P901
ECCMID 2008

JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

bla$_{VIM-2}$-carrying Integron among Enterobacteriaceae Isolates in Mexico: Report from the SENTRY Antimicrobial Surveillance Program

L DESHPANDE, RN JONES, R MORFIN-OTERO, E RODRIGUEZ-NORIEGA, HS SADER
JMI Laboratories, North Liberty, IA, USA;
Instituto de Patologia Infecciosa y Experimental, Guadalajara, Mexico

SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objectives: To characterize metallo-beta-lactamase (MBL)-producing Enterobacteriaceae (ENT) strains isolated in a Mexican hospital and to evaluate the genetic context of the MBL-encoding genes. Carbapenems are generally very active against ENT but resistance has been emerging and has increased rapidly in some geographic areas due to the dissemination of MBL-carrying integrons.

Methods: ENT collected as part of the SENTRY Antimicrobial Surveillance Program were susceptibility (S) tested against >25 antimicrobials by CLSI broth microdilution methods. Isolates with imipenem and/or meropenem MIC at ≥2 mg/L were screened for MBL production by the disk approximation test; positive isolates were further characterized by PCR and gene sequencing. Primers targeting the class 1 integron conserved structures anchoring MBL genes were used to reveal integron structure. Amplicons generated were sequenced on both strands and MBL-producing isolates were evaluated for possible clonality by PFGE.

Results: Three isolates, all from one hospital in Mexico, exhibited positive MBL-screening test results. Two *E. cloacae* (2005 and 2007) and one *K. oxytoca* (2006) yielded a PCR product with *bla*$_{VIM}$ primers. The MBL gene was embedded in a 2.8-Kb class 1 integron in all strains. Sequencing revealed that *bla*$_{VIM-2}$ was located in the first position of the integron followed by 2 gene cassettes: an open reading frame (orf) and an aminoglycoside acetyl transferase gene (aacA7). The 400-bp orf showed low homology with other DNA sequences; however, the deduced amino acid sequence showed active domains of a chorismate mutase enzyme from *Pseudomonas fluorescens*. The degree of carbapenem resistance varied significantly among these VIM-2-producing isolates. The *E. cloacae* isolates were epidemiologically unrelated and one strain was S to both imipenem and meropenem (ertapenem also S; 0.12 or 0.25 mg/L) based on current CLSI breakpoints of ≤4 mg/L (see Table 1).

Conclusions: The finding of distinct ENT strains over a period of 3 years harboring the same *bla*$_{VIM-2}$-carrying integron indicates that this mobile genetic structure has become locally endemic. MBL-producing ENT may not be detected by clinical microbiology laboratories due to S-level carbapenem and other ß-lactam MIC results, requiring new detection strategies or revised S breakpoints.

INTRODUCTION

The VIM-2 metallo-ß-lactamase (MßL) was first reported from a *Pseudomonas aeruginosa* isolate recovered in a hospital located in Marseille, France (1996). A retrospective epidemiological study evaluating *P. aeruginosa* isolates from this hospital showed that VIM-2-producing strains were discovered in different wards since 1995, suggesting that this enzyme was the first VIM-variant to emerge. Among VIM enzymes, VIM-2 appears to be the most dominant and has been described in 23 countries in at least 10 different bacterial species.

The VIM-2 encoding gene is usually embedded in class 1 integrons with distinct gene cassette arrays. The *bla*$_{VIM-2}$-carrying integrons can be plasmid or chromosomally located, showing that this MßL gene was mobilized to distinct genetic structures on different occasions. Moreover, *bla*$_{VIM-2}$-carrying strains are considered to be the source of outbreaks in hospitals located in various geographic locations, including Greece, Italy and more recently, in the United States (USA).

In the Americas, VIM-2-producing isolates have been found in Canada, USA, Brazil, Argentina, and other Latin American countries, but not in Mexico. In this report, we describe the characterization of three VIM-2-producing Enterobacteriaceae strains isolated in a Mexican hospital participating in the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial isolates: In the 2005-2007 period, 120 *Klebsiella* spp. and 54 *Enterobacter cloacae* were consecutively collected in medical centers located in Mexico and were tested as part of the SENTRY Program. These isolates were recovered from bloodstream, respiratory tract and skin and soft tissue 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: All 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-S18 (CLSI, 2008). *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 mg/L) were screened for production of carbapenemases. Indole-positive *Proteae* and *Proteus mirabilis* were screened only when frankly resistant (MIC, ≥16 mg/L) to one of these compounds since these species are inherently less susceptible to carbapenems. Disk approximation screen tests for MßL production were performed using imipenem, meropenem and ceftazidime as substrates and EDTA as well as 2-mercaptopropionic acid as inhibitors.

MßL detection and class 1 integron analysis: Isolates exhibiting positive MßL screening results were evaluated for the presence of *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{SPM-1}$ by PCR. Class 1 integrons carrying MßL genes 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) and compared to available sequences via NCBI BLAST search (http://www.ncbi.nlm.nih.gov/blast/).

Molecular typing: Isolates belonging to the same species were evaluated for clonality by pulsed-field gel electrophoresis (PFGE). Genomic DNA was prepared in agarose blocks and digested with Spe I (New England Biolabs, Beverly, MA). Electrophoresis was performed on the CHEF-DR II (BioRad, Richmond, CA), 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.

Plasmid and hybridization analysis: Plasmid extractions were performed using the Plasmid MIDI kit (QIAGEN, Hilden, Germany). Plasmid sizes were determined by comparison with plasmids harbored by *Escherichia coli* NCTC 50192. Plasmid DNA was transferred from 1% agarose gel to nylon membranes by southern blotting. A digoxigenin (Roche Diagnostics GmbH, Mannheim, Germany) labeled *bla*$_{VIM-2}$-specific probe was used to identify the location of the MßL gene.

RESULTS

- Among 174 Enterobacteriaceae isolates tested, eight strains (4.6%; 2 *E. cloacae* and 6 *Klebsiella* spp.) with elevated carbapenem MIC values were screened for MßL production.
- Two *E. cloacae* and one *K. oxytoca* exhibited positive MßL screening test results and yielded a PCR product with *bla*$_{VIM}$ primers. Sequencing showed that all three isolates carried *bla*$_{VIM-2}$. These isolates were originated from a medical center located in Guadalajara, Mexico. The two *E. cloacae* were recovered in 2005 and 2007 and the *K. oxytoca* in 2006.
- The *E. cloacae* isolates exhibited distinct PFGE patterns and one strain was susceptible to the carbapenems tested (Table 1) based on current CLSI breakpoints of ≤4 mg/L.
- In all three isolates, the MßL gene was embedded in a 2.8-Kb class 1 integron containing the key genetic elements: the integrase gene (*intI1*) containing the attachment site (*attI1*) in the 5'-CS, and *qacEΔ1/sul1* in the 3'-CS.

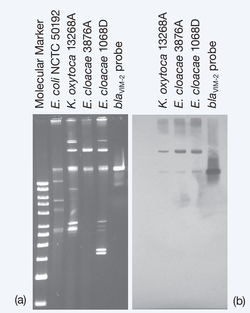
Table 1. Antimicrobial susceptibility patterns of VIM-2 producing Enterobacteriaceae isolates from the medical center in Guadalajara, Mexico.

	Organism (MIC range mg/L)		
	K. oxytoca	*E. cloacae*	
Antimicrobial agent	13268A	3876A	1068D
Imipenem	>8	4	8
Meropenem	>8	1	0.25
Ertapenem	>8	0.25	0.12
Piperacillin/tazobactam	32	64	64
Aztreonam	0.5	≤0.12	>16
Cefepime	4	2	2
Gentamicin	≤2	≤2	≤2
Amikacin	≤4	4	8
Tobramycin	4	8	16
Ciprofloxacin	1	≤0.03	0.5
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	>2
Polymyxin B	≤0.5	>4	≤0.5
Colistin	≤0.5	>4	≤0.5
Tetracycline	4	≤2	>8
Tigecycline	0.25	0.5	0.5

- Sequencing revealed that *bla*$_{VIM-2}$ was located in the first position of the integron followed by two gene cassettes: an open reading frame (*orf*) and an aminoglycoside acetyl transferase gene (*aac*A7; Figure 1). This gene cassette array was identical in all strains and is unique among the *bla*$_{VIM-2}$-integrons previously described in peer-reviewed publications.
- The 400-bp *orf* showed no similarity with DNA sequences reported before; however, the ORF putative protein showed the conserved enzymatic motifs of a chorismate mutase from *P. fluorescens* (involved in the biosynthetic pathway of amino acid formation).
- Plasmid preparations showed a distinct array of plasmids with various molecular weights in each of the isolates (Figure 2).
- Hybridization with *bla*$_{VIM-2}$ probe identified a single pattern, showing that all isolates carried the MßL gene on a 450-Kb plasmid.

Figure 1: Schematic representation of the structure of the 2.8-Kb integron carrying *bla*$_{VIM-2}$ 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.

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



CONCLUSIONS

- A plasmid carrying *bla*$_{VIM-2}$ was identified in three distinct Enterobacteriaceae isolates, showing that these mobile genetic structures most likely played an important role in the dissemination of MßL genes.
- MßL-producing Enterobacteriaceae showing carbapenem MIC values within the CLSI susceptible range (≤4 mg/L) may not be detected by clinical microbiology laboratories, posing a great risk for inadequate antimicrobial selection with subsequent treatment failures and delay in the implementation of infection control measures.
- Worldwide antimicrobial resistance surveillance programs, such as the SENTRY Program, contribute to the detection of emergent resistance mechanisms globally, producing important information that can guide therapeutic decisions, modify infection control practices and alter susceptibility guidelines to further recognize MßL strains.

SELECTED REFERENCES

1. Clinical and Laboratory Standards Institute. (2006). *M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition.* Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute. (2008). *M100-S18, Performance standards for antimicrobial susceptibility testing, 18th informational supplement.* Wayne, PA: CLSI.
3. Deshpande LM, Jones RN, Fritsche TR, Sader HS (2006). Occurrence and characterization of carbapenemases produced by Enterobacteriaceae: Report from the SENTRY Antimicrobial Surveillance Program (2000-2004). *Microb Drug Resist* 12: 223-230.
4. Fritsche TR, Sader HS, Toleman MA, Walsh TR, Jones RN (2005). Emerging metallo-ß-lactamase-mediated resistances: A summary report from the worldwide SENTRY antimicrobial surveillance program. *Clin Infect Dis* 41 Suppl 4: S276-278.
5. Moland ES, Hong SG, Cleary T, Morris MI, Hanson ND, Thomson KS (2006). US isolate of *Klebsiella pneumoniae* (KP) producing VIM metallo-ß-lactamse (MßL) and SHV-5-like ESBL, Abstr. C1-50. 46th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), San Francisco, CA. American Society for Microbiology, 69.
6. Nordmann P, Poirel L (2002). Emerging carbapenemases in Gram-negative aerobes. *Clin Microbiol Infect* 8: 321-331.