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# Transferable Cefoxitin Resistance in Enterobacteria from Greek Hospitals and Characterization of a Plasmid-Mediated Group 1 β-Lactamase (LAT-2)

MARIA GAZOULI, LEONIDAS S. TZOUVELEKIS, EFTHIMIOS PRINARAKIS, VIVI MIRIAGOU, AND EVA TZELEPI*

*Department of Bacteriology, Hellenic Pasteur Institute, Athens 11521, Greece*

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**Cefoxitin resistance in *Klebsiella pneumoniae* and *Escherichia coli* strains isolated in Greek hospitals was found to be due to the acquisition of similar plasmids coding for group 1 β-lactamases. The plasmids were not self-transferable but were mobilized by conjugative plasmids. These elements have also been spread to *Enterobacter aerogenes*. The most common enzyme was a *Citrobacter freundii*-derived cephalosporinase (LAT-2) which differed from LAT-1 by three amino acids.**

Emergence of plasmid-mediated group 1 β-lactamase has been recently reported in several studies. The enzymes resemble chromosomal class C β-lactamases in that they confer resistance to cephalosporins, including cefoxitin, and are not inhibited by clavulanate (3). Their presence appears to be sporadic and limited mainly to *Klebsiella pneumoniae* (2, 7, 9, 11, 12, 18, 25). Genetic data show that plasmid-mediated group 1 β-lactamases have been derived from *Citrobacter freundii* (5, 24), *Pseudomonas aeruginosa* (7, 9), and *Enterobacter cloacae* (18). The encoding plasmids differ in molecular mass, transfer proficiency, and cotransferred resistance markers (20). These facts suggest that *ampC* genes can translocate from the chromosomes of several species into various plasmids. A considerable number of *K. pneumoniae* isolates from Greek hospitals exhibit resistance to cefoxitin and β-lactam–inhibitor combinations (13, 27). We show here that this phenotype is due to the acquisition of plasmids coding for *C. freundii*-derived cephalosporinases. We also found that such plasmids have been spread to *Escherichia coli* and *Enterobacter aerogenes*.

Twelve *K. pneumoniae*, two *E. coli*, and two *E. aerogenes* clinical strains resistant to cefoxitin (MIC ≥ 32 μg/ml) were examined. The latter strains were collected as imipenem resistant and were included in the study after they were found to be able to transfer cefoxitin resistance. The *K. pneumoniae* and *E. coli* strains studied represent all the cefoxitin-resistant strains of these species isolated during October 1994 in five Athens hospitals. *E. coli* strains used as recipients and strains producing standard β-lactamases have been described previously (25). Typing of *K. pneumoniae* was done by a randomly amplified polymorphic DNA PCR (4).

Susceptibilities to β-lactams were evaluated by the E test (AB Biodisk, Solna, Sweden). Susceptibilities to other antibiotics were evaluated by standard antibiograms (16). Transfer of resistance characters was carried out by conjugation using a recipient *E. coli* 26R793 ($Lac^-$ $Rif^r$) (26). Transconjugants were selected on media containing cefoxitin (20 μg/ml) or ampicillin (40 μg/ml) plus rifampin (200 μg/ml). Analysis of plasmid patterns was performed with the QIAGEN Plasmid Kit (QIAGEN, Hilden, Germany). For transformation of *E. coli* C600 and XL1-Blue cells, plasmid DNA preparations or plasmids isolated from low-melting-point agarose gels were used (21). Plasmid restriction patterns were obtained after digestion with several endonucleases. Endonuclease-treated plasmids were transferred to nitrocellulose and hybridized with a $bla_{LAT-1}$ probe and an *E. cloacae* MHN1 *ampC* probe under high-stringency conditions (20× SSC [1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate], 65°C). The former probe was a 288-bp *Sac*II-*Mlu*I fragment from plasmid pBL23 (22), and the latter was a 514-bp *Sph*I-*Nru*I fragment from plasmid pEC1E (8). Both probes were internal to the respective cephalosporinase coding regions. They were labeled by random primer incorporation of digoxigenin-dUTP (Boehringer, Mannheim, Germany).

For β-lactamase studies, cell sonic extracts clarified by centrifugation were used. The β-lactamase content was assessed by measuring cephaloridine hydrolysis rates spectrophotometrically at 295 nm and expressed as units of activity (1 U = 1 μmol of cephaloridine hydrolyzed per min per mg of protein at pH 7.0 and 37°C). Hydrolytic activity against β-lactams was evaluated by UV spectrophotometry. Determinations were performed at pH 7.0 and 37°C. The substrates, used at various concentrations, and the respective wavelengths were as follows: penicillin G (233 nm), ampicillin (235 nm), cephalothin (265 nm), cephaloridine (295 nm), ceftazidime (260 nm), cefotaxime (264 nm), cefpirome (258 nm), and ceftibuten (260 nm). The maximum rate of hydrolysis ($V_{max}$) and $K_m$ values were determined by Lineweaver-Burk plots. The $V_{max}$ values were expressed as hydrolysis rates relative to that of cephaloridine set at 100. The concentrations of clavulanate, aztreonam, and cefoxitin that inhibited 50% of the enzymatic activity against nitrocefin were assessed as described previously (25). Analytical isoelectric focusing was performed on polyacrylamide gels. The ampholytes covered a pH range from 3.5 to 9.5 (Pharmacia-LKB, Uppsala, Sweden).

Nucleotide sequencing was performed by the dideoxy chain termination method (23) with a Sequenase 2.0 kit (U.S. Biochemical Corp., Cleveland, Ohio). Three oligonucleotides that correspond to positions 561 to 578 and 845 to 866 (sense) and 1101 to 1122 (antisense) of the *C. freundii* OS60 *ampC* gene (14), as well as other universal primers, were used.

All cefoxitin-resistant *E. coli* ($n$ = 2) and *K. pneumoniae* ($n$ = 12) strains were isolated from hospitalized patients. They represented 1% of the *E. coli* strains and 14% of the *K. pneumoniae* strains isolated throughout the period of surveillance.

* Corresponding author. Mailing address: Department of Bacteriology, Hellenic Pasteur Institute, Vass. Sofias 127, Athens 11521, Greece. Phone: 01130-1-6462281. Fax: 01130-1-6423498.

TABLE 1. E test MICs of β-lactam antibiotics, β-lactamase contents, and additional non-β-lactam resistance markers of the clinical strains and representative *E. coli* 26R793 transconjugants and *E. coli* C600 transformants

| Strain and trc(s) and/or trf[a] | E test MIC (μg/ml)[b] | | | | | β-Lactamase content | | Non-β-lactam resistance markers[e] |
|---|---|---|---|---|---|---|---|---|
| | Fox | Caz | Ctx | Atm | Amc | pI(s)[c] | Units[d] | |
| *E. coli* S208 | >256 | >256 | 128 | >256 | 64 | 5.4, 8.2, (8.5), 9.4 | 636 | Gm Tb Cm Tp Tc |
| trc S208-F[f] | 256 | >256 | 128 | 128 | 128 | 8.2, (8.5), 9.4 | 518 | Gm Tb Tp Tc |
| trf S208-F | 256 | >256 | 64 | 64 | 128 | (8.5), 9.4 | 404 | None |
| *E. coli* S95 | 256 | >256 | 128 | 48 | 64 | 8.9 | 400 | None |
| trf S95-F | 256 | >256 | 64 | 64 | 128 | (8.5), 8.9 | 411 | None |
| *K. pneumoniae* N1 | >256 | >256 | 32 | >256 | 32 | 5.4, (7.6), 8.2, 9.1 | 466 | Gm Tb An Nt Cm Tp |
| *K. pneumoniae* N2 | >256 | >256 | 24 | >256 | 48 | 5.4, (7.6), 8.2, 9.1 | 325 | Gm Tb An Nt Cm Tp |
| *K. pneumoniae* N3 | 128 | >256 | 64 | >256 | 12 | 5.4, (7.6), 8.2, 9.1 | 228 | Gm Tb An Nt Tp Tc |
| *K. pneumoniae* N4 | >256 | 256 | 24 | >256 | 48 | 5.4, (7.6), 8.2, 9.1 | 302 | Gm Tb An Nt Tp |
| *K. pneumoniae* N5 | >256 | >256 | >256 | >256 | 64 | 5.4, (7.6), 8.2, 9.1 | 501 | Gm Tb An Nt Cm Tp |
| trc N5-A1[f] | 4 | 0.5 | 0.1 | 0.125 | 12 | 5.4, (8.5) | 60 | Gm Tb |
| trc N5-A2 | 4 | 128 | 24 | 128 | 6 | 8.2, (8.5) | 140 | Gm Tb An Nt Tp |
| trc N5-F1 | 256 | >256 | >256 | 64 | 128 | 5.4, 8.2, (8.5), 9.1 | 520 | Gm Tb An Nt |
| trc N5-F2 | 256 | >256 | 48 | 48 | 128 | (8.5), 9.1 | 399 | None |
| trf N5-F | 256 | >256 | 64 | 64 | 128 | (8.5), 9.1 | 422 | None |
| *K. pneumoniae* N6 | >256 | >256 | 24 | >256 | 48 | (7.6), 8.2, 9.1 | 330 | Gm Tb An Nt Cm Tp |
| *K. pneumoniae* N7 | >256 | >256 | 24 | >256 | 32 | 5.4, (7.6), 8.2, 9.1 | 315 | Gm Tb An Nt Cm Tp Tc |
| *K. pneumoniae* N8 | >256 | >256 | 24 | >256 | 32 | 5.4, (7.6), 8.2, 9.1 | 307 | Gm Tb An Nt Cm Tp |
| *K. pneumoniae* N9 | >256 | >256 | 16 | >256 | 32 | 5.4, (7.6), 8.2, 9.1 | 288 | Gm Tb An Nt Tp |
| *K. pneumoniae* N10 | 64 | >256 | 16 | 64 | 32 | (7.6), 8.2, 9.1 | 122 | Gm Tb An Nt Tp |
| trf N10-F | >256 | >256 | 32 | 24 | 128 | (8.5), 9.1 | 410 | None |
| *K. pneumoniae* T80 | 96 | 1.5 | 1 | 0.5 | 16 | (7.6), 9.1 | 43 | Cm Tp Tc |
| *K. pneumoniae* L67 | 32 | 8 | 3 | 3 | 32 | (7.6), 9.1 | 44 | None |
| trf L67-F | 256 | >256 | 64 | 64 | 128 | (8.5), 9.1 | 430 | None |
| *E. aerogenes* Y15 | >256 | 32 | 24 | 24 | 32 | (8.2), 9.1 | 722 | Tb An Nt Cm Tp Tc |
| trf Y15-F | >256 | >256 | 24 | 24 | 128 | (8.5), 9.1 | 394 | None |
| *E. aerogenes* Y25 | >256 | >256 | 256 | 256 | 256 | (8.2), 9.1 | 1,423 | Tb An Nt |
| trc Y25-F | 256 | 256 | 32 | 48 | 64 | (8.5), 9.1 | 377 | None |
| trf Y25-F | 256 | >256 | 64 | 64 | 128 | (8.5), 9.1 | 417 | None |
| *E. coli* 26R793 | 4 | 0.38 | 0.05 | 0.125 | 4 | (8.5) | ND[g] | None |
| *E. coli* C600 | 4 | 0.25 | 0.05 | 0.09 | 4 | (8.5) | ND | None |

[a] trc, *E. coli* 26R793 transconjugant; trf, *E. coli* C600 transformant.
[b] Fox, cefoxitin; Caz, ceftazidime; Ctx, cefotaxime; Atm, aztreonam; Amc, amoxicillin plus clavulanate (2:1).
[c] β-Lactamase bands presumed to be the chromosomal enzymes of the host strains are in parentheses.
[d] Units are as described in the text.
[e] Gm, gentamicin; Tb, tobramycin; An, amikacin; Nt, netilmicin; Cm, chloramphenicol; Tp, trimethoprim; Tc, tetracycline.
[f] F and A, selection with cefoxitin and ampicillin, respectively.
[g] ND, not determined because the hydrolysis rate was too low.

The *E. coli* strains were from separate hospitals. The *E. aerogenes* strains ($n = 2$) were isolated in the intensive care unit of a hospital during the same period. Typing of *K. pneumoniae* isolates by randomly amplified polymorphic DNA PCR revealed that they were scattered in five types (data not shown). This, taken together with the differences in biotypes and plasmid patterns, indicated that they were distinct. Differences in the respective biotypes were also observed for the *E. coli* and *E. aerogenes* isolates.

Susceptibilities to antibiotics and β-lactamase contents of the strains are presented in Table 1. The *E. coli* strains S95 and S208 were resistant to amoxicillin-clavulanate, cefoxitin, aztreonam, and oxyimino cephalosporins (ceftazidime and cefotaxime). This phenotype was observed for 10 *K. pneumoniae* strains (N1 to N10). The remaining *K. pneumoniae* strains, T80 and L67, were resistant only to cefoxitin and amoxicillin-clavulanate. The *E. aerogenes* strains Y15 and Y25 were resistant to all β-lactams tested. Most of the strains were also resistant to other non-β-lactam antibiotics. Isoelectric focusing showed that the *K. pneumoniae* and *E. aerogenes* strains possessed a β-lactamase focused at 9.1. The *E. coli* strains S95 and S208 exhibited β-lactamase bands with pIs of 8.9 and 9.4, respectively (Fig. 1). In all *K. pneumoniae* strains, except L67 and T80, and in the *E. coli* strain S208, the above-described enzymes were accompanied by additional bands focused at pIs of 8.2 and 5.4. It is likely that the latter bands represent the SHV-5 and TEM-1 β-lactamases that are encountered frequently in enterobacteria isolated in Greek hospitals (26, 27).

Conjugal transfer of β-lactam resistance was observed with one *E. coli* strain (S208), one *E. aerogenes* strain (Y25), and five

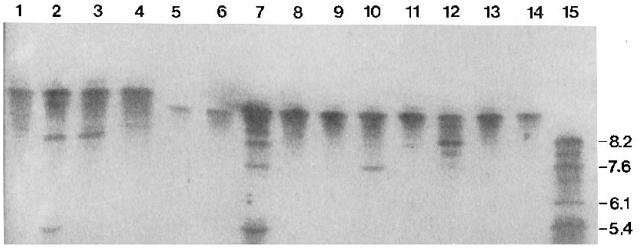


FIG. 1. Isoelectric focusing of β-lactamases in representative clinical strains and the respective *E. coli* 26R793 transconjugants (trc) and *E. coli* C600 transformants (trf). Lanes: 2 to 4, *E. coli* S208 and trc S208-F; 5 and 6, *E. coli* S95 and trf S95-F; 7 to 9, *K. pneumoniae* N5, trc N5-F2, and trf N5-F; 10 and 11, *K. pneumoniae* L67 and trf L67-F; 12 to 14, *E. aerogenes* Y25, trc Y25-F, and trf Y25-F. LAT-1 β-lactamase (pI = 9.4) is in lane 1. The pIs of the enzymes TEM-1, PSE-2, SHV-1, and SHV-5 in lane 15 are indicated on the right.

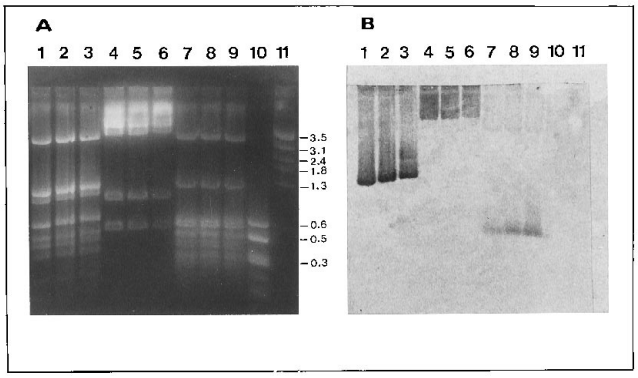


FIG. 2. (A) Comparison of restriction patterns of three 8.3-kb plasmids derived from *K. pneumoniae* N5 (lanes 1, 4, and 7), *E. coli* S208 (lanes 2, 5, and 8), and *E. coli* S95 (lanes 3, 6, and 9). The endonucleases used were *Rsa*I (lanes 1 to 3), *Dra*I (lanes 4 to 6), and *Hae*III (lanes 7 to 9). Molecular weight markers are in lanes 10 and 11 (sizes in kilobase pairs are on the right). (B) The same preparations after hybridization with the LAT-1 probe.

TABLE 2. Substrate profiles of LAT-2 and reference β-lactamases (BIL-1 and LAT-1)

| Substrate | Relative $V_{max}$ by β-lactamase[a]: | | Result for β-lactamase LAT-2[b] | |
|---|---|---|---|---|
| | BIL-1[c] | LAT-1[b] | Relative $V_{max}$[a] | $K_m$ (μM) |
| Cephaloridine | 100 | 100 | 100 | 10 |
| Cephalothin | 1.2 | 140 | 169 | 54.5 |
| Penicillin G | ND[d] | 3 | 3 | 12 |
| Ampicillin | ND | 1 | 1 | 17 |
| Ceftibuten | ND | 3 | 6.2 | 188 |
| Cefpirome | ND | ND | 14 | 360 |
| Ceftazidime | ND | 2 | 2.2 | ND |
| Cefotaxime | ND | <1 | 1 | ND |

[a] $V_{max}$ values are relative to that of cephaloridine set at 100.
[b] For LAT-1 and LAT-2 enzymes, the respective values were determined in parallel experiments.
[c] $V_{max}$ values for BIL-1 are from reference 19.
[d] ND, not determined.

*K. pneumoniae* (N1 to N5) strains. Transfer frequencies ranged from $10^{-8}$ to $10^{-7}$ per donor cell when cefoxitin was used for selection. The frequencies were higher with ampicillin ($10^{-6}$ to $10^{-4}$). Transconjugants selected with cefoxitin exhibited the β-lactam resistance phenotype of the donors and had acquired the β-lactamase with a pI of 9.1 or that with a pI of 9.4 when the donor was *E. coli* S208. A small proportion of clones selected on ampicillin were resistant to cefoxitin and expressed the β-lactamase that focused at a pI of 9.1 or that which focused at a pI of 9.4. The rest were resistant only to ampicillin or to ampicillin, oxyimino cephalosporins, and aztreonam and expressed β-lactamases focusing at 8.2 and 5.4. Other resistance markers were also transferred. The resistance phenotypes observed along with the isoelectric points suggest that the latter clones had acquired SHV-5 and TEM-1. None of the cefoxitin-susceptible transconjugants acquired any of the β-lactamases that focused at pIs of 9.1 or 9.4. *E. coli* S95, exhibiting the β-lactamase with a pI of 8.9, as well as *E. aerogenes* Y15 and *K. pneumoniae* N6 to N10, was not able to transfer cefoxitin resistance by conjugation.

Plasmid analysis showed that small plasmids of 8.3 kb were consistently present in all clinical strains and cefoxitin-resistant transconjugants. Plasmid preparations from the clinical strains were run in low-melting-point agarose. The 8.3-kb bands were excised from the gel, purified, and used to transform *E. coli* C600 and XL1-Blue cells. In all cases, cefoxitin-resistant transformants were obtained. They were also resistant to oxyimino cephalosporins, aztreonam, and amoxicillin-clavulanate. Analysis of β-lactamase patterns by isoelectric focusing showed that the transformants derived by using *K. pneumoniae* and *E. aerogenes* plasmid preparations expressed the enzyme that focused at a pI of 9.1. Transformants derived from plasmids from *E. coli* strains S95 and S208 displayed the same resistance phenotype and expressed β-lactamases with pIs of 8.9 and 9.4, respectively. Notably, when plasmid preparations from *K. pneumoniae* strains T80 and L67 were used, the *E. coli* transformants obtained were also resistant to oxyimino cephalosporins and aztreonam and expressed levels of β-lactamase higher than those expressed by the respective clinical strains (Table 1). None of the *E. coli* C600 transformants were able to transfer β-lactam resistance by conjugation. However, when transformants of *E. coli* XL1-Blue, which contain the F′ factor, were used as donors, β-lactam-resistant transconjugants were obtained. All 8.3-kb plasmids gave similar restriction profiles (Fig. 2A). These data indicated that closely related plasmids coding for basic β-lactamases (with pIs of 8.9, 9.1, and 9.4) and conferring a cephalosporinase phenotype have been spread to the examined clinical strains. The plasmids were not self-transmissible but were mobilized by conjugative plasmids.

The β-lactamase displaying a pI equal to 9.1 was selected for further characterization. Sonic extracts from transformant trf N5-F (Table 1) were used for hydrolysis studies. As shown in Table 2, the enzyme hydrolyzed cephaloridine and cephalothin more efficiently than it hydrolyzed penicillin G and ampicillin. It hydrolyzed ceftibuten and cefpirome but displayed low affinity for the antibiotic. The hydrolysis rate of ceftazidime was higher than that of cefotaxime. Hydrolysis of cefoxitin and aztreonam was very slow. Both antibiotics were, however, effective inhibitors of the hydrolysis of nitrocefin as shown by their low 50% inhibitory concentrations for this activity (Table 3). Clavulanate was ineffective as an inhibitor.

Fragments of plasmids digested with endonuclease reacted with a $bla_{LAT-1}$ probe (Fig. 2B). The fragment of the $bla_{LAT-1}$ gene used had a high degree of homology (96%) with the respective *Sac*II-*Mlu*I region of *C. freundii* OS60 (14) but less than 80% homology with the corresponding regions of the *E. cloacae* (6), *Serratia marcescens* (17), and *P. aeruginosa* (15) *ampC* genes. None of the plasmids reacted with the *E. cloacae* MHN1 *ampC* probe. It was estimated that under the stringent conditions employed, more than 80% homology was required to obtain a positive signal. Thus, the detected enzymes might be LAT-like and derived from *C. freundii*. The enzyme that focused at 9.1 was designated LAT-2, and the respective 8.3-kb plasmid from trf N5-F was designated pMEL. The $bla_{LAT-1}$

TABLE 3. Inhibition profiles of LAT-2 and reference β-lactamases (BIL-1 and LAT-1)

| β-Lactamase | $IC_{50}$ (μM) of inhibitor[a]: | | |
|---|---|---|---|
| | Clavulanate | Cefoxitin | Aztreonam |
| BIL-1 | 362 | 4.1 | ND[b] |
| LAT-1 | >250 | 0.8 | 0.03 |
| LAT-2 | 250 | 0.65 | 0.02 |

[a] $IC_{50}$, concentration that inhibited 50% of the enzymatic activity against nitrocefin. Values for BIL-1 are from reference 19. For LAT-1 and LAT-2 enzymes, the respective values were determined in parallel experiments.
[b] ND, not determined.

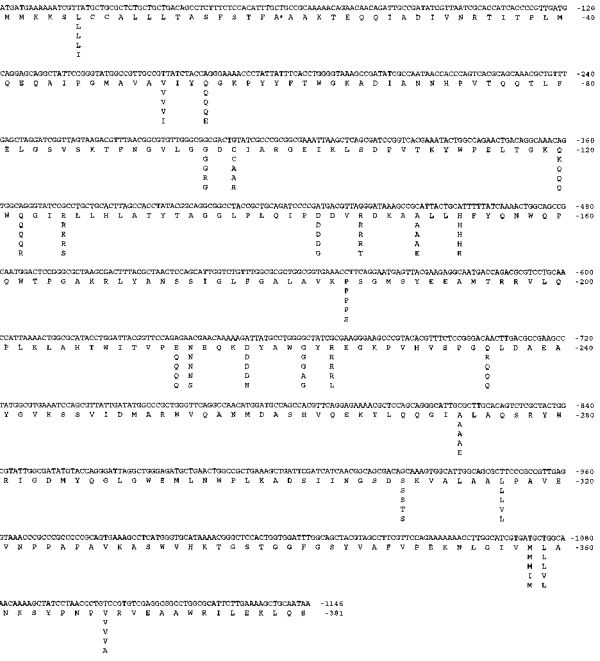

FIG. 3. The DNA sequence of $bla_{\text{LAT-2}}$ and the deduced amino acid sequence aligned with those of LAT-1, BIL-1, CMY-2, and *C. freundii* OS60 AmpC.

gene from pMEL was cloned into pBCSK(+), and its sequence was determined and found to be highly homologous with those of $bla_{\text{LAT-1}}$ (24), $bla_{\text{CMY-2}}$ (2), $bla_{\text{BIL-1}}$ (5), and the *C. freundii* OS60 *ampC* gene (14). The respective homologies of the deduced amino acid sequences were 99.2, 99.4, 97.8, and 95.3% (Fig. 3).

In this report we show that the cause of cefoxitin resistance in *E. coli* and *K. pneumoniae* strains isolated in Athens hospitals is the acquisition of similar non-self-transmissible but mobilizable plasmids that encode group 1 β-lactamases possibly derived from *C. freundii*. The plasmids are not confined to these species but have also been disseminated to *E. aerogenes*. Plasmid-mediated cephalosporinases in *K. pneumoniae* and *E. coli* may be detected by the phenotype they produce. Their presence in enterobacters, as observed here, and citrobacters (1) is phenotypically hidden because these species are able to express large amounts of chromosomal cephalosporinase (22). Hence, the spread of plasmid-mediated group 1 enzymes may be wider than previously thought.

The enzyme described here behaved as a typical AmpC β-lactamase. The hydrolysis rates of cefoxitin and aztreonam were low. However, LAT-2 displayed high affinity for these β-lactams. It is likely that high affinity compensates for the slow hydrolysis, thus explaining the MICs of both antibiotics. In fact, cefoxitin hydrolysis under the conditions that prevail in the periplasmic space (high enzyme and low drug concentrations) should be very efficient, since the respective MICs were higher than those of the other cephalosporins. Hybridization provided indications that the enzyme described above is closely related to the previously reported LAT-1 and may be derived from *C. freundii* (24). This notion was confirmed by sequencing of the $bla_{\text{LAT-2}}$ gene. The amino acid composition of LAT-2 was closely related to those of LAT-1, CMY-2, BIL-1, and *C. freundii* OS60 AmpC. LAT-2 differed from LAT-1 by three amino acids: Gln for Lys at position 120, Glu for Gln at position 213, and Gln for Arg at position 235.

In two *K. pneumoniae* strains the β-lactamase that could confer resistance to cephalosporins was LAT-2. An enzyme focusing at the pI of SHV-5 was concomitantly expressed in the other *K. pneumoniae* strains. This may account for the different MICs of oxyimino cephalosporins and aztreonam. The latter strains were resistant to these drugs, whereas the former displayed decreased susceptibility. However, acquisition of LAT-2-encoding plasmids by *E. coli* rendered strains resistant to aztreonam and oxyimino cephalosporins. This result was apparently due to the production of larger amounts of the enzyme, suggesting differences in the interaction of the two species with the LAT-2-encoding plasmids. It is unlikely that there is any direct regulation of plasmid β-lactamase expression by the host's genes. One possible explanation for the differential enzyme production could be that the copy number of the plasmids is higher in the *E. coli* hosts. Overexpression of common plasmid-mediated β-lactamases has little effect on resistance to cephalosporins (10). In contrast, the effect of "gene dosage" seems important for plasmid-mediated group 1 β-lactamases and production of large amounts of the enzymes is required to confer a derepressed-like phenotype.

Group 1 plasmid-mediated β-lactamases have been derived from several species, and the respective plasmids seem to be different. Despite the apparent variety of translocation events that have led to the emergence of plasmids expressing these enzymes, their presence so far appears to be sporadic and confined mostly to *K. pneumoniae*. To date only one outbreak of *K. pneumoniae* strains expressing MIR-1 in a U.S. hospital has been reported (18). However, as has been shown here, plasmid-mediated cephalosporinases, derived from *C. freundii*, are established in the flora of Greek hospitals. The conjugation experiments showed that the LAT-2-encoding plasmids are mobilized by enterobacterial self-transmissible R plasmids. Coexistence with such elements, frequently encountered in enterobacteria (26, 27), could explain, at least partly, their wide spread.

Epidemics of R plasmids are mainly due to the heavy antibiotic pressure in the hospital setting. However, we cannot provide a straightforward explanation to associate antibiotic usage with the spread of these determinants. *E. aerogenes* strains expressing LAT-2 were stable overproducers of the chromosomal cephalosporinase, and therefore the enzyme does not provide any particular selective advantage. In *K. pneumoniae*, production of LAT-2 expands resistance toward cefamycins, but these antibiotics are not frequently prescribed in Greek hospitals. The increasing use of β-lactam–inhibitor combinations may explain the dissemination of plasmid-mediated group 1 enzymes in the latter species.

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