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Escherichia coli with a Self-Transferable, Multiresistant Plasmid Coding for Metallo-β-Lactamase VIM-1

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An *Escherichia coli* strain exhibiting decreased susceptibility to carbapenems was isolated from a hospitalized patient in Greece. The strain carried a self-transferable plasmid coding for metallo-β-lactamase VIM-1. bla_{VIM-1}, along with *aacA7*, *dhfrI*, and *aadA*, was included as a gene cassette in a novel class 1 integron. A *Citrobacter freundii ampC*-derived gene, not associated with the integron, was also located in the same plasmid.

The clinical significance of the carbapenem-hydrolyzing metallo-β-lactamases (MBLs) of the IMP and VIM types is increasing. The respective *bla* genes are spread among various gram-negative microorganisms in the Far East and Europe (8). The VIM-type MBLs (VIM-1 through -3) have been found mostly in *Pseudomonas aeruginosa* and also in *P. putida*, *P. stutzeri*, *Acinetobacter* spp., *Achromobacter xylosoxidans*, and *Serratia marcescens* (2, 3, 9, 10, 15–17). In this report, we describe an *Escherichia coli* strain with reduced susceptibility to imipenem due to acquisition of a self-transferable, VIM-1-encoding plasmid.

E. coli V541 was isolated in November 2001 from a urine specimen of a patient treated in the "Tzanion" general hospital in Piraeus, Greece. *E. coli* K-12 strain 26R793 (Rif^r) was used as the recipient in conjugation experiments. *E. coli* DH5α was used as the host for transformation. *E. coli* DH5α strains producing the β-lactamases VIM-2 (5) and LAT-1 (13) were used as controls.

MICs of β-lactams were determined by an agar dilution technique (6). Susceptibility to β-lactams in the presence of Ro 48-1220, a penicillanic acid sulfone exhibiting potent inhibitory activity against both class A and class C β-lactamases (12), was also determined. Susceptibility to other antimicrobials was assessed by disk diffusion (7). To detect MBL production, a synergy test using imipenem- and EDTA-containing disks was employed (1).

Conjugation was carried out in mixed broth cultures. Transconjugant clones were selected on Mueller-Hinton agar containing ampicillin (50 μg/ml) plus rifampin (200 μg/ml). Extraction of plasmid DNA and transformation experiments were performed by standard techniques (11).

bla_{VIM} genes were detected by PCR with primers VIM-F (5′-AGTGGTGAGTATCCGACAG-3′) and VIM-R (5′-ATG AAAGTGCGTGGAGAC-3′), corresponding to nucleotides (nt) 1339 to 1357 and 1599 to 1582, respectively, of the bla_{VIM-1} integron (GenBank accession no. Y18050). Detection of *Citrobacter freundii ampC*-derived genes by PCR was performed as described previously (14). DNA fragments obtained in these assays were labeled with a digoxigenin DNA labeling and detection kit (Roche Diagnostics, Mannheim, Germany) and used as probes in hybridization experiments. Mapping of class 1 integrons was performed by PCR with a set of primers including 5′-CS, 3′-CS (4), and INT-F (5′-CGTTCCATACA GAAGCTG-3′), which corresponds to an *intI1* sequence preceding the promoter region of class 1 integrons (from nt 657 to nt 674 of the bla_{VIM-1} integron). Nucleotide sequences of the PCR products were determined on both strands with an ABI Prism 377 DNA sequencer (Perkin-Elmer Applied Biosystems Division, Foster City, Calif.).

β-Lactamases were extracted by ultrasonic treatment of bacterial cell suspensions and clarified by centrifugation. The rate of imipenem hydrolysis was measured by UV spectrophotometry (2) and expressed as units of activity (1 U was defined as the amount of enzyme hydrolyzing 1 pmol of substrate/min/μg of protein at 30°C). Inhibition of imipenem hydrolysis by Ro 48-1220 (10 μg/ml) and EDTA (2 mM) was also assessed. Hydrolysis and inhibition experiments were performed in triplicate. Analytical isoelectric focusing was performed with polyacrylamide gels containing ampholytes (pH range, 3.5 to 9.5; AP Biotech, Piscataway, N.J.). β-Lactamase activity was visualized with nitrocefin (Oxoid Ltd., Basingstoke, United Kingdom) and by the iodine-starch method with imipenem as the substrate (9).

E. coli V541 was collected because of its decreased susceptibility to imipenem. It was resistant to penicillins, penicillin-inhibitor combinations, cefoxitin, and expanded-spectrum cephalosporins. MICs of aztreonam, imipenem, and meropenem, although below the respective resistance breakpoints, indicated decreased susceptibility to these agents (Table 1). The strain also exhibited resistance to aminoglycosides, trimethoprim, and sulfonamides. Resistance was readily transferable to *E. coli* 26R793 recipients at a relatively high frequency (2×10^{-2} transconjugant per donor cell). Transconjugants exhibited a resistance phenotype similar to that of the donor. Plasmid DNA analysis indicated transfer of a plasmid approximately 50 kb in size that was designated p541. Transformation of *E. coli* DH5α with purified p541 produced resistant clones that were able to transfer resistance by conjugation. All p541-carrying strains were positive by the EDTA disk synergy test.

DNA preparations from *E. coli* V541 and the p541-contain-

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TABLE 1. MICs of β-lactam antibiotics tested against VIM-1-producing and control *E. coli* strains

Antibiotic(s)[a]	MIC (μg/ml) for *E. coli* strain[b]:						
	V541 wt	26R793 trc (VIM-1 + AmpC)	26R793	DH5α trf (VIM-1 + AmpC)	DH5α (VIM-2)	DH5α (LAT-1)	DH5α
Ampicillin	>256	>256	2	>256	>256	>256	1
Ticarcillin	>256	>256	1	>256	>256	>256	0.5
Ticarcillin + CLA	>256	>256	1	>256	>256	>256	1
Piperacillin	>256	>256	1	>256	128	>256	0.5
Piperacillin + TAZ	>256	>256	0.5	>256	128	64	0.25
Piperacillin + Ro	>256	256	1	256	128	2	0.5
Cefoxitin	>128	>128	2	>128	>128	>128	2
Cefoxitin + Ro	>128	64	2	64	>128	2	2
Cefotaxime	128	>128	0.12	128	32	32	≤0.06
Cefotaxime + Ro	128	128	≤0.06	64	32	≤0.06	≤0.06
Cefazidime	>128	>128	0.12	>128	64	128	0.12
Ceftazidime + Ro	>128	>128	0.12	>128	64	0.5	0.12
Cefepime	64	32	≤0.06	32	2	0.5	≤0.06
Cefepime + Ro	64	32	≤0.06	32	1	≤0.06	≤0.06
Aztreonam	8	4	≤0.06	4	0.25	16	≤0.06
Aztreonam + Ro	0.25	0.12	0.12	0.12	0.25	0.12	≤0.06
Imipenem	8	8	≤0.06	8	2	0.5	≤0.06
Imipenem + Ro	8	8	≤0.06	8	2	≤0.06	≤0.06
Meropenem	2	2	≤0.06	1	1	0.12	≤0.06
Meropenem + Ro	2	2	≤0.06	1	1	≤0.06	≤0.06

[a] CLA, clavulanic acid (2 μg/ml); TAZ, tazobactam (4 μg/ml); Ro, Ro 48-1220 (10 μg/ml).
[b] wt, wild-type; trc, transconjugant; trf, transformant. The β-lactamas(es) produced by each strain is in parentheses.

ing laboratory strains were positive by the bla_{VIM}-specific and *C. freundii ampC*-specific PCR assays. Also, p541 hybridized with the bla_{VIM} and *ampC* probes (data not shown). PCR mapping indicated that bla_{VIM} was part of a class 1 integron. DNA sequencing showed a gene cassette array that included (from 5′ to 3′) bla_{VIM-1}, *aacA7*, *dhfrI*, and *aadA*. This structure was preceded by a strong P1 promoter ($TTGACAN_{17}TAAACT$) and the inactive form of P2 ($TTGTTAN_{14}TACAGT$) located at the 5′ end of an integrase 1 gene (Fig. 1). The bla_{VIM-1} cassette was identical to that originally described in *P. aeruginosa* (2). The DNA sequence of a 325-bp *ampC* amplicon was 98% homologous with the respective segment of the bla_{CMY-2} gene (nt 2679 to 3003; GenBank accession no. X91840).

In the p541-containing strains, Ro 48-1220 decreased the MICs of aztreonam by five doubling dilutions. A decrease in the MICs of piperacillin, cefoxitin, and cefotaxime was also observed, while susceptibility to carbapenems was not affected by this inhibitor, as was observed for the VIM-2-producing *E. coli* control strain. In contrast, Ro 48-1220 rendered the cephalosporinase-producing *E. coli* control strain susceptible to β-lactams (Table 1).

Two β-lactamase species focusing at pH values of 9.0 (AmpC) and 5.2 (VIM-1) were observed by isoelectric focusing. VIM-1 activity was detectable by the iodine-starch method but not with nitrocefin (data not shown). β-Lactamase preparations from *E. coli* V541 exhibited imipenem-hydrolyzing activity (532 ± 21 U) that was inhibited by EDTA (<10 U) but was not significantly reduced by Ro 48-1220 (480 ± 35 U).

Production of a VIM MBL by an *E. coli* strain is documented here for the first time. Notably, VIM-1 was encoded by a self-transferable plasmid. Conjugal transfer of bla_{VIM} was suggested in *P. aeruginosa* strains from Korean hospitals, but the respective plasmids could not be identified (3). bla_{VIM-1}, along with *aacA7*, *dhfrI*, and *aadA*, constitutes the gene cassette region of a class 1 integron the structure of which was

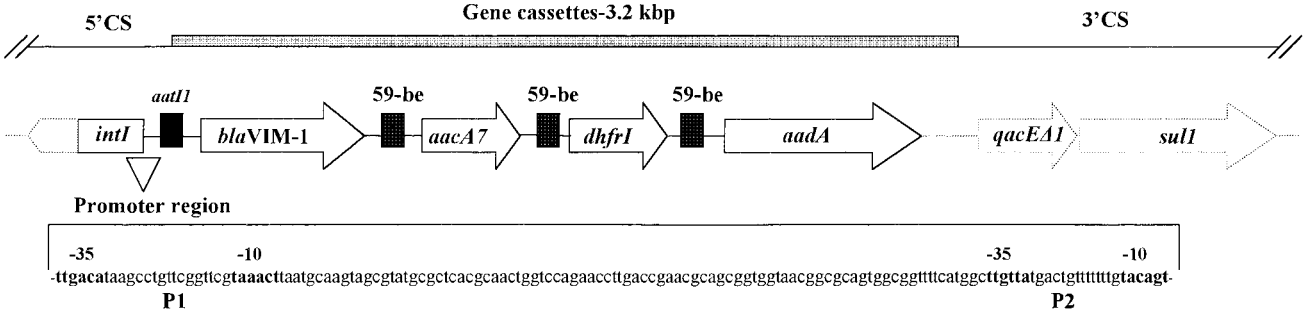

FIG. 1. Schematic presentation of the bla_{VIM-1}-containing class 1 integron carried by plasmid p541. Part of the variable region and the 3′ conserved sequence (dashed lines) were postulated on the basis of PCR assays. The sequence of the promoter region is also shown. CS, conserved segment.

different from that of the previously described VIM-encoding integrons. Also, the putative promoter was a strong P1 while the bla_{VIM-1} genes described previously in *P. aeruginosa* and *A. xylosoxidans* were under the control of a weak P1 promoter and a hybrid P1 promoter, respectively (2, 10). The different phylogeny and diverse genetic locations of these integrons suggest a widespread environmental reservoir of the bla_{VIM} genes. Acquisition of these determinants by self-transferable plasmids that can be established in enterobacteria will facilitate their further dissemination.

Plasmid p541 also encoded a class C β-lactamase that, most likely, did not substantially contribute to the levels of resistance to carbapenems, as indicated by susceptibility testing and hydrolysis experiments with the inhibitor Ro 48-1220. The carbapenem MICs for *E. coli* V541 were below the resistance breakpoints, as has been observed in *E. coli* strains expressing cloned bla_{VIM} genes (2, 5, 9, 15). Detection of such strains may be facilitated by EDTA synergy tests.

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