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# Multiplicity of TEM-Derived β-Lactamases from *Klebsiella pneumoniae* Strains Isolated at the Same Hospital and Relationships between the Responsible Plasmids

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**Five plasmid-mediated β-lactamases conferring high-level resistance to ceftazidime were isolated from *Klebsiella pneumoniae* strains in the same hospital. These enzymes had isoelectric points ranging from 5.3 to 6.5 (CAZ-1, 5.55; CAZ-2, 6.0; CAZ-3, 5.3; CAZ-6, 6.5; and CAZ-7, 6.3). All isolates and their *Escherichia coli* transconjugants were highly resistant to amoxicillin (MICs, >4,096 μg/ml), piperacillin (64 to 256 μg/ml), cephalothin (32 to 256 μg/ml), and ceftazidime (32 to 512 μg/ml) but remained moderately susceptible to cefotaxime (0.5 to 8 μg/ml). Only CAZ-6- and CAZ-7-producing strains were highly resistant to aztreonam (64 to 128 μg/ml). All the isolates remained susceptible to moxalactam and imipenem. The reduced activity of piperacillin, cefotaxime, ceftazidime, or aztreonam was restored by 2 μg of clavulanate, sulbactam, tazobactam, or brobactam per ml for *E. coli* producing CAZ-2, CAZ-3, and CAZ-7. Sulbactam had a lower protective effect than other inhibitors for *E. coli* harboring CAZ-1 and especially CAZ-6. Except for CAZ-1, which was mediated by a 150-kilobase (kb) plasmid (pCFF14), the other ceftazidimases were mediated by plasmids of 85 kb with *Eco*RI digestion patterns similar to that of pCFF04 encoding CTX-1 β-lactamase. A TEM probe hybridized with a 19-kb *Eco*RI fragment of all these closely related plasmids.**

Since the outbreak of infections caused by CTX-1-producing *Klebsiella pneumoniae* and other species of the family *Enterobacteriaceae* (17, 18), we have observed in the same hospital the advent of several novel extended-spectrum β-lactamases in *K. pneumoniae* isolates. These isolates were markedly more resistant to ceftazidime than to cefotaxime. On the basis of this resistance phenotype, we designated these β-lactamases ceftazidimases (CAZ).

Since the original CAZ-1 (16)- and CAZ-2 (7)-producing strains were isolated in January and July 1987, respectively, three other extended-spectrum β-lactamases were identified at the end of 1987: CAZ-3, CAZ-4, and CAZ-5 (D. Sirot, C. Chanal, R. Labia, M. Meyran, J. Sirot, and R. Cluzel, J. Antimicrob. Chemother., in press; R. Labia, A. Morand, K. Tiwari, C. Chanal, J. Sirot, and J. S. Pitton, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 481, 1988). These enzymes were either TEM derivatives (CAZ-1, CAZ-2, and CAZ-3) or SHV derivatives (CAZ-4 and CAZ-5).

Since 1988, two novel TEM-derived, extended-spectrum β-lactamases conferring a similar resistance phenotype have been detected in *K. pneumoniae* isolates. They were designated CAZ-6 and CAZ-7.

In this study, we describe these two novel enzymes and compare them with other TEM-derivative β-lactamases previously observed in the same hospital.

## MATERIALS AND METHODS

**Bacterial strains and plasmids.** Two clinical isolates of *K. pneumoniae* resistant to ceftazidime were studied: CF1104 (producing CAZ-6) and CF1304 (producing CAZ-7), isolated in 1988 from the respiratory tract and a blood sample, respectively, of two patients hospitalized in intensive care units in Clermont-Ferrand hospitals. These patients were under ceftazidime treatment when CAZ-6- and CAZ-7-producing strains were detected. The plasmid-mediated β-lactamases CAZ-6 and CAZ-7 were transferred by conjugation (13) into *Escherichia coli* K-12 C600 resistant to nalidixic acid (1). Transconjugants were selected on Mueller-Hinton agar containing nalidixic acid (150 μg/ml) plus ceftazidime (4 μg/ml). *E. coli* K-12 C600 containing the previously described extended-spectrum β-lactamases CTX-1 (pCFF04) (18), CAZ-1 (pCFF14) (16), CAZ-2 (pCFF34) (7), CAZ-3 (pCFF44) (Labia et al., 28th ICAAC; Sirot et al., in press), and TEM-2 (pCFF24) were included in this study for comparison. Plasmid pBR322 was used as a source of a TEM-specific DNA probe for DNA hybridization.

**Antibiotics.** Disks for the agar diffusion test were purchased from Diagnostics Pasteur. Antibiotic powders were provided as follows: amoxicillin, clavulanic acid, and ticarcillin, Beecham Laboratories; cephalothin and moxalactam, Eli Lilly & Co.; cefoxitin and imipenem, Merck Sharp & Dohme; ceftazidime, Glaxo Pharmaceuticals, Ltd.; cefotaxime, Hoechst-Roussel Pharmaceuticals, Inc.; aztreonam, E. R. Squibb & Sons; cefoperazone and sulbactam, Pfizer Inc.; tazobactam and piperacillin, Lederle Laboratories; brobactam (6-β-bromopenicillanic acid), Léo S. A. (22).

**Susceptibility testing.** Agar disk diffusion susceptibility tests were done on Mueller-Hinton agar. The double-disk synergy test was performed as previously described (9). The MICs of β-lactams were determined in Mueller-Hinton broth by a microdilution technique (Autodiluter II; Dynatech Laboratories, Inc.). Inocula of $10^5$ to $10^6$ CFU per ml were distributed with a multipoint inoculator (MIC 2000; Dynatech).

**β-Lactamase preparation.** Sonic extracts were prepared from cultures in Trypticase soy broth as previously described (10). Induction of β-lactamases was attempted by

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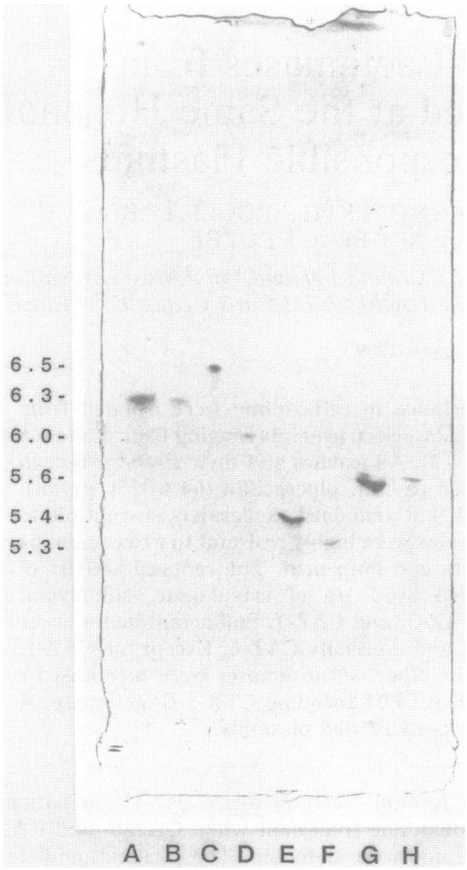


FIG. 1. Analytical isoelectric focusing (pH range, 3.5 to 10) of β-lactamases from *E. coli* transconjugants. Lanes: A, CF204 (CTX-1; pI, 6.3); B, CF1404 (CAZ-7; pI, 6.3); C, CF1204 (CAZ-6; pI, 6.5); D, CF804 (CAZ-2; pI, 6.0); E, *E. coli* R111 (TEM-1; pI, 5.4); F, CF2204 (CAZ-3; pI, 5.3); G, CF604 (CAZ-1; pI, 5.55); H, *E. coli* RP4 (TEM-2; pI, 5.6). The enzymes were located in the polyacrylamide gel by using chromogenic cephalosporin CM 32150.

growing the cultures in broth containing subinhibitory concentrations of ceftazidime or cefotaxime. β-Lactamase activity was revealed by adding 10 μl of crude bacterial extract to 10 μl of a solution (500 μg/ml) of the chromogenic cephalosporin CM32150 (Sanofi Clin-Midy).

**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 Instruments, Inc.). Electrofocusing was carried out by the procedure recommended by the manufacturer, with the LKB 2117 Multiphor apparatus. β-Lactamases TEM-1 (pI, 5.4) and TEM-2 (pI, 5.6) were focused in parallel with the extracts. The enzyme activities were located in the gels with chromogenic cephalosporin CM32150.

**Determination of β-lactamase kinetic constants ($K_m$, $V_{max}$).** The $K_m$ and $V_{max}$ constants of the β-lactamases were obtained by the computerized microacidometric method described by Labia et al. (11). The $K_m$ and $V_{max}$ values of β-lactamases CAZ-6 and CAZ-7 were determined by using partially (close to 50-fold) purified (by preparative gel electrofocusing) extracts (2) obtained from *E. coli* transconjugants. The relative $V_{max}$ rates of hydrolysis for cefotaxime, ceftazidime, and aztreonam were compared with that for benzylpenicillin, which was taken as 100%.

**Tests for incompatibility.** Tests for incompatibility were performed as previously described (6), with *E. coli* BM21 harboring plasmid pIP135 ($Tra^+$; incompatibility group 7 or M; Gm Sm Sp Su Tc; 79.3 kilobases [kb]) (12).

**Plasmid DNA isolation.** Plasmid DNA of clinical isolates and transconjugants was extracted as described elsewhere (15) by a protocol which is a modification of the method initially described by Birnboim and Doly (4). 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 recommendations of the manufacturer (Bethesda Research Laboratories, Inc.). After digestion, the samples were applied to 1% agarose gels, electrophoresed, stained, and photographed as described above.

**Hybridization.** A TEM-specific DNA probe was obtained by purification of a 560-base-pair fragment of pBR322 after cleavage with *Pst*I and *Ssp*I. DNA was labeled with $^{32}P$ by nick translation (15), and hybridization was carried out under stringent conditions (14) after transfer to nitrocellulose by the method of Southern (20).

## RESULTS

**Identification of the β-lactamases.** As revealed by isoelectric focusing (Fig. 1), all these β-lactamases had acid isoelectric points ranging from 5.3 to 6.5. The two novel enzymes CAZ-6 and CAZ-7 focused at pI 6.5 and 6.3, respectively. As previously reported (7, 16, 18; Sirot et al., in press; Labia et al., 28th ICAAC), the β-lactamases CTX-1, CAZ-1, CAZ-2, and CAZ-3 had pIs of 6.3, 5.55, 6.0, and 5.3, respectively.

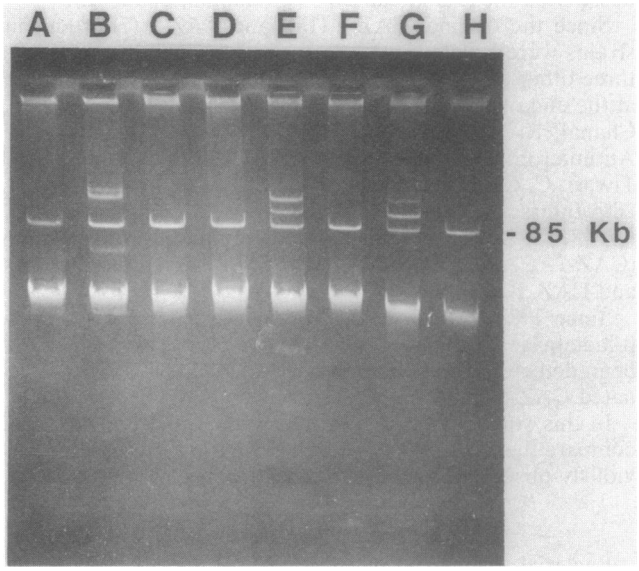


FIG. 2. Agarose gel electrophoresis of plasmid DNA from *K. pneumoniae* and *E. coli* transconjugants. Lanes: A and B, *E. coli* transconjugant and *K. pneumoniae* CF2104 (CAZ-3), respectively; C, *E. coli* transconjugant CF804 (CAZ-2); D and E, *E. coli* transconjugant and *K. pneumoniae* CF1304 (CAZ-7), respectively; F and G, *E. coli* transconjugant and *K. pneumoniae* CF1104 (CAZ-6), respectively; H, *E. coli* transconjugant CF204 (CTX-1).

TABLE 1. MICs of β-lactam antibiotics against *E. coli* K-12 harboring the different TEM-derivative ceftazidimases

| Antibiotic | MIC (μg/ml) for: | | | | | |
|---|---|---|---|---|---|---|
| | CAZ-1 | CAZ-2 | CAZ-3 | CAZ-6 | CAZ-7 | *E. coli* K-12 |
| Amoxicillin | >4,096 | >4,096 | >4,096 | >4,096 | >4,096 | 8 |
| Ticarcillin | >4,096 | >4,096 | >4,096 | >4,096 | >4,096 | 8 |
| Piperacillin | 64 | 64 | 128 | 64 | 256 | 2 |
| Cephalothin | 256 | 32 | 32 | 256 | 128 | 16 |
| Cefoperazone | 32 | 16 | 8 | 32 | 32 | 0.25 |
| Cefotaxime | 8 | 2 | 0.5 | 8 | 4 | 0.06 |
| Ceftazidime | 128 | 128 | 32 | 512 | 256 | 0.5 |
| Aztreonam | 8 | 8 | 2 | 128 | 64 | 0.06 |
| Cefoxitin | 8 | 4 | 8 | 4 | 8 | 2 |
| Moxalactam | 1 | 0.5 | 0.25 | 1 | 0.5 | 0.12 |
| Imipenem | 0.25 | 0.25 | 0.25 | 0.5 | 0.5 | 0.25 |

**Susceptibility patterns conferred by extended-spectrum β-lactamases.** The resistance phenotypes of the clinical isolates of *K. pneumoniae* producing CAZ-6 or CAZ-7 were determined by the disk diffusion assay (results not shown). These two isolates were highly resistant to amoxicillin, ticarcillin, piperacillin, cephalothin, ceftazidime, and aztreonam and were moderately susceptible to cefotaxime (with diameters of 19 and 26 mm). However, the double-disk synergy test (9) between cefotaxime or ceftazidime and amoxicillin-clavulanic acid (Augmentin [20 μg of amoxicillin plus 10 μg of clavulanic acid]; Beecham Laboratories) was positive. The two strains were susceptible to moxalactam and imipenem.

The MICs of different β-lactam antibiotics for *E. coli* K-12 harboring the different plasmid-mediated β-lactamases are shown in Table 1. In all transconjugants, the MICs of ceftazidime (32 to 512 μg/ml) were higher than the MICs of cefotaxime (0.5 to 8 μg/ml). The MICs of aztreonam were higher for *E. coli* harboring CAZ-6 and CAZ-7 (128 and 64 μg/ml, respectively) than for *E. coli* producing the ceftazidimases CAZ-1 (8 μg/ml), CAZ-2 (8 μg/ml), or CAZ-3 (2 μg/ml). For transconjugants harboring the different ceftazidimases, the MICs of moxalactam (0.25 to 1 μg/ml) and imipenem (0.25 to 0.5 μg/ml) were close to those for *E. coli* K-12. Similar MICs were observed for *K. pneumoniae* isolates and their *E. coli* transconjugants with all β-lactams except cefoxitin and moxalactam for CAZ-1-producing strains. In this case, the wild type was more resistant than its transconjugant (data not shown).

**Susceptibility to β-lactamase inhibitors.** The reduced activities of piperacillin, cefoperazone, and cefotaxime were restored equally well by 2 μg of clavulanate, sulbactam, tazobactam, or brobactam per ml for *E. coli* derivatives producing the different ceftazidimases (Table 2). The four β-lactamase inhibitors potentiated equally well the activities of ceftazidime (≤1 μg/ml) and aztreonam (≤0.12 μg/ml) for *E. coli* harboring CAZ-2 and CAZ-3. In contrast, they had a lower protective effect on ceftazidime for *E. coli* producing CAZ-1, CAZ-6 and CAZ-7. Moreover, the activities of ceftazidime and aztreonam were weakly potentiated by sulbactam for *E. coli* producing CAZ-6 (32 and 2 μg/ml, respectively).

**Kinetic constants.** $K_m$ and $V_{max}$ values of the different ceftazidimases for cefotaxime, ceftazidime, and aztreonam are reported in Table 3. These enzymes can be separated into two groups. The first includes the enzymes CAZ-3 and CAZ-7, characterized by a fairly weak activity, with rates of hydrolysis always lower than those for benzylpenicillin. While the $V_{max}$ for ceftazidime was similar to that for cefotaxime with CAZ-3 (10.2 and 18.4, respectively), the ceftazidimase CAZ-7 hydrolyzed 10-fold more ceftazidime ($V_{max}$, 98) than did cefotaxime ($V_{max}$, 9.8). These two enzymes weakly hydrolyzed aztreonam ($V_{max}$, 2.3 and 28, respectively).

In contrast, the second group includes very active enzymes CAZ-1, CAZ-2, and CAZ-6, which hydrolyzed cefo-

TABLE 2. Potentiation effect of clavulanate, sulbactam, tazobactam, and brobactam in combination with β-lactam antibiotics against *E. coli* derivatives producing the different ceftazidimases

| Antibiotic (plus inhibitor)[a] | MIC (μg/ml) for *E. coli* producing: | | | | |
|---|---|---|---|---|---|
| | CAZ-1 | CAZ-2 | CAZ-3 | CAZ-6 | CAZ-7 |
| Amoxicillin | >4,096 | >4,096 | >4,096 | >4,096 | >4,096 |
| + CA | 32 | 16 | 64 | 32 | 32 |
| + Sul | 512 | 16 | 32 | 128 | 64 |
| + Taz | 128 | 16 | 32 | 64 | 16 |
| + Bro | 32 | 16 | 32 | 16 | 32 |
| Piperacillin | 64 | 64 | 128 | 64 | 256 |
| + CA | 2 | 1 | 1 | 4 | 2 |
| + Sul | 2 | 1 | 2 | 4 | 2 |
| + Taz | 2 | 1 | 2 | 2 | 2 |
| + Bro | 2 | 1 | 2 | 2 | 2 |
| Cefoperazone | 32 | 16 | 8 | 32 | 32 |
| + CA | 0.5 | 0.5 | 0.5 | 0.5 | 0.12 |
| + Sul | 1 | 0.25 | 0.25 | 1 | 0.25 |
| + Taz | 0.5 | 0.12 | 0.25 | 0.25 | 0.12 |
| + Bro | 0.5 | 0.12 | 0.25 | 0.25 | 0.12 |
| Cefotaxime | 8 | 2 | 0.5 | 8 | 4 |
| + CA | 0.25 | 0.12 | 0.12 | 0.25 | 0.06 |
| + Sul | 0.25 | 0.06 | 0.06 | 0.25 | 0.12 |
| + Taz | 0.12 | 0.06 | 0.06 | 0.12 | 0.06 |
| + Bro | 0.12 | 0.06 | 0.06 | 0.12 | 0.06 |
| Ceftazidime | 128 | 128 | 32 | 512 | 256 |
| + CA | 2 | 0.5 | 0.5 | 4 | 2 |
| + Sul | 8 | 0.5 | 0.5 | 32 | 2 |
| + Taz | 4 | 0.5 | 0.5 | 8 | 1 |
| + Bro | 2 | 1 | 0.5 | 4 | 2 |
| Aztreonam | 8 | 8 | 2 | 128 | 64 |
| + CA | 0.25 | 0.12 | 0.12 | 0.25 | 0.25 |
| + Sul | 0.5 | 0.12 | 0.12 | 2 | 0.25 |
| + Taz | 0.25 | 0.12 | 0.12 | 0.5 | 0.12 |
| + Bro | 0.12 | 0.12 | 0.12 | 0.25 | 0.12 |

[a] Clavulanic acid (CA), sulbactam (Sul), tazobactam (Taz), and brobactam (Bro) were each used at a concentration of 2 μg/ml.

TABLE 3. Kinetic constants of ceftazidimases from *E. coli* transconjugants[a]

| Substrate | CAZ-1 | | CAZ-2 | | CAZ-3 | | CAZ-6 | | CAZ-7 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $V_{max}$[b] | $K_m$ (μM) | $V_{max}$ | $K_m$ (μM) | $V_{max}$ | $K_m$ (μM) | $V_{max}$ | $K_m$ (μM) | $V_{max}$ | $K_m$ (μM) |
| Benzylpenicillin | 100 | 4.2 | 100 | 6 | 100 | 6.3 | 100 | 5 | 100 | 7.8 |
| Cefotaxime | 150 | 28 | 640 | 75 | 18.4 | 310 | 208 | 48.5 | 9.8 | 43.8 |
| Ceftazidime | 490 | 120 | 261 | 145 | 10.2 | 240 | 848 | 251 | 98 | 125.2 |
| Aztreonam | 116 | 100 | 207 | 62 | 2.3 | 158 | 134.2 | 29 | 28 | 31.2 |

[a] Specific activities (mU/mg): CAZ-1, 34; CAZ-2, 22; CAZ-3, 24; CAZ-6, 82; CAZ-7, 580. In each case, 50 mU was used.
[b] Values are relative to those for benzylpenicillin (taken as 100%).

taxime efficiently, with $V_{max}$ values ranging from 150 to 640. CAZ-1 and CAZ-6 demonstrated higher rates of hydrolysis for ceftazidime ($V_{max}$, 490 and 848, respectively) than for cefotaxime ($V_{max}$, 150 and 208, respectively). With the enzyme CAZ-2, the opposite effect was observed. The ceftazidimases CAZ-1, CAZ-2, and CAZ-6 were markedly active against aztreonam, with $V_{max}$ values ranging from 116 to 207.

**Characterization of the specifying plasmids.** Plasmid DNA from the *K. pneumoniae* isolates and from the corresponding *E. coli* K-12 transconjugants was analyzed by agarose gel electrophoresis (Fig. 2). The novel β-lactamases CAZ-6 and CAZ-7 were both mediated by approximately 85-kb plasmids (pCFF74 and pCFF84, respectively) as previously reported for the β-lactamases CTX-1 (pCFF04), CAZ-2 (pCFF34), and CAZ-3 (pCFF44) (7, 18; Labia et al., 28th ICAAC; Sirot et al., in press). Incompatibility tests with *E. coli* BM21 harboring pIP135 revealed that all the 85-kb plasmids belonged to incompatibility group M or 7. The enzyme CAZ-1 (16) was mediated by a larger plasmid of approximately 150 kb. All these plasmids were transferable to *E. coli* with a frequency ranging from $10^{-4}$ to $10^{-6}$ (Table 4).

In *E. coli* transconjugants harboring CAZ-2, CAZ-6, and CAZ-7, β-lactam resistance was cotransferred with resistance to aminoglycosides (amikacin, kanamycin, netilmicin, tobramycin), sulfonamide, and tetracycline. Resistance markers cotransferred with CAZ-1 were kanamycin, neomycin, streptomycin, sulfonamide, and tetracycline. In *E. coli* producing CAZ-3, β-lactam resistance was associated with resistance to streptomycin, sulfonamide, tetracycline, and trimethoprim.

The plasmid pCFF84 (CAZ-7) showed an *Eco*RI restriction pattern similar to those of plasmids pCFF04 (CTX-1), pCFF34 (CAZ-2), and pCFF44 (CAZ-3) (Fig. 3A). The *Eco*RI restriction pattern of plasmid pCFF74 (CAZ-6) differed from these only by one additional fragment. All these plasmids seemed to be closely related to pIP135. In contrast, plasmid pCFF14 (CAZ-1) showed a very different restriction pattern. A TEM probe hybridized with the largest *Eco*RI fragment (19 kb) of plasmids pCFF04 (CTX-1), pCFF34 (CAZ-2), pCFF44 (CAZ-3), pCFF74 (CAZ-6), pCFF84 (CAZ-7), and of pCFF24 (TEM-2) used as a positive control. Hybridization was also observed with a fragment larger than 19 kb of plasmid pCFF14 (CAZ-1) (Fig. 3B).

## DISCUSSION

After the outbreak of infections caused by CTX-1-producing members of the family *Enterobacteriaceae* (17), we observed in the same hospital the advent of five different TEM-derived broad-spectrum β-lactamases: CAZ-1, CAZ-2, CAZ-3, CAZ-6, and CAZ-7. These enzymes were designated ceftazidimases (CAZ) as previously used for enzymes conferring a markedly higher level of resistance to ceftazidime than to cefotaxime (Table 5), although the hydrolysis ($V_{max}$) of ceftazidime was not always higher than that of cefotaxime. Like other TEM-derived ceftazidimases previously described, they were characterized by acid isoelectric points while SHV-derived ceftazidimases showed pIs ranging from 7 to 8.2.

The enzymes CAZ-1, CAZ-6, and CAZ-7 had a greater hydrolytic activity against ceftazidime than against cefotaxime. Conversely, CAZ-2 showed a higher $V_{max}$ for cefotaxime than for ceftazidime. CAZ-3 showed low and similar rates of hydrolysis of ceftazidime and cefotaxime. It was, nevertheless, interesting to observe that for all these enzymes, except CAZ-7, the efficiency of hydrolysis ($V_{max}$:$K_m$ ratio) of ceftazidime remained always slightly lower than that of cefotaxime. Conversely, MICs of ceftazidime were always higher than those of cefotaxime. The increased resistance to ceftazidime could be explained by other factors, such as antibiotic diffusion across the outer membrane (23). Plasmids encoding the two novel enzymes CAZ-6 and CAZ-7 and the previously described ceftazidimase CAZ-2 coded for the same resistance-associated markers as those of pCFF04 encoding CTX-1 enzyme: aminoglycosides via a 6'-acetyltransferase IV, sulfonamide, and tetracycline. Interestingly, plasmids pCFF74 (CAZ-6), pCFF84 (CAZ-7), and pCFF34 (CAZ-2) had the same molecular mass as pCFF04 (CTX-1) first had in the same hospital. Moreover,

TABLE 4. Characteristics of TEM-derived ceftazidimases specifying plasmids and comparison with pCFF04 encoding for CTX-1

| Enzyme | pI | Plasmid (incompatibility group) | Plasmid size (kb) | Resistance markers cotransferred[a] | Frequency of transfer |
|---|---|---|---|---|---|
| CAZ-1/TEM-5 | 5.55 | pCFF14 | 150 | Km Nm Sm Su Tc | $10^{-6}$ |
| CAZ-2 | 6.0 | pCFF34 (M or 7) | 85 | Ak Km Nt Su Tc Tm | $10^{-4}$ |
| CAZ-3 | 5.3 | pCFF44 (M or 7) | 85 | Sm Su Tc Tp | $10^{-4}$ |
| CAZ-6 | 6.5 | pCFF74 (M or 7) | 85 | Ak Km Nt Su Tc Tm | $10^{-6}$ |
| CAZ-7 | 6.3 | pCFF84 (M or 7) | 85 | Ak Km Nt Su Tc Tm | $10^{-6}$ |
| CTX-1/TEM-3 | 6.3 | pCFF04 (M or 7) | 85 | Ak Km Nt Su Tc Tm | $10^{-4}$ |

[a] Abbreviations: Ak, amikacin; Km, kanamycin; Nt, netilmicin; Sm, streptomycin; Su, sulfonamides; Tc, tetracyclines; Tm, tobramycin; Tp, trimethoprim.

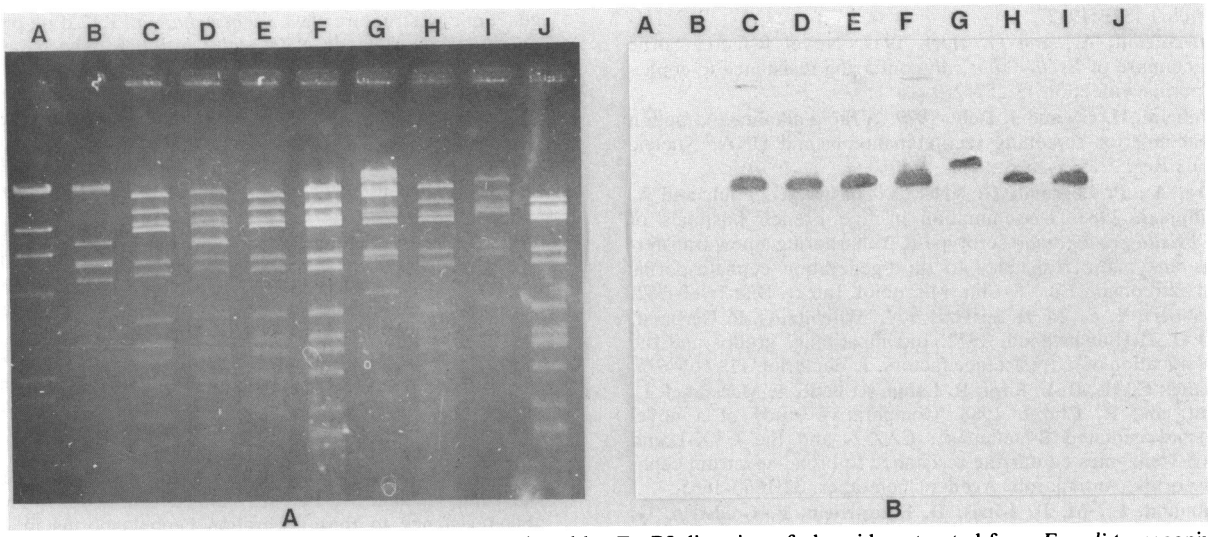


FIG. 3. (A) Agarose gel electrophoresis of fragments produced by *Eco*RI digestion of plasmids extracted from *E. coli* transconjugants. Lanes: C, pCFF84 (CAZ-7); D, pCFF74 (CAZ-6); E, pCFF44 (CAZ-3); F, pCFF34 (CAZ-2); G, pCFF14 (CAZ-1); H, pCFF04 (CTX-1); I, pCFF24 (TEM-2); J, pIP135. Lambda DNA digested with *Hin*dIII (lane A) and with *Eco*RI (lane B) were used as molecular standards. (B) Corresponding hybridization with a TEM probe.

the *Eco*RI restriction patterns of pCFF84 and pCFF34 were identical (or closely related, for pCFF74) to that of pCFF04. Since it has been demonstrated that CTX-1 derived from TEM-2 by two point mutations (19), further studies are in progress to confirm the hypothesis that some new extended broad-spectrum β-lactamases isolated in the same hospital could derive from one another by a similar evolutionary process. Interestingly, the first isolates of *K. pneumoniae* producing CAZ-2, CAZ-6, and CAZ-7 were detected under ceftazidime treatment in patients who had previously harbored CTX-1-producing strains. Moreover, the enzymes CAZ-6 and CAZ-7 had isoelectric points close or identical to that of CTX-1. Although the associated resistance markers were different, the plasmid pCFF44 (CAZ-3) also seemed similar to pCFF04.

TABLE 5. Plasmid-mediated ceftazidimases

| Ceftazidimase | pI | Reference |
|---|---|---|
| TEM derivatives | | |
| CAZ-1 (TEM-5) | 5.55 | 16 |
| TEM-6[a] | 5.85 | 3 |
| TEM-7 | 5.41 | 8 |
| RHH-1 (TEM-9) | 5.5 | 21 |
| CAZ-2 | 6.0 | 7 |
| CAZ-3 | 5.3 | Labia et al.[b]; Sirot et al.[c] |
| TEM-10 | 5.57 | Quinn et al.[d] |
| CAZ-6 | 6.5 | This report |
| CAZ-7 | 6.3 | This report |
| SHV derivatives | | |
| CAZ-4/SHV-5 | 8.2 | Labia et al.[b]; Sirot et al.[c]; Gutmann et al.[e] |
| CAZ-5/SHV-4 | 7.8 | 5; Labia et al.[b]; Sirot et al.[c]; Barthelemy et al.[f] |

[a] Not named in the original publication, the enzyme has subsequently been designated TEM-6.
[b] Labia et al., 28th ICAAC.
[c] Sirot et al., in press.
[d] J. P. Quinn, D. Miyashiro, B. Edlin, R. Flamm, and K. Bush, 28th ICAAC, abstr. no. 484, 1988.
[e] L. Gutmann, F. W. Goldstein, B. Ferre, N. Ritzk, M. H. Nicolas, J. F. Acar, and E. Collatz, 28th ICAAC, abstr. no. 869, 1988.
[f] M. Barthelemy, J. Peduzzi, J. Chenon, C. Nicolas, A. Thabaut, K. Tiwari, A. Morand, and R. Labia, 28th ICAAC, abstr. no. 480, 1988.

The restriction patterns and hybridization with a TEM probe on the same *Eco*RI fragment of all these 85-kb plasmids suggest a common origin. The 150-kb plasmid pCFF14 (CAZ-1) harbored resistance markers different from those associated with the 85-kb plasmids. It showed an *Eco*RI restriction pattern clearly different from the others. It has been demonstrated that the enzyme CAZ-1 (TEM-5) derived from the β-lactamase TEM-1 (A. Petit, S. Goussard, W. Sougakoff, D. Sirot, and P. Courvalin, 28th ICAAC, abstr. no. 861, 1988). These results confirmed that the genes encoding CTX-1 and CAZ-1 had separate origins.

Since the first ceftazidimase-producing *K. pneumoniae* strains were isolated, 11 more CAZ-1-producing, 14 more CAZ-2-producing, 9 more CAZ-6-producing, and 5 more CAZ-7-producing strains have been encountered in different species of *Enterobacteriaceae*. The CAZ-6 enzyme was observed in the same intensive care unit as CAZ-1. Neither enzyme spread to other units. CAZ-7-producing strains were isolated from patients hospitalized in another intensive care unit. In contrast, CAZ-2-producing strains were observed in nine different wards of Clermont-Ferrand hospitals. The CAZ-3 enzyme was detected in only one *K. pneumoniae* isolate. The extensive use of ceftazidime in our hospitals during the years 1986 and 1987 could be, in part, responsible for the emergence and dissemination of strains producing these different plasmid-mediated ceftazidimases.

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