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The Emergence of VIM-2 in Latin American Hospitals: Report from the SENTRY Antimicrobial Surveillance Program

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ABSTRACT

Background: The VIM metallo-ß-lactamases (MßL) are emerging mobile resistance determinants recently described and reported in various European countries and in the Far East. We report the detection and characterization of *Pseudomonas* isolates producing VIM-2 causing infections in Latin American (LA) hospitals.
Methods: As part of the SENTRY Program, MßL production was screened by Etest (AB BIODISK) MßL strip or the disk approximation test, then confirmed by hydrolyses assays and PCR for bla_{IMP}, bla_{VIM} and bla_{SPM} followed by gene sequencing. Integrons were detected with primer sets specific to the 5'CS and 3'CS of Class 1 integrons. The PCR products were sequenced bidirectionally using DuPont Automated systems and the sequences analyzed by DNAstar.
Results: Two isolates were selected for characterization. Strain 43-14926A was a *P. fluorescens-putida* isolated in Chile from bloodstream infection in a 19 yo female who underwent bone marrow transplantation. Strain 49-4597C was a *P. aeruginosa* isolated in Venezuela from a 52 yo male who developed sepsis and nosocomial pneumonia as a complication of a surgical abdominal infection. Both patients received imipenem (IMP) therapy and other ß-lactams previous to the isolation of the carbapenem-resistant (R) strain. Both isolates were R to IMP, meropenem and ceftazidime. Sequence analysis revealed a MßL gene bla_{VIM-2} in both isolates. Upstream from the MßL (43-14926A) bla_{VIM-2}, lies a class 1 integron. Downstream of bla_{VIM-2} was found a gene cassette *aacA4* followed by *aadA1* and *aadA2*.
Conclusions: We documented the emergence of VIM-2 in two LA hospitals. Global surveillance networks, such as the SENTRY Program, play an important role in detecting the regional emergence and dissemination of novel MßL mechanisms.

INTRODUCTION

Carbapenems, mainly imipenem and meropenem, are potent agents for the treatment of infections caused by multi-resistant *Pseudomonas*. However, the emergence of carbapenem-resistant strains in this genus has been increasing worldwide. High-level resistance to carbapenems (> 32 mg/L) is still uncommon in *Pseudomonas* spp., but can occur due to the presence of Ambler class B ß-lactamases – metallo-ß-lactamases (MßLs).

Four different clinically relevant types of mobile MßLs have been described in the literature: IMP, VIM, SPM and GIM. The VIM-family has been reported mostly in European and Eastern countries, although a distantly related MßL (VIM-7) has been characterized from the USA. We are not aware of VIM-type MßLs being reported from Latin American. In this study, we describe the presence of bla_{VIM-2} among *Pseudomonas* spp. isolates obtained from the SENTRY Antimicrobial Surveillance Program participant medical centers located in Chile and Venezuela. We also characterized the genetic context of the genes.

MATERIAL AND METHODS

Bacterial Strains: Four isolates of *Pseudomonas* spp., one *P. fluorescens* (43-14926) from blood culture isolated in December 2002 in Santiago, Chile and three *P. aeruginosa* (49-4583, 49-4596 and 49-4597) recovered from the respiratory tract between August and September 2002 from the same hospital in Caracas, Venezuela were collected as part of the SENTRY Program. The isolates were selected based on resistance to imipenem (MIC, ≥16 mg/L), meropenem (MIC, ≥16 mg/L), and ceftazidime (MIC, ≥32 mg/L).

Susceptibility testing**.** Antimicrobial susceptibility testing was performed using the reference broth microdilution method as described by the National Committee for Clinical Laboratory Standards (NCCLS). Antimicrobial agents were obtained from the respective manufacturers dispensed into 96-well panels, and quality control was performed by testing *Escherichia coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213.

Phenotypic detection of ß-lactamases: Production of MßLs was screened by the disk approximation test. Briefly, a 100mm Mueller-Hinton agar plate was inoculated using a 0.5 McFarland suspension from fresh cultures. Imipenem, meropenem, and ceftazidime disks were strategically aligned around disks contained either EDTA (750 µg) or thiolactic acid (360 µg). The test was read after 18-20 hrs of incubation at 35ºC. The appearance of either an elongated or a phantom zone between the carbapenems and/or ceftazidime and either one of the disks containing a MßL inhibitor (EDTA or thiolactic acid) was considered a positive test. *Acinetobacter baumannii* 54/97 was used as a positive control. MßL Etest strips (AB Biodisk, Solna, Sweden) were used to confirm the disk approximation test results, each containing imipenem with and without EDTA.

MßL hydrolytic activity assay**.** MßLs carrying strains were grown overnight at 37ºC in 10 ml of nutrient broth. The cells were harvested by centrifugation (4,000 x *g* for 20 min at 4ºC) and ressuspended in 2 ml of assay buffer (50 mM Cacodylate, 100 mM Sodium chloride, 100 mM Zinc chloride, pH 7.0). The cells were lysed by sonication and cell debris removed by centrifugation. MßL activity was determined through spectophotometric assays using 100 µl of the resulting supernatant and 2 ml of the imipenem solution. Hydrolysis was measured in a Lambda 35 spectrophotometer (Perkin-Elmer, Cambridge, UK) by observing the changes in absorption as a result of the opening the ß-lactam ring at 299 nm and the results expressed in absorbance per minute. Assays were performed with and without the presence of EDTA (20mM) to confirm the inhibition of MßLs by chelating agents.

DNA sequencing: Amplification with primers for the internal region of bla_{VIM}-like genes and 5'conserved sequence (CS) and 3'CS from class 1 integron and subsequent sequencing were performed as described earlier. Primers used for amplification and sequencing of the bla_{VIM} gene were: VIM-F (5'-AAAGTTATGCCGCACTCACC-3') and VIM-R (5'-TGCAACTTCATGTTATGCCG-3').

Plasmid analyzes, conjugation and transformation experiments: Plasmid extraction was carried out with QIAprep Spin Mini prep kit (Quiagen, West Sussex, UK). Conjugation and transformation experiments were performed as previously described with rifampin-resistant (Rif^r) *E. coli* K-12 and *E. coli* DH5α, respectively. Strains harboring possible plasmids were selected by plating onto nutrient agar plates containing ceftazidime (5 mg/L) or ceftazidime and rifampin (500 mg/L).

COMMENTS

- The isolates were resistant to all ß-lactams, aminoglycosides, quinolones and many other antimicrobial agents tested (Table 1). Only polymyxin B was active against all of the isolates.
- MßLs phenotypic tests were positive as judged by the disk approximation method and the Etest MßL strips. These results were confirmed by spectrophotometric assays measuring imipenem hydrolysis. The rates of imipenem hydrolysis were inhibited 88-97% for each of the strains by EDTA (20mM) (Table 2).
- Sequencing results of the PCR amplicons revealed the presence of bla_{VIM-2} gene in the first position of a class 1 integron, which has *qac*EΔ/*sul*1 downstream of the MßL gene.
- This integron gene arrangement has been previously reported in France from *P. aeruginosa* isolated from a patient in 1996 (Figure 1). Other cases have been described in Greece from *P. aeruginosa* in 2000, and in Korea from *Serratia marcescens* in 2002. Class 1 integrons carrying bla_{VIM}-type genes are now reported to contain genes encoding aminoglycoside modifying enzymes and it is uncertain whether the arrangement of the integron without the additional genes is the progenitor.
- Despite repeated attempts of plasmid extraction, the isolates did not show any plasmid and transformation experiments were unsuccessful. Conjugation experiments between the clinical isolates and *E. coli* K-12 Rif^r did not yield any transconjugant. These data infer that the bla_{VIM-2} found in these isolates, are likely to be chromosomally encoded.
- Automated ribotyping and PFGE showed that the three isolates from Venezuela were identical suggesting local clonal dissemination. The strain from Chile was unique and a different species from the MßL-producing strains observed in Venezuela.

Table 1. Antimicrobial susceptibility profile of the bla_{VIM-2} carrying *Pseudomonas* spp. isolates.

	MIC (mg/L)			
	P. fluorescens	*P. aeruginosa*		
Antimicrobial agents	43-14926	49-4583	49-4596	49-4597
ß-lactams				
Aztreonam	>16	>16	16	16
Ceftazidime	>16	>16	>16	>16
Cefepime	16	>16	>16	>16
Imipenem	>8	>8	>8	>8
Meropenem	>8	>8	>8	>8
Pip/Taz[a]	>64	64	64	64
Quinolones				
Ciprofloxacin	>4	>4	>4	>4
Gatifloxacin	>4	>4	>4	>4
Levofloxacin	>4	>4	>4	>4
Aminoglycosides				
Amikacin	8	>32	>32	>32
Gentamicin	>8	>8	>8	>8
Netilmicin	>32	>32	>32	>32
Tobramycin	>16	>16	>16	>16
Others				
Polymyxin B	≤1	≤1	≤1	≤1
Tetracycline	>8	>8	>8	>8
Trim/Sulfa[b]	>2	>2	>2	>2

a. Piperacillin/Tazobactam
b. Trimethoprim/Sulfamethoxazole

Table 2. MIC from Etest MßL strips and imipenem hydrolytic activities with and without the EDTA of the bla_{VIM-2} carrying *Pseudomonas* spp. isolates.

	Etest MßL (mg/L)		Hydrolytic activities (Abs/min)[a]		
	Imipenem	Imipenem + EDTA	Imipenem	Imipenem + EDTA	% of inhibition
Strains					
43-14926	>256	≤1	0.07194	0.00384	94.6%
49-4583	>256	4	0.04519	0.00251	94.5%
49-4596	>256	6	0.10144	0.00253	97.5%
49-4597	>256	8	0.04359	0.00500	88.5%

a. Absorbance per minute.

Figure 1. Schematic representation of the bla_{VIM-2} *P. aeruginosa* containing integron (49-4597) isolated from Venezuela. This structure is identical to the previously described In56, which consists of a class 1 integrase gene (*intI 1*) and its combination site together with the bla_{VIM-2} gene and *qac*EΔ/*sul*1cassette. Arrows indicate the direction of the transcription of the genes. Red and yellow ellipses represent the attachment site and the 59 base elements, respectively.

CONCLUSIONS

- This is the first report of VIM-2 producing *Pseudomonas* strains in Latin America.
- Clonal dissemination of multi-drug resistant strains coupled with the plasticity of class 1 integrons indicates that resistance to the main anti-*Pseudomonas* ß-lactams, such as cephalosporins, ß-lactam-ß-lactamase inhibitor combinations, and carbapenems, will continue to increase.
- These results highlight the importance that global surveillance networks, such as the SENTRY Program, may play in the detection and control of the dissemination of new resistance mechanisms.

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