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Detection of VIM-34, a novel VIM-1 variant identified in the intercontinental ST15 *Klebsiella pneumoniae* clone

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Sir,

Enterobacteriaceae producing metallo-β-lactamases (MBLs), and particularly VIM-type MBLs, have frequently been implicated in hospital outbreaks across Europe,[1] bla_{VIM} genes having been linked to Tn*402* derivatives, epidemic plasmids (IncN, IncI1, IncHI2) and occasionally with particular Enterobacteriaceae clones.[1–4] VIM enzymes have been classified in three clusters (VIM-1, VIM-2 and VIM-7) according to their amino acid sequences (http://www.lahey.org/studies), VIM-1 and VIM-2 being the most widespread variants.[5] In this study, we report the molecular epidemiology and the antibiotic susceptibility profiles of *Klebsiella pneumoniae* clinical isolates producing VIM-34, a novel VIM-1 variant identified in Portugal.

In October 2011 and October 2012, two *K. pneumoniae* isolates (strains K43 and K47, respectively) showing reduced susceptibility to carbapenems (MICs 0.38–1.0 mg/L) were recovered from urine samples of hospitalized patients in a general hospital in northern Portugal (Hospital Pedro Hispano). They are the only carbapenemase-producing Enterobacteriaceae isolates identified in this hospital since the beginning of 2011, when reference protocols for carbapenemase detection were adopted.

Antimicrobial susceptibility tests were performed using the Etest for β-lactams and disc diffusion for all other antimicrobial agents. These showed that all isolates were resistant to diverse cephalosporins, aztreonam, β-lactam/β-lactamase inhibitor combinations (Table 1), nalidixic acid, ciprofloxacin, chloramphenicol, gentamicin, kanamycin, netilmicin, streptomycin, tobramycin and sulphonamides, but susceptible to trimethoprim and amikacin (http://www.eucast.org/).[6] Standard disc diffusion phenotypic tests using different β-lactams and β-lactamase inhibitors (cefotaxime, ceftazidime, imipenem; 0.2 mM EDTA, clavulanic acid),[6] isoelectric focusing, PCR and sequencing[7] demonstrated the production of VIM-34 (pI = 5.4) (GenBank accession number JX013656), a novel VIM-type enzyme differing from VIM-1 by one amino acid change (V113I, according to MBL standard numbering scheme) and co-production of SHV-1 (pI = 7.6) and SHV-12 (pI = 8.2) extended-spectrum β-lactamase. We could not identify the origin of these isolates but as both patients had multiple previous hospitalizations (including in other hospitals) and carried the same novel bla_{VIM} type, a common nosocomial source seems more plausible than community acquisition.

Table 1. MICs of different β-lactam antibiotics for VIM-34-producing wild-type isolates and recombinant strains encoding VIM-34 or VIM-1

	MIC (mg/L)			
		E. coli DH5α		
Antibiotic	*Klebsiella pneumoniae* K43 (VIM-34)[a]	pBGS18	pBGS18/VIM-1	pBGS18/VIM-34
Amoxicillin/clavulanate	24	2	24	12
Ticarcillin/clavulanate	>256	2	>256	>256
Piperacillin/tazobactam	>256	0.75	6	6
Cefalotin	>256	2	64	32
Ceftazidime	>256	0.19	12	8
Cefotaxime	32	0.125	2	2
Cefepime	12	0.016	0.75	0.5
Cefpirome	32	0.032	1.5	2
Cefoxitin	32	4	12	4
Aztreonam	32	0.023	0.023	0.016
Ertapenem	0.38[b]	0.006	0.008	0.008
Imipenem	1.0[b]	0.125	0.38	0.38
Meropenem	0.5[b]	0.016	0.032	0.023

[a]K47 isolate exhibited identical antibiotic susceptibility profiles.
[b]MIC values interpreted as susceptible by both EUCAST and CLSI guidelines, but above the epidemiological cut-off values defined for *K. pneumoniae* (http://www.eucast.org).[6]

The bla_{VIM-34} from the K47 isolate was cloned in the pBGS18 (kanamycin resistance) plasmid using primers VIM-EcoRI (5′-GGGAATTCGCAGTCGCCCTAAAACAAAG-3′) and VIM-PstI (5′-AACTGCAGCCGCTCCAACGATTTGTTAT-3′) (restriction sites are underlined), and the expression vector (pBGS18/VIM-34) was further introduced into *Escherichia coli* DH5α, as previously reported.[8] MICs of different β-lactams were determined using the Etest (in triplicate) and compared with those corresponding to a bla_{VIM-1}-carrying clone obtained in the same conditions (Table 1). The VIM-34-producing *E. coli* recombinant yielded β-lactam MIC values similar to those observed in the VIM-1-encoding transformant (with the exception of cefoxitin; Table 1). Because our experiments were performed in an isogenic context and identical standard experimental conditions, we are able to hypothesize that the substitution V113I has a low influence on the MICs of carbapenems, although further studies of enzymatic activity are required to confirm this observation.

The isolates exhibited identical XbaI-PFGE profiles and clonal identification by multilocus sequence typing (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html) revealed that they belong to the intercontinental ST15 *K. pneumoniae* clone, widely disseminated in different European countries and associated with the spread of extended-spectrum β-lactamases (CTX-M-15; diverse SHV types) and/or MBLs (VIM-1, NDM-1).[2,3,7,9,10] Conjugation assays performed by broth and/or filter mating methods using *E. coli* HB101 (azide and kanamycin resistant, Lac-, plasmid free) as

recipient at 22°C and 37°C (selection of transconjugants in MacConkey agar with 2 mg/L of ceftazidime and 130 mg/L of azide)[7] failed to yield transconjugants either for bla_{VIM-34} or bla_{SHV-12}. The location of *bla* (bla_{VIM-34}, bla_{SHV-12}) genes and plasmid characterization were accomplished by S1- and I-CeuI-PFGE, and identification of incompatibility groups.[7] In both isolates, bla_{VIM-34}, bla_{SHV-12} and repHI2 probes hybridized in the same chromosomal band (I-CeuI-PFGE) whereas no signals were observed in the S1 gel, suggesting the acquisition of both *bla* genes by an IncHI2 plasmid and subsequent plasmid (whole or in part) integration. A chromosomal location for *bla* genes, including bla_{VIM}, has been occasionally observed in different Enterobacteriaceae species.[1]

The linkage of bla_{VIM-34} to class 1 integrons and Tn*402* derivatives was investigated by PCR (*intI1*, 5′CS-3′CS region, *orf5*, *orf6*, IS*1326*, IS*1353*, IS*6100*) and sequencing.[4,11] bla_{VIM-34} was located within an ~6 kb class 1 integron named In817 by INTEGRALL (http://integrall.bio.ua.pt/) (GenBank accession number JX185132), with an original array of gene cassettes comprising bla_{VIM-34}, *aacA4′*, *aphA15*, *aadA1b* and *catB2* (Figure S1; available as Supplementary data at *JAC* Online). The absence of tni_{402} sequences and the high similarity detected with In70 and In113, identified in VIM-1-producing *Achromobacter xylosoxidans*, *K. pneumoniae* and *E. coli* isolates, suggests that the In817 integron might have arisen by both recombination and *in vivo* evolution events (Figure S1; available as Supplementary data at *JAC* Online).[4]

In summary, we present the first report of VIM-34, a VIM-1-like variant embedded in the novel integron type In817 on the chromosome of the intercontinental ST15 *K. pneumoniae* clone, associated with carbapenem susceptibility profiles similar to those observed for VIM-1. This study highlights the risk of further dissemination of the multidrug-resistant ST15 *K. pneumoniae* clone and genetic backgrounds containing metallo-β-lactamase genes in our country, which deserves future monitoring.

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Transparency declarations

None to declare.

Supplementary data

Figure S1 is available as Supplementary data at *JAC* Online (http://jac.oxfordjournals.org/).

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Mutant prevention concentrations of colistin for *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* clinical isolates

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