidixic acid.

mating experiment, colonies present on selective medium with Caz were transferred by replicating on agar plates containing Cm plus Caz and Tc plus Caz. Colonies growing on Cm plus Caz and not on Tc plus Caz were studied for the resistance phenotype and plasmid content to detect transconjugants harboring a >150-kb plasmid encoding CAZ-7 plus Ak^r. Colonies present on selective medium with Gm were transferred by replicating on agar plates containing Tc plus Gm and Cm plus Gm. We studied colonies growing on Tc plus Gm and not on Cm plus Gm to select transconjugants harboring an 85-kb plasmid encoding TEM-1 plus Gm^r.

This last series of crosses, in which *E. coli* HB101 harboring the 85- and >150-kb plasmids was used as donor, showed an exchange of markers between the 85- and the >150-kb plasmids. The genes encoding TEM-1 plus Gm^r initially located on the >150-kb plasmid were found on the 85-kb plasmid. Inversely, the genes encoding CAZ-7 plus Ak^r initially located on the 85-kb plasmid were found on the >150-kb plasmid.

These results show that two different translocatable sequences were present in our strains: one sequence encoding CAZ-7 plus Ak^r and another encoding TEM-1 plus Gm^r. The frequency of translocational events differed according to the direction of the transfer. In the last cross experiment, the frequency of translocation of resistance markers CAZ-7 plus Ak^r or TEM-1 plus Gm^r from >150-kb plasmids to 85-kb plasmids was about 10^{-6} per donor cells, and the frequency from 85-kb plasmids to >150-kb plasmids was $\sim 10^{-9}$.

We did not obtain transconjugants harboring a single plasmid of 85 or >150 kb encoding CAZ-7 plus Ak^r and TEM-1 plus Gm^r.

The same translocations of β-lactamases associated with aminoglycoside resistance determinants were obtained in a reverse transfer experiment between transconjugants TR5 and TR3. The transfer of resistance determinants from a >150-kb plasmid to an 85-kb plasmid was always obtained with higher frequency than in the opposite transfer.

DISCUSSION

There have been outbreaks of enterobacteria producing different ESbla in our hospital since 1984 (6, 19, 21). The ceftazidimase CAZ-7 (5) was the first enzyme that we found encoded by two different plasmids harbored by two different species, *K. pneumoniae* and *E. coli*, both strains isolated from the same patient. A short time after this observation, the CTX-1 enzyme was also found to be encoded by two different plasmids as reported in a recent epidemiological study of the dissemination of ESbla in our hospital (6).

Using agarose gel electrophoresis and genetic procedures of mating, digestion, and hybridization, we showed that there is a common *Eco*RI fragment of ~20 kb in both plasmids of 85-kb (Inc7 or M) and plasmids of >150 kb (Inc6 or C) encoding CAZ-7 associated with amikacin resistance, as in the plasmids encoding the β-lactamase TEM-1 associated with gentamicin resistance. Hybridizations obtained with bla_{tem}, *aacA4*, and IS*15* probes confirmed that resistance determinants (CAZ-7 and amikacin) and IS*15* elements were clustered on this ~20-kb DNA fragment, as previously described (19).

It was also observed that the multiple resistance determinants, CAZ-7 plus amikacin and TEM-1 plus gentamicin, initially encoded by 85- and >150-kb plasmids, respectively, were translocated in vitro from one plasmid (85 or >150 kb) to another (>150 or 85 kb) and vice versa. As this translocation of sequences was obtained in a host devoid of the normal *recA* function (*E. coli* HB101), homologous recombination was not involved in the process of gene exchange between the plasmids.

It is possible that TEM-1 plus Gm^r and CAZ-7 plus Ak^r units occur together on a single plasmid (85 or >150 kb). As we did not find such transconjugants, the translocational event may have occurred in or near the gene encoding the aminoglycoside resistance.

As in our study, Rubens et al. (21) have shown that a transposable DNA sequence encoding resistance to aminoglycosides and to β-lactams, located on a 105-MDa (>150 kb) plasmid isolated from *Klebsiella* strains, is capable of transposing to other plasmids that exist within the same cell during a prolonged epidemic of nosocomial infection.

There are two possible explanations of the in vivo translocation of the ESbla CAZ-7. The multiple resistance sequence encoding CAZ-7 and AAC(6′) transposed in *K. pneumoniae* from the 85-kb plasmid to the coresident >150-kb plasmid, which was then transferred to *E. coli*. The transposition occurred in *E. coli* after it had received the 85-kb plasmid encoding CAZ-7 from *K. pneumoniae*. This transposition may have occurred as a consequence of the genetic transfer.

To verify the above hypothesis, it would be necessary to determine the structure of this multiple resistance sequence by using hybridizations with a series of intragenic DNA probes encoding most of transposase, resolvase, and inverted repeats of different transposons and by using complementation of transposition functions. According to Levesque and Jacoby (15), several of the multiresistance β-lactamase transposons are related to Tn*21*, a 20-kb transposon carrying streptomycin/spectinomycin, sulfonamide, and mercuric ion resistance (7), or to Tn*21* group (13).

Several AAC(6) resistance determinants have been cloned and mapped (18, 26, 27). The multiresistant Tn*2424*, of 25 kb, related to Tn*21* does not encode a β-lactamase (18). The close association with a TEM-type β-lactamase gene has been described for Tn*1331*, a 7.5-kb multiresistance trans-

poson identified as part of a *K. pneumoniae* plasmid and belonging to the Tn*3* family (26).

It has been shown (27) that the expression of the AAC(6')4 gene is under the control of the regulatory sequences of the β-lactamase encoded by Tn*3*. In a recent study (8), the *tnpR* gene of Tn*3* was seen to hybridize with plasmids pCFF04 (CTX-1) and pCFF34 (CAZ-2) and 85-kb plasmids Inc7 or M, which are very closely related to pCFF84 encoding CAZ-7 (5). Tn*21* and Tn*3* are the two main members of the class 2 transposons, which have transposition functions in the central regions.

IS*15* can promote its own transposition and the transposition of DNA fragments that it flanks (11). Because of the presence of this element, the transposable sequence encoding the CAZ-7 β-lactamase could also be a member of class 1, which includes composite transposons in which IS elements flank a segment of DNA carrying genes unrelated to transposition (9, 13). DNA sequencing of regions flanking the CAZ-7 gene is in progress to elucidate how the CAZ-7 β-lactamase gene became associated with the transposable sequence.

We have previously defined outbreaks of nosocomial infections due to ESbla-producing members of the family *Enterobacteriaceae* in terms of strain or plasmid epidemics (6, 19, 23). The more recent observation described in this article may represent a typical example of gene dissemination in which transposable elements translocate resistance genes among different plasmids, resulting in a rapid spread of resistance to different species of enterobacteria in response to antibiotic selection pressure.

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