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First Occurrence of an *Escherichia coli* Clinical Isolate Producing the VIM-1/VIM-2 Hybrid Metallo-β-Lactamase VIM-12▽

Gram-negative bacteria producing VIM-type metallo-β-lactamases (MBLs) are increasingly isolated worldwide (8). The VIM group includes at least 13 variants, clustered into three evolutionary lineages, driven by VIM-1, VIM-2, and VIM-7 (www.lahey.org/studies/other.asp#table1). bla_{VIM-12} is a recently identified $bla_{VIM-1/VIM-2}$ hybrid gene, originally found in 2005 in a *Klebsiella pneumoniae* clinical isolate from Greece and ranked as intermediate between bla_{VIM-1} and bla_{VIM-2} (6). Its gene cassette resides in a class 1 integron, designated In-h12 (6); it has not since been detected in other gram-negative species. Kinetic parameters of the purified VIM-12 enzyme have not been described. We document herein the identification of the bla_{VIM-12} gene in an *Escherichia coli* clinical isolate.

E. coli strain 28 was recovered in February 2006 from a decubitus ulcer infection of an 85-year-old male hospitalized at Hippokration General Hospital, Thessaloniki, Greece, for lower respiratory tract infection. MICs of several β-lactams, aminoglycosides, ciprofloxacin, tetracycline, and cotrimoxazole were determined by using Etest (AB Biodisk, Solna, Sweden). The isolate was phenotypically screened for MBL production by using Etest MBL (AB Biodisk) and the imipenem-EDTA double disc synergy test (DDST) (5). *Pseudomonas aeruginosa* ATCC 27853 was used as a control in all susceptibility assays.

PCR detection of various genes encoding MBLs, extended-spectrum beta-lactamases, and AmpC enzymes, including bla_{VIM}, bla_{IMP}, bla_{SPM}, bla_{TEM}, bla_{SHV}, bla_{GES}, bla_{CTX-M} and $bla_{CMY/LAT}$, and PCR integron mapping were performed as described previously (6), followed by DNA sequencing.

Filter mating experiments were performed using *E. coli* 26R793 (Rif^r) as the recipient. Selection of transconjugants was made on MacConkey agar plates containing rifampin (100 μg/ml) and ceftazidime (2 to 4 μg/ml). Plasmid isolation was performed using the ChargeSwitch Plasmid ER minikit (Invitrogen Corporation, Carlsbad, CA) and a standard alkaline lysis protocol, using *E. coli* 39R861 as the standard plasmid control. Restriction fragment length polymorphism (RFLP) of the plasmid extract of *E. coli* strain 28 and the transconjugant derived from *Klebsiella pneumoniae* 2873 carrying VIM-12 in plasmid p2873 (6) was performed using BamHI. The location of the bla_{VIM} gene was determined as described previously (3) by Southern blotting of unsheared genomic DNA, followed by gene-specific hybridization using a digoxigenin-labeled bla_{VIM-1} probe (7).

TABLE 1. Susceptibilities of strain EC28 and its respective transconjugant carrying pEC28

Antibiotic(s)[a]	MIC (μg/ml) of antibiotic(s) for:		
	E. coli 28 (VIM-12)	Transconjugant strain (pEC28)	*E. coli* 26R793
Imipenem	1	0.25	0.5
Meropenem	0.25	0.023	0.032
Ertapenem	0.5	0.094	0.064
Aztreonam	1	0.5	0.094
Cefotaxime	>32	8	0.094
Cefepime	12	1	0.094
Ceftazidime	>256	4	0.75
Cefoxitine	>256	32	8
Amoxicillin	>256	32	4
Amoxicillin + CLA	>256	16	4
Amikacin	128	48	2
Gentamicin	>256	32	0.75
Piperacillin + TZB	96	12	0.75
Ciprofloxacin	0.047	0.047	0.047
Tetracycline	>256	1	1
Trimethoprim-sulfamethoxazole	0.094	0.016	0.012

[a] CLA, clavulanic acid (2 μg/ml); TZB, tazobactam (4 μg/ml).

E. coli strain 28 was susceptible to imipenem and meropenem (MICs, 1 and 0.25 μg/ml, respectively), aztreonam, ciprofloxacin, and cotrimoxazole but resistant to all other antimicrobials tested (Table 1). The presence of MBL was indicated by positive DDST and Etest MBL. PCR for β-lactamase genes showed that *E. coli* strain 28 was positive for bla_{VIM} and $bla_{CMY/LAT}$ genes but negative for the remaining genes. PCR assays using primers 5′CS and 3′CS in various combinations with primers bla_{VIM} and *aacA* and nucleotide sequencing revealed an integron structure identical with that of integron In-h12, originally found in the bla_{VIM-12}-producing *K. pneumoniae* (GenBank accession number DQ143913) (6).

Repeated mating experiments yielded, at a median frequency of 1.8×10^{-2} per donor cell, transconjugant colonies that had elevated MICs of penicillins, cephalosporins, and aminoglycosides but not of carbapenems and tetracycline (Table 1). Plasmid analysis of both clinical and transconjugant colonies showed a single plasmid of approximately 70 kb. PCR specific for bla_{VIM} and $bla_{CMY/LAT}$ in several transconjugant colonies was positive only for the latter gene. RFLP analysis with BamHI showed that the conjugative plasmid of *E. coli* strain 28 had a different restriction pattern from that of p2873 (6), tested in parallel. The location of In-h12 in the chromosome was indicated by hybridization of the unsheared genomic DNA with the bla_{VIM-1} probe.

Production of VIM-12, a VIM-1/VIM-2 hybrid MBL, is documented here for the first time for an *E. coli* isolate after its original detection in *K. pneumoniae*. It is noteworthy that several bla_{VIM-12}-producing *K. pneumoniae* strains have been identified in the same hospital since the original detection in 2005 (unpublished data), indicating a rather wide dissemination of this gene among our *K. pneumoniae* isolates. *E. coli* strain 28 carried an integron identical to In-h12, although not lying in the transferable plasmid p2873, which harbored bla_{VIM-12} in *K. pneumoniae* (6). It could be speculated here that the integron In-h12 containing the $bla_{VIM-1/VIM-2}$ hybrid gene bla_{VIM-12} has arisen within our hospital settings, where bla_{VIM-1}- and bla_{VIM-2}-carrying gram-negative pathogens are common (1, 2, 4, 7). Alternatively, the bla_{VIM-12} gene might be independent and have a wide natural distribution in bacterial populations, as was observed previously for other bla_{VIM} genes (8).

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