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Genetic Characterization of the New *bla*$_{VIM-18}$ and Comparative Antimicrobial Susceptibility Profile of VIM-18 with Highest Homologous VIM-variant, VIM-2: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objective: To characterize the genetic context of a new *bla*$_{VIM}$-variant from India; and to compare the antimicrobial susceptibility (S) profiles of *E. coli* carrying recombinant plasmids harboring *bla*$_{VIM-18}$ and *bla*$_{VIM-2}$. We recently found that VIM-producing *P. aeruginosa* were highly prevalent in Indian medical centres (55% of the carbapenem [CARB]-resistant [R] isolates) and VIM-2 was the most common VIM-variant (38.6%).

Methods: As part of the SENTRY Program, CARB-R isolates were screened for metallo-ß-lactamases (MßL). Positive isolates were then amplified and sequenced with MßL internal primers and primers targeting conserved structures of class 1 integrons. *bla*$_{VIM-2}$ and the new *bla*$_{VIM}$ were amplified, cloned into PCRScript plasmid and transformed in *E. coli* XL1 Blue. Colonies were selected with chloramphenicol (30 mg/L) and ceftazidime (4 mg/L). The presence and orientation of *bla*$_{VIM}$ was confirmed by PCR and sequencing. S testing was performed by E-test (AB BIODISK, Solna, Sweden). The region flanking the MßL integron was amplified using a degenerate primer approach. Plasmid contents analysis was also carried out.

Results: *P. aeruginosa* isolate 243-31C was recovered from a sputum specimen in Kolkatta, India (2006). PCR and sequencing showed the presence of new *bla*$_{VIM}$ variant, named *bla*$_{VIM-18}$ (Lahey Clinic). The deduced VIM-18 protein comprised 262 amino acids showing 74.3 to 99.2% identity to other VIM enzymes. VIM-18 was identical to VIM-6, except for the deletion of four amino acids. The MßL active sites were all present. The *E. coli* carrying *bla*$_{VIM-18}$ showed lower MIC values for ampicillin, cefoxitin, cephalothin and ertapenem (3-, 6-, 4- and 4-fold, respectively) when compared to those obtained from the *E. coli* carrying *bla*$_{VIM-2}$, while similar MIC values were noted for imipenem, meropenem, piperacillin, cefotaxime, ceftazidime and cefepime. *bla*$_{VIM-18}$ was the only R gene cassette in a class 1 integron that was flanked upstream by a resolvase gene (*tnp*R) from Tn*5051*-like. Further analysis showed that this isolate carried no plasmid.

Conclusions: VIM-18 displayed reduced activity to some beta-lactams, while retaining similar activity against anti-pseudomonal agents when compared to VIM-2. Thus, the 12-bp deletion in VIM-18 seems to have little effect in the activity against target CARB and anti-pseudomonal cephems. VIM-18 was found in one *P. aeruginosa* isolate among numerous VIM-2-producing strains, and it appears to be an evolutionary derivative.

INTRODUCTION

Metallo-ß-lactamases (MßLs) hydrolyze the ß-lactam ring using a divalent cation as cofactor. MßLs can degrade all classes of ß-lactams except monobactams, but they are particularly worrisome because they efficiently hydrolyze carbapenems. These antimicrobial agents are stable against the vast majority of serine-ß-lactamases produced by resistant organisms and are among the few back up agents for use against multidrug-resistant Gram-negative pathogens. Moreover, MßLs are not susceptible to clinically available ß-lactamase inhibitors.

The emergence in the past decade of acquired MßLs, encoded by genes carried on mobile DNA elements (among important gram-negative pathogens, including members of the Enterobacteriaceae family, *Pseudomonas aeruginosa,* and *Acinetobacter* spp.) has highlighted this significant clinical problem. The worldwide dissemination of MßLs and the diversity of these enzymes has been dramatic, as acquired MßLs have been detected in clinical isolates from Asia, Europe and North and South America.

Currently, the most prevalent and widespread acquired MßLs are the VIM enzymes, of which numerous variants are known. We recently found a high prevalence of VIM-types in India, with VIM-2 being the most prevalent, followed by VIM-6. Three other VIM-types were also found in this country: VIM-5, VIM-11 and a new VIM variant.

In this study, we describe the genetic characterization of the gene encoding VIM-18 and the ß-lactam susceptibility profile of VIM-18 compared to VIM-2 (the most common VIM-variant) residing in the same genetic background.

MATERIALS AND METHODS

Bacterial isolates. During 2006, 282 *P. aeruginosa* isolates were collected from 10 Indian medical center sites participating in the SENTRY Program. These isolates were susceptibility tested against more than 25 antimicrobial agents by the broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI, 2008). Among those isolates, 96 (34.0%) showed elevated MIC values for carbapenems (MIC, ≥8 mg/L) and were tested with a multiplex PCR using MßL generic primers in a Real-Time platform. Amplicons obtained were sequenced on both strands. The nucleotide sequences and deduced amino acid sequences were analyzed using Lasergene software package (DNASTAR, Madison, WI) and compared with sequences available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

Cloning of *bla*$_{VIM}$ genes. Amplicons containing the complete sequence of the *bla*$_{VIM-18}$ and *bla*$_{VIM-2}$ were cloned into pPCRScriptCam SK+ (Stratagene Cloning Systems, La Jolla, CA). The colonies obtained after transformation in XL10-Gold® Kan ultracompetent *E. coli* were selected on plates containing 30 mg/L chloramphenicol and 4 mg/L ceftazidime. The presence and orientation of inserts was confirmed by sequencing. The MIC for ß-lactam antimicrobial agents was determined by E-test® (AB BIODISK, Solna, Sweden) for the *E. coli* host carrying recombinant plasmids harboring *bla*$_{VIM}$ genes and for the host strain carrying the same plasmid without an insert.

PCR experiments and DNA sequencing. Primers targeting the 5' conserved sequence (CS) and the 3' CS of class 1 integrons were used in combination with *bla*$_{VIM}$ primers to amplify and sequence the variable region of the MßL-harboring integron. The region upstream of the *bla*$_{VIM}$-carrying integron was characterized by PCR and sequencing using degenerate random primers in combination with primers anchored in the integrase gene.

Genetic analysis and hybridization. Plasmid DNA was extracted from the VIM-18 producing clinical strain using the Plasmid DNA Midi Kit (Qiagen, Hilden, Germany). Agarose plugs containing total DNA were digested with I-CeuI and DNA separation was performed on the CHEF-DRII apparatus (BioRad, Richmond, CA). Transference of plasmid and total DNA to a nylon membrane was performed by southern blot as described elsewhere. The *bla*$_{VIM-18}$ probe generated by PCR was labeled and membranes were hybridized with nonradioactive DIG high Prime DNA labeling and Detection Kit (Roche Diagnostics GmbH, Mannheim, Germany).

Nucleotide sequence accession number. The nucleotide sequence of *bla*$_{VIM-18}$ has been submitted to GenBank nucleotide database and assigned accession number AM778091.

RESULTS

- Among 53 MßL carrying isolates detected in India during the SENTRY Program 2006, one isolate was found to carry a new VIM variant. This enzyme, named VIM-18, was identified in a *P. aeruginosa* (243-31C) strain isolated from a sputum specimen from a patient hospitalized in Kolkatta, India.
- *P. aeruginosa* isolate 243-31C showed resistance to all antimicrobial agents tested, including all ß-lactams, aminoglycosides and fluoroquinolones, and was only susceptible to polymyxin B.
- VIM-18 showed the highest homology with VIM-6 (99.6%) followed by VIM-2, -3 and -11 (99.2%) and displayed the lowest similarity with VIM-7 (74.3%).
- VIM-18 possessed 262 amino acids and contained a deletion of 4 amino acids in the position 140, when compared with other VIM enzymes (Figure 1). This MßL variant showed all zinc ligand aminoacid residues conserved in MßLs (His116, His118, Asp120, His196, Cys221 and His263).

Table 1. ß-lactam susceptibility profile of the *E. coli* strain carrying the recombinant plasmid vector PCRScript with *bla*$_{VIM-18}$ and *bla*$_{VIM-2}$ inserts, and the *E. coli* strain carrying the plasmid vector without insert.

	E. coli pPCRScript carrying *bla*$_{VIM-18}$	*E. coli* pPCRScript carrying *bla*$_{VIM-2}$	*E. coli* pPCRScript
Imipenem	0.38	0.5	0.25
Meropenem	0.047	0.094	0.023
Ertapenem	0.016	0.19	0.006
Ampicillin	48	>256	0.023
Piperacillin	6	4	1.5
Cefoxitin	6	>256	3
Cefazolin	24	>256	2
Cefotaxime	1.5	3	0.047
Ceftazidime	16	4	0.094
Cefepime	0.125	0.38	0.032
Aztreonam	0.032	0.032	0.032

- Comparison of the MICs of VIM-18 and VIM-2 expressed in the same *E. coli* background (Table 1) showed lower MIC values for ampicillin, cefoxitin, cephalothin and ertapenem (3-, 6-, 4- and 4-fold, respectively).
- MIC values for imipenem, meropenem, piperacillin, cefotaxime, ceftazidime and cefepime were similar for both MßL enzymes (Table 1).
- As expected, the comparison of the *E. coli* host carrying the plasmid vector without insert and the recombinant strains producing VIM-2 and VIM-18 showed that the MßL carrying isolates showed higher MICs for most ß-lactam agents.

Figure 1. Alignment of the amino acid sequences of VIM-18 and other VIM variants found in India during the SENTRY Program (2006). The deletion of 4 amino acids in the VIM-18 structure is shown in the orange box. The amino acid residues involved in the ligation of the zinc cations (HXHXD116 to 120, H196, C221 and H263) are underlined in the VIM-2 sequence and are conserved for all VIM enzymes represented.

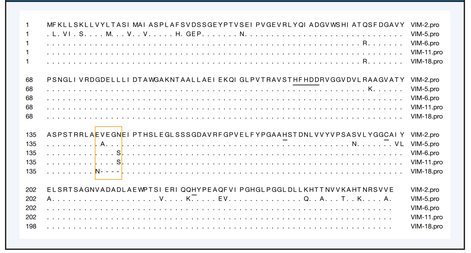

Figure 2. Schematic representation of the *bla*$_{VIM-18}$-carrying integron detected in carbapenem-resistant *P. aeruginosa* isolate 243-31C from India. Genes are represented as open arrows and the direction of the arrows indicates the transcription orientation. Filled circle represents the 59-base element recombination site.

- *bla*$_{VIM-18}$ was found to be a single gene cassette in a class 1 integron that carried the integrase gene in the 5'CS and the *qacEΔ1/sul1* in the 3'CS. This integron was flanked upstream by a resolvase gene (*tnp*R) from a Tn*5051*-like transposon.
- Isolate 243-31C appeared to be without plasmid DNA and hybridization experiments of the I-CeuI digested total DNA showed that *bla*$_{VIM-18}$ was chromosomally located.

CONCLUSIONS

- A high diversity of VIM enzymes, including VIM-2, VIM-5, VIM-6, VIM-11 and VIM-18, was recently found in India. VIM-18 showed high homology to VIM-6 and VIM-2, which were highly prevalent in this geographic region, and which could be the ancestral genes.
- VIM-18 possesses a deletion of four amino acids when compared to other VIM enzymes. Apparently, this deletion did not decrease its activity against anti-pseudomonal agents.
- Structural studies based on the deletion found on VIM-18 may help to elucidate the evolution of MßL enzymes and their interactions with ß-lactam compounds.

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