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Characterization of Three New VIM Enzymes, VIM-35, VIM-36 and VIM-37 Identified in European Countries: Report of the SENTRY Antimicrobial Surveillance Programme

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AMENDED ABSTRACT

Objective: To characterize three new VIM variants identified in *P. aeruginosa* (PSA) and *K. oxytoca* (KOX) recovered in Europe during 2011. VIM enzymes were the second type of acquired metallo-ß-lactamases identified and these enzymes are widespread in several European countries among Enterobacteriaceae and PSA isolates.

Methods: Screening for carbapenemase-encoding genes was performed for carbapenem-non-susceptible Enterobacteriaceae and PSA strains collected during 2011 and submitted to the SENTRY Programme. All amplicons were sequenced. Genes encoding new VIM variants, VIM-1 and VIM-2 were cloned into PCRScript CamR(+) and transformants were susceptibility tested. Primer walking targeting integron (INT)-related structures and bla_{VIM} were used to reveal the genetic environment of the new genes.

Results: Three isolates from Poland (1 KOX and 1 PSA) and Belgium (1 PSA) carried new bla_{VIM} variants. Isolates displayed elevated MICs against ß-lactams (including aztreonam), fluoroquinolones, amikacin and tobramycin. One PSA (Belgium) and the KOX were susceptible to gentamicin. The new VIM enzymes were named VIM-35, VIM-36 and VIM-37. VIM-36 was detected in the PSA from Belgium and displayed one amino acid (aa) alteration (Q59R) when compared to VIM-2. VIM-35 and VIM-37 were identified in KOX and PSA from Poland, respectively. VIM-35 displayed one aa alteration compared to VIM-1 (A235T; 99.6% homology) and VIM-37 showed 99.2% similarity with VIM-1, displaying two aa differences: A57S and S228R. Recombinant bla_{VIM} plasmids carried in the same *E. coli* background showed that VIM-35 had similar ß-lactam resistance profile to VIM-1, whereas VIM-37 had much lower in vitro activity against cefepime and ceftazidime when compared to VIM-1 (MIC, 0.25 vs. 4 and 8 vs. >32 mg/L, respectively). VIM-36 had ceftazidime MIC values eight-fold lower than VIM-2, its closest ancestor. Integrons carrying bla_{VIM-35} and bla_{VIM-37} were identical and carried *aacA(6')-Ib*, followed by bla_{VIM} and the 3'-conserved sequence (CS). A duplication of 155-bp of bla_{VIM} 3'-end was detected between this gene and the 3'CS. bla_{VIM-36} was embedded in an integron carrying *aacA29* in the first position followed by this gene.

Conclusions: We identified three new VIM variants that seem to have more limited activity against cefepime and/or ceftazidime when compared to ancestors. Furthermore, different genes carried in similar genetic structures were detected in the same hospital in two bacterial species.

INTRODUCTION

The emergence of acquired metallo-ß-lactamases (MßLs) among important Gram-negative pathogens, including members of the Enterobacteriaceae family, *Pseudomonas aeruginosa,* and *Acinetobacter* spp. has highlighted this significant clinical problem. MßLs can hydrolyze the vast majority of ß-lactam agents available for clinical use and are not inhibited by ß-lactamase inhibitors currently marketed or in clinical development. Several types of MßLs have been reported; IMP-, VIM- and NDM-types have been found worldwide.

The first VIM-type MßL, VIM-1, was described in 1999 in a multidrug-resistant *P. aeruginosa* isolate from Verona, Italy. A more divergent subtype of this enzyme, VIM-2, was later identified in strains isolated as early as 1995 (Portugal) and 1996 (France). Since these initial reports, VIM enzymes have become the most widespread MßL type with 34 variants described to date. Moreover, VIM-producing isolates were reported to cause several outbreaks in hospitals in Europe, Central America and in the Asia-Pacific region.

In this study, we describe three new VIM-variants from *P. aeruginosa* and *K. oxytoca* recovered in European countries during 2011 and their genetic environment. New enzymes were cloned in an *Escherichia coli* host and compared to VIM-1 and VIM-2 in the same genetic background.

MATERIALS AND METHODS

Bacterial isolates. Two *P. aeruginosa* and one *K. oxytoca* clinical isolates initially susceptibility tested by the reference broth microdilution method according to CLSI guidelines (M07-A9) displayed elevated MIC results to imipenem or meropenem (MIC, ≥2 or ≥8 mg/L for Enterobacteriaceae and *P. aeruginosa*, respectively). These isolates were screened for bla_{KPC}, bla_{SME}, bla_{GES}, bla_{NMC-A}, bla_{IMI}, bla_{IMP}, bla_{VIM}, bla_{SPM-1}, bla_{GIM-1}, bla_{SIM-1}, bla_{AIM-1}, bla_{KHM-1}, bla_{NDM}, bla_{DIM-1} and bla_{BIC-1} in four separate multiplex polymerase chain reactions. All PCR experiments included reactions containing target DNA templates for each screening primer set utilized. Amplicons generated were sequenced on both strands; nucleotide and deduced amino acid sequences were analyzed using the Lasergene software package (DNASTAR, Madison, Wisconsin, USA). Amino acid sequences were compared with those available through the internet using NCBI/BLAST.

Cloning of bla_{VIM}-variants. Amplicons containing the open reading frame and promoter region of bla_{VIM} were cloned into pPCRScriptCam SK+ (Stratagene, California, USA). The colonies obtained after transformation in XL10-Gold® Kan ultracompetent *E. coli* were selected on plates containing 30 mg/L chloramphenicol. The presence and orientation of inserts was confirmed by PCR and sequencing. MIC testing was performed as described above.

Class 1 integron characterization. Primers designed in the 5' and 3' conserved sequence (CS) regions of class 1 integrons were used in combination with the MßL primers to determine the size and structure of the integron. Additional primers targeting the genes detected in the integron were used to complete sequencing. Amplicons were sequenced as described above.

RESULTS

- One *K. oxytoca* and one *P. aeruginosa* from Poland and one *P. aeruginosa* from Belgium carried new VIM-encoding genes. Resistance to ß-lactams was elevated in all three strains and aztreonam values were modestly high (MIC, 8-16 mg/L).
- The *K. oxytoca* from Poland and *P. aeruginosa* isolates from Belgium were susceptible to colistin, tigecycline and gentamicin (CLSI breakpoints; **Table 1**).
- The *P. aeruginosa* isolate from Poland was highly resistant to most agents tested (**Table 1**), except colistin (MIC, 2 mg/L).
- The VIM-variant carried by *K. oxytoca* isolate was named VIM-35 and displayed one amino acid alteration compared to VIM-1 (A235T; 99.6% homology; **Figure 1**).
- VIM-36 was detected in the *P. aeruginosa* isolate from Belgium and displayed one amino acid alteration (Q59R) when compared to VIM-2.
- The *P. aeruginosa* isolate from Poland carried bla_{VIM-37}, displaying two amino acid differences (A57S and S228R) compared to VIM-1 (99.2% similarity).
- VIM-36, when expressed in a similar background, displayed similar MIC values compared to VIM-1, its closest ancestor (**Table 1**). In comparison, VIM-37 that is also closely related to VIM-1, displayed lower doripenem, meropenem, ceftriaxone, ceftazidime and cefuroxime MIC values, but higher ertapenem (MIC, 16 vs. 0.12 mg/L for VIM-37 and VIM-1, respectively).
- VIM-36 had comparable MIC values to VIM-2 (99.6% homologous) for most ß-lactams, but ceftazidime and cefuroxime MIC values were lower for the new variant (**Table 1**).
- Integrons carrying bla_{VIM-35} and bla_{VIM-37} were identical and carried *aacA(6')-Ib*, followed by bla_{VIM} and the 3' conserved sequence (CS). A duplication of 155-bp of bla_{VIM} 3'-end was detected between this gene and the 3'CS.
- VIM-36 encoding gene was located in an integron carrying *aacA29* and the MßL gene.

Figure 1. Alignment of the amino acid sequences of (A) VIM-35 and VIM-37 detected in Poland compared to its closest variant VIM-1 and (B) VIM-36 detected in Belgium when compared to VIM-2.

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(A)
VIM-1.pro   MLKVI SSLLVYMTASVMAVASPLAHSGEPSGEYPTVNEI PVGEVRLYQI ADGVWSHI ATQSFDGAVYPSNGLI VRDGDELLLI DTAWGA  89
VIM-35      MLKVI SSLLVYMTASVMAVASPLAHSGEPSGEYPTVNEI PVGEVRLYQI ADGVWSHI ATQSFDGAVYPSNGLI VRDGDELLLI DTAWGA  89
VIM-37      MLKVI SSLLVYMTASVMAVASPLAHSGEPSGEYPTVNEI PVGEVRLYQI ADGVWSHI STQSFDGAVYPSNGLI VRDGDELLLI DTAWGA  89

VIM-1.pro   KNTAALLAEI EKQI GLPVTRAVSTHFHDDRVGGVDVLRAAGVATYASPSTRRLAEAEGNEI PTHSLEGLSSSGDAVRFGPVELFYPGAA  178
VIM-35      KNTAALLAEI EKQI GLPVTRAVSTHFHDDRVGGVDVLRAAGVATYASPSTRRLAEAEGNEI PTHSLEGLSSSGDAVRFGPVELFYPGAA  178
VIM-37      KNTAALLAEI EKQI GLPVTRAVSTHFHDDRVGGVDVLRAAGVATYASPSTRRLAEAEGNEI PTHSLEGLSSSGDAVRFGPVELFYPGAA  178

VIM-1.pro   HSTDNLVVYVPSANVLYGGCAVHELSSTSAGNVADADLAEWPTSVERI QKHYPEAEVVI PGHGLPGGLDLLQHTANVVKAHKNRSVAE.  267
VIM-35      HSTDNLVVYVPSANVLYGGCAVHELSSTSAGNVTDADLAEWPTSVERI QKHYPEAEVVI PGHGLPGGLDLLQHTANVVKAHKNRSVAE.  267
VIM-37      HSTDNLVVYVPSANVLYGGCAVHELSRTSAGNVADADLAEWPTSVERI QKHYPEAEVVI PGHGLPGGLDLLQHTANVVKAHKNRSVAE.  267

(B)
VIM-2.pro   MFKLLSKLLVYLTASI MAI ASPLAFSVDSSGEYPTVSEI PVGEVRLYQI ADGVWSHI ATQSFDGAVYPSNGLI VRDGDELLLI DTAWGA  89
VIM-36      MFKLLSKLLVYLTASI MAI ASPLAFSVDSSGEYPTVSEI PVGEVRLYQI ADGVWSHI ATRSFDGAVYPSNGLI VRDGDELLLI DTAWGA  89

VIM-2.pro   KNTAALLAEI EKQI GLPVTRAVSTHFHDDRVGGVDVLRAAGVATYASPSTRRLAEVEGNEI PTHSLEGLSSSGDAVRFGPVELFYPGAA  178
VIM-36      KNTAALLAEI EKQI GLPVTRAVSTHFHDDRVGGVDVLRAAGVATYASPSTRRLAEVEGNEI PTHSLEGLSSSGDAVRFGPVELFYPGAA  178

VIM-2.pro   HSTDNLVVYVPSASVLYGGCAI YELSRTSAGNVADADLAEWPTSI ERI QQHYPEAQFVI PGHGLPGGLDLLKHTTNVVKAHTNRSVVE  266
VIM-36      HSTDNLVVYVPSASVLYGGCAI YELSRTSAGNVADADLAEWPTSI ERI QQHYPEAQFVI PGHGLPGGLDLLKHTTNVVKAHTNRSVVE.  267
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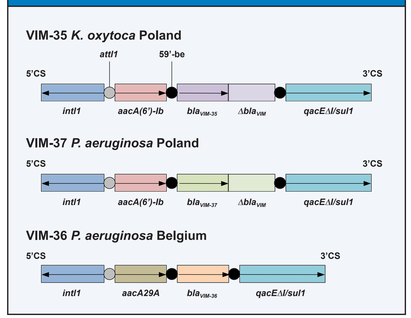
Table 1. Susceptibility results for clinical and recombinant isolates carrying genes encoding VIM-35, VIM-36, VIM-37. The susceptibility profile of recombinant strains carrying new VIMs were compared to those of recombinant strains carrying VIM-1 and VIM-2 genes.

	MIC (mg/L):							
Antimicrobial agent	VIM-35-producing *K. oxytoca* clinical isolate	*E. coli* XL1Blue PCRScript (bla_{VIM-35})	VIM-36-producing *P. aeruginosa* clinical isolate	*E. coli* XL1Blue PCRScript (bla_{VIM-36})	VIM-37-producing *P. aeruginosa* clinical isolate	*E. coli* XL1Blue PCRScript (bla_{VIM-37})	*E. coli* XL1Blue PCRScript (bla_{VIM-1})	*E. coli* XL1Blue PCRScript (bla_{VIM-2})
Doripenem	>8	1	>8	0.12	>8	0.12	1	0.12
Imipenem	>32	4	>32	2	>32	1	2	1
Meropenem	16	0.25	>32	≤0.06	>32	≤0.06	0.25	0.12
Ertapenem	32	0.5	>32	0.5	>32	16	0.12	0.25
Cefoxitin	>256	>256	>256	>256	>256	>256	>256	>256
Cefuroxime	256	128	>256	16	>256	8	64	>256
Ceftriaxone	64	8	>256	2	>256	2	>8	4
Ceftazidime	>256	128	>256	16	32	8	64	>256
Cefepime	64	2	16	≤0.5	64	≤0.5	4	1
Aztreonam	8	0.25	16	≤0.12	16	≤0.12	0.25	0.25
Ampicillin	>256	>256	>256	>256	>256	>256	>256	>256
Ampicillin/Sulbactam	>32	>32	>32	>32	>32	>32	>32	>32
Amoxicillin/Clavulanate	>8	>8	>8	>8	>8	>8	>8	>8
Piperacillin	128	16	>256	>256	>256	16	>256	8
Piperacillin/Tazobactam	64	16	64	>256	>256	16	>256	4
Amikacin	16	-[a]	>32	-	>32	-	-	-
Tobramycin	>16	-	>16	-	>16	-	-	-
Gentamicin	2	-	4	-	>8	-	-	-
Ciprofloxacin	>4	-	>4	-	>4	-	-	-
Tigecycline	1	-	>4	-	>4	-	-	-
Colistin	0.5	-	2	-	2	-	-	-

a. "-"=Not tested.

Figure 2. Schematic representation of the novel bla_{VIM}-carrying integrons detected in carbapenem-resistant isolates recovered in Poland and Belgium. Genes are represented as open arrows and the direction of the arrows indicates the transcription orientation. Δbla_{VIM} indicates the 155-bp repetition of bla_{VIM} 3'-end sequence.



CONCLUSIONS

- Three new VIMs were detected in Poland and Belgium. The genes from two different species from Poland were similar to bla_{VIM-1} and were located in an identical integron structure that included a signature duplication of the bla_{VIM} gene.
- A high prevalence of MßL-encoding genes was noticed in several European countries, including Poland and Belgium (see Poster #1339). These strains seem to be endemic in some countries and might limit the use of carbapenems and newer ß-lactam/ß-lactamase inhibitor combinations that only target serine-enzymes.

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