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Genetic Characterization and Comparative Antimicrobial Susceptibility of a New *bla*$_{VIM}$-variant

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SENTRY ANTIMICROBIAL SURVEILLANCE

Abstract

Background: VIM-2-producing strains have been reported in Mexico. We characterized the genetic context of a new *bla*$_{VIM}$-variant (*bla*$_{VIM-23}$) and compared antimicrobial susceptibility profiles of *bla*$_{VIM-2}$- and *bla*$_{VIM-23}$-carrying *Escherichia coli*.

Methods: Enterobacteriaceae were tested for susceptibility by CLSI methods during SENTRY Program. Isolates with imipenem or meropenem MIC values at ≥2 μg/mL were screened for carbapenemase production by Modified Hodge Test (MHT) and PCR (KPC, IMP, VIM, SME, IMI, NMC-A, GES, OXA-48). Primers targeting *bla*$_{VIM}$ and class 1 integron (INT1) regions were used. *bla*$_{VIM-23}$ and *bla*$_{VIM-2}$ were cloned and transformants tested for susceptibility by Etest. Gene location was assessed by Southern blot and hybridization.

Results: *E. cloacae* 6196A was recovered from a 18-year-old male burn patient in Guadalajara. Strain 6196A was resistant to penicillins and cephalosporins, but not aztreonam (MIC, 0.5 μg/mL); and showed elevated carbapenem MIC results (2 – 8 μg/mL). Sequencing revealed a new *bla*$_{VIM-23}$ in a INT1, which had the same cassette arrangement (a unique *orf* and *aacA7*) as *bla*$_{VIM-2}$-containing INT1 reported earlier in the same hospital. VIM-23 was identical to VIM-2, except for R228S, also present in VIM-1. *bla*$_{VIM-23}$-carrying *E. coli* showed similar MIC values for imipenem, meropenem, ceftazidime and piperacillin (≤2-fold variations) compared to *bla*$_{VIM-2}$-carrying *E. coli*, while lower MIC results for ampicillin (≥8-fold), cefepime (4-fold), cefoxitin (≥64-fold) and ertapenem (4-fold) were noted. *bla*$_{VIM-23}$ was plasmid located, but distinct from previous *bla*$_{VIM-2}$-carrying plasmids.

Conclusions: *bla*$_{VIM-23}$ has the same genetic context as previous *bla*$_{VIM-2}$-carrying INT1 detected at this site, suggesting in vivo mutation; but VIM-23 appears not to confer further resistance compared to VIM-2. R228S is located adjacent to the active site (loop L3) and may influence substrate binding, explaining unique MIC differences.

Introduction

The first VIM-type metallo-β-lactamase (MβL), VIM-1, was described in 1999 in a multidrug-resistant *Pseudomonas 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). Thus, VIM-2 was the first VIM-variant to emerge and currently this enzyme has become the most dominant among 25 VIM-subtypes described.

The VIM-2-encoding gene is usually embedded in class 1 integrons with distinct gene cassette arrays. Integrons carrying *bla*$_{VIM-2}$ can be plasmid or chromosomally located, indicating that this MβL gene has been mobilized to distinct genetic structures on different occasions. Moreover, *bla*$_{VIM-2}$ has been detected in at least 10 different bacterial species in over 25 countries. In addition, VIM-2-producers have caused outbreaks in hospitals located in various geographic locations.

In the Americas, VIM-2-producing isolates have been reported in Canada, the United States, Brazil, Argentina, and other Latin American countries. In Mexico, the dissemination of *bla*$_{VIM-2}$-carrying integron in genetically distinct Enterobacteriaceae isolates (*Klebsiella oxytoca* and *Enterobacter cloacae*) has been described. In this study we characterized a novel VIM-2-variant produced by an *E. cloacae* recovered from a hospital in Mexico evaluated during the SENTRY Antimicrobial Surveillance Program.

Methods

Bacterial isolates: A total of 110 Enterobacteriaceae isolates were consecutively collected from medical centers located in Mexico as part of the SENTRY Program (2008). These isolates were recovered from bloodstream, respiratory tract and skin and skin-structure infections according to defined protocols. Only clinically significant isolates were included in the study; one per patient episode. Species identification was confirmed by standard biochemical tests and use of the Vitek System (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing: Isolates were susceptibility tested against more than 25 antimicrobials by reference broth microdilution using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were as described in M100-S20-U (CLSI, 2010). *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were concurrently tested for quality assurance; all the results were within published ranges.

Screening for carbapenemases: All Enterobacteriaceae isolates with reduced susceptibility to imipenem or meropenem (MIC, ≥2 μg/mL) were screened for production of carbapenemases. Indole-positive *Proteae* and *Proteus mirabilis* were screened only when frankly resistant (MIC, ≥16 μg/mL) to one of these compounds since these species are inherently less susceptible to carbapenems. Modified Hodge test (MHT) was performed to detect carbapenemase production using imipenem and meropenem as substrates.

Isolates were also evaluated for the presence of *bla*$_{IMP}$, *bla*$_{VIM}$, *bla*$_{SPM-1}$, *bla*$_{KPC}$, *bla*$_{SME}$, *bla*$_{IMI}$, *bla*$_{NMC-A}$, *bla*$_{GES}$ and *bla*$_{OXA-48}$ by PCR.

Class 1 integron analysis: Class 1 integrons were amplified by targeting the 5'- and 3'-conserved sequences (CS). PCR products were sequenced on both strands and nucleotide sequences obtained were analyzed using Lasergene® software package (DNAStar; Madison, Wisconsin, USA) and compared to available sequences via NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/).

Plasmid and hybridization analysis: Plasmid extractions for VIM-2 carrying strains previously identified in Mexico and *E. cloacae* strain 6196A were performed using the Plasmid MIDI kit (QIAGEN; Hilden, Germany). Plasmid DNA was transferred from 1% agarose gel to nylon membranes by southern blotting. A digoxigenin (Roche Diagnostics GmbH; Mannheim, Germany) labeled *bla*$_{VIM-2}$ probe was used to identify the location of the MβL gene.

Molecular typing: Pulsed-field gel electrophoresis (PFGE) was used to evaluate clonality among *E. cloacae* strains producing VIM-2 (2005 and 2007) and VIM-23 (2008). Genomic DNA was prepared in agarose blocks and digested with Spe I (New England Biolabs; Beverly, Massachusetts, USA). Electrophoresis was performed on the CHEF-DR II (BioRad, Richmond, California, USA), with the following conditions: 0.5 x TBE, 1% agarose, 13°C , 200V, for 23 h with the switch time ramped from 5 to 60 seconds.

Results

- Among four carbapenem-non-susceptible *E. cloacae* isolates collected in 2008 (MIC, ≥2 μg/mL for imipenem or meropenem), only one (6196A) was MHT positive and yielded a positive PCR result for *bla*$_{VIM}$.
- *E. cloacae* 6196A was recovered from a 18-year-old male burn patient in Guadalajara. This strain was resistant to most β-lactams (Table 1), including carbapenems, but susceptible to aztreonam (MIC, 0.5 μg/mL). Additionally, the isolate was susceptible to gentamicin (MIC, ≤2 μg/mL), tetracycline (MIC, ≤2 μg/mL) and tigecycline (MIC, 0.5 μg/ml).
- Sequencing revealed that this strain carried a MβL gene distinct from *bla*$_{VIM-2}$ by a single point mutation. Deduced amino acid sequence revealed a R228S modification (standard MβL numbering), also present in VIM-1. This novel variant was named VIM-23.
- *bla*$_{VIM-23}$ was embedded in a class 1 integron that carried an open reading frame (*orf*) and *aacA7*. This cassette arrangement was identical to *bla*$_{VIM-2}$-containing integrons detected among one *K. oxytoca* and two *E. cloacae* recovered in the 2005-2007 period from the same hospital (Figure 1).
- *bla*$_{VIM-23}$-carrying *E. coli* recombinant strain showed similar MIC results for imipenem, meropenem, piperacillin alone and combined with tazobactam (≤2-fold variations) when compared to *bla*$_{VIM-2}$-carrying *E. coli*.
- Compared to VIM-2-producing *E. coli*, the recombinant strain producing VIM-23 had lower MIC results for cefepime, ceftazidime, cefotaxime and cefoxitin (≥4-fold; Table 1).
- Plasmid profiles were performed for all four VIM-producing isolates recovered from this medical site (three VIM-2 and the VIM-23 producer). Several plasmid bands were observed in all isolates and hybridization signals were noted from one high molecular weight plasmid band present in each of the four isolates (Figure 2).
- *E. cloacae* 6196A PFGE pattern was distinct from those of the two genetically unique VIM-2-producing *E. cloacae* isolates from the same hospital (data not shown).

Figure 1. Schematic representation of the structure of the 2.8-Kb integron carrying *bla*$_{VIM-2}$ or *bla*$_{VIM-23}$ identified in Enterobacteriaceae (*K. oxytoca* and *E. cloacae*) isolates from Guadalajara, Mexico. Genes are represented as open arrows and the direction of the arrows indicate the transcription orientation. Filled circles represent the 59-be recombination sites.

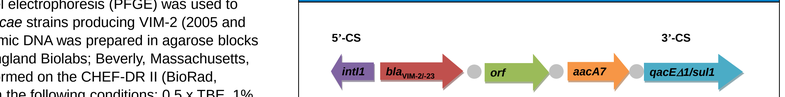
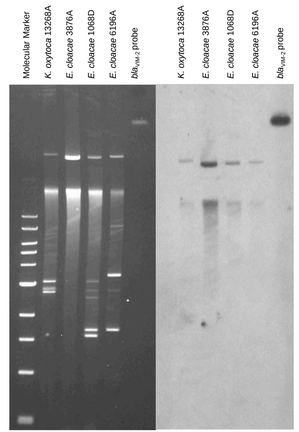


Table 1. Antimicrobial susceptibility profile of the VIM-23-producing *E. cloacae* clinical isolate and β-lactam susceptibility profiles of the *E. coli* strain carrying the recombinant plasmid vector PCRScript with *bla*$_{VIM-23}$ and *bla*$_{VIM-2}$ inserts and without insert.

Antimicrobial agent	MIC (μg/mL)			
	E. cloacae 6196A	*E. coli* pPCRScript carrying *bla*$_{VIM-23}$	*E. coli* pPCRScript carrying *bla*$_{VIM-2}$	*E. coli* pPCRSript
Imipenem	8	0.5	1	0.5
Meropenem	4	0.06	0.12	0.03
Doripenem	8	0.12	0.12	0.06
Ertapenem	2	0.06	0.25	0.012
Ampicillin	>256	>256	>256	4
Piperacillin	>128	4	8	1
Piperacillin/Tazobactam	>64	4	4	1
Cefoxitin	>256	4	>256	4
Cefotaxime	>32	1	4	0.06
Ceftazidime	>16	2	8	0.25
Cefepime	16	0.12	0.5	0.03
Cefuroxime	>256	>256	>256	4
Aztreonam	0.5	0.03	0.5	0.06
Amikacin	16	NA[a]	NA	NA
Gentamicin	≤2	NA	NA	NA
Tobramycin	16	NA	NA	NA
Ciprofloxacin	1	NA	NA	NA
Tetracycline	≤2	NA	NA	NA
Polymyxin B	≤0.5	NA	NA	NA
Tigecycline	0.5	NA	NA	NA
Trimethoprim/sulfamethoxazole	>2	NA	NA	NA

a. NA = not applicable

Figure 2. Plasmid and hybridization signal profiles of the *bla*$_{VIM-2}$- and *bla*$_{VIM-23}$-carrying Enterobacteriaceae isolates from the medical center in Mexico; (a) Agarose gel electrophoresis of the plasmid preparation and (b) hybridization membrane probed with labeled *bla*$_{VIM-2}$ fragment.



Conclusions

- Since 2005, a *bla*$_{VIM-2}$-carrying integron has been reported in different Enterobacteriaceae isolates (at least two different species) in a monitored Mexican hospital. In this study, we described the same integron carrying a gene encoding a new VIM-variant.
- The amino acid change in VIM-23 (R228S) appears not to confer greater resistance when compared to VIM-2. MIC values for cephalosporins were significantly lower for the new variant (expressed in an *E. coli* background) when compared to its ancestor.
- This report emphasizes the ability of class I integrons to persist and disseminate within hospital environments for extended periods to time and to evolve new gene contexts.

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