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-lactamas (MBLs), and particularly VIM-type MBLs, have frequently been implicated in hospital outbreaks across Europe,1,2 bl a VIM-34 genes having been linked to Tn402 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 sequencing7 demonstrated the production of VIM-34 (pI = 5.6) (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 bl a VIM-34 type, a common nosocomial source seems more plausible than community acquisition.

The bl a VIM-34 from the K47 isolate was cloned in the pBGS18 (kanamycin resistance) plasmid using primers VIM-EcoRI (5′-GGGAATT CGGAGTCGCCCTAAAACAAAG-3′) and VIM-PstI (5′-CTGCAGCCGCTCCA ACGATTGTGTAT-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 bl a 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 wild-type 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/PPB8/mist/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 recipients with K. pneumoniae MBLs as donors led to the identification of VIM-34 in all recipient strains.

In conclusion, this report describes the identification of a novel VIM-1 variant (VIM-34) in K. pneumoniae from a hospital in Portugal, and confirms the possible dissemination of this enzyme in the intercontinental ST15 K. pneumoniae clone.

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

| Antibiotic             | Klebsiella pneumoniae K43 (VIM-34) | E. coli DH5a pBGS18/ VIM-1 | pBGS18/ VIM-34 |
|------------------------|-----------------------------------|-----------------------------|-----------------|
| Amoxicillin/ clavulanate | 24                                | 2                           | 26              |
| Ticarcillin/ clavulanate| >256                               | 2                           | >256            |
| Piperacillin/ tazobactam| >256                               | 0.75                        | 6               |
| Cefalotin              | >256                               | 2                           | 64              |
| Cefotaxime             | >256                               | 0.19                        | 12              |
| Ceftazidime            | 12                                 | 0.125                       | 2               |
| Cefepime               | 12                                 | 0.016                       | 0.75            |
| Cefpirome              | 32                                 | 0.032                       | 1.5             |
| Cefoxitin              | 32                                 | 4                           | 12              |
| Aztreonam              | 32                                 | 0.023                       | 0.023           |
| Ertopenem              | 0.38b                              | 0.006                       | 0.008           |
| Imipenem               | 1.0b                               | 0.125                       | 0.38            |
| Meropenem              | 0.5b                               | 0.016                       | 0.032           |

6K47 isolate exhibited identical antibiotic susceptibility profiles.
7MIC 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).8

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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) 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. In both isolates, bla_{VIM-34}, bla_{SHV-12} and repH12 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 InH12 plasmid and subsequent plasmid (whole or in part) integration. A chromosomal location for bla genes, including bla_{VIM-34}, has been occasionally observed in different Entero bacteriaceae species. The linkage of bla_{VIM-34} to class 1 integrons and Tn402 derivatives was investigated by PCR (intI1, 5′-CS-3′CS region, arf5, arf6, IS1326, IS1353, IS6100) and sequencing. \(^6\) \(^7\) \(^8\) 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}, aac(A)4, aphA15, adaA1b and catB2 (Figure S1; available as Supplementary data at JAC Online). The absence of tniQ22 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). 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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