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Dissemination of *bla*VIM Among Clonally Unrelated Clinical Strains Isolated in an USA Medical Center

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ABSTRACT

Background: VIM-7 was the first mobile metallo-ß-lactamase (MßL) described in the USA. *bla*VIM-7 was initially detected in May, 2001 in a *P. aeruginosa* (PSA) isolated from a 58 y-o female with pneumonia. We report the results of the characterization of two other MßL-producing PSA strains isolated in the same medical center 2-3 years later.

Methods: Two carbapenem (CARB)-resistant (R) PSA strains were collected from the MD Anderson Cancer Center, in 2003 (strain # 4623) and 2004 (1-1852), as part of MYSTIC Program. The patients had clinical history of malignancies, transplantation, neutropenia and multiple courses of broad-spectrum antimicrobial treatment. The isolates were tested for susceptibility to >30 agents by CLSI broth microdilution methods, screened for MßL production by the disk approximation (DA) test and ribotyped. MßL production was confirmed by PCR using primers for *bla*VIM-family followed by sequencing of PCR products. Results were compared to the index VIM-7-producing strain (07-406).

Results: Both isolates were R to all antimicrobials tested except polymyxin B, and were DA test positive (enzyme inhibited by EDTA and 2-MPA). The isolates had distinct ribotypes, which were also distinct from strain 07-406. PCR results were positive for *bla*VIM in both strains. PCR product from strain 4623 showed 100% homology with *bla*VIM-7 while the PCR product from 1-1852 showed several differences compared to *bla*VIM-7.

Conclusions: The dissemination of the initial *bla*VIM-7-producing strain was successfully controlled and no other case was detected for nearly 2 years. However, *bla*VIM-7 re-emerged in a clonally unrelated strain and a second distinct *bla*VIM emerged in the same medical center. These results emphasize the potential for dissemination and the difficulty for complete eradication of these mobile MßL genes in the clinical environment, especially among an at-risk patient population.

INTRODUCTION

In 1999, a novel family of class B metallo-ß-lactamases (MßL), the VIM family (VIM-1 to VIM-11 enzymes) was initially described in *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolated in Europe. The VIM family was subsequently found in strains of *Serratia marcescens* and *Acinetobacter* spp. in Korea (VIM-2) as well as, *P. aeruginosa* (VIM-3), *Pseudomonas putida*, and *Pseudomonas stutzeri* (VIM-2) in Taiwan. More recently, VIM variants have been found in *Escherichia coli* (VIM-1) and *P. aeruginosa* (VIM-4) in Greece, in *Klebsiella pneumoniae* (VIM-5) in Turkey, and in *P. putida* (VIM-6) in Singapore.

The *bla*VIM gene, like the *bla*IMP gene, is carried on mobile gene cassettes inserted into class 1 integrons and located chromosomally or on resident plasmids. The class 1 integrons are the most common mechanisms by which bacteria are able to disseminate resistant gene cassettes across species or genera. The process involves recombination between 59-bp elements on the gene cassette and *attI*1 sites on the integron.

VIM-7 was described in a clinical strain of *P. aeruginosa* collected at the MD Anderson Cancer Center (Houston, Texas), as part of the CANCER Surveillance Program in 2001 and represented the first MßL described in the United States. In the present study, we characterized two other VIM-producing *P. aeruginosa* strains collected in the same institution in 2003 and 2004.

MATERIALS AND METHODS

Bacterial Isolates: The isolates were sent to JMI Laboratories (North Liberty, IA) as part of various antimicrobial resistance surveillance programs. Among other selected pathogens, *P. aeruginosa* strains resistant to imipenem (MIC, ≥ 16 µg/ml), meropenem (MIC, ≥ 16 µg/ml), and ceftazidime (MIC, ≥ 32 µg/ml) have been routinely screened for MßL genes. Two *P. aeruginosa* strains collected from patients hospitalized at the MD Anderson Cancer Center in 2003 (strain # 4623) and 2004 (1-1852) had a positive result for the MßL screen test and were further characterized in the present study.

Case one: Strain # 4623 was recovered from a 60-year-old white male with a diagnosis of stage IV non-small-cell lung carcinoma with metastatic lesions to the brain and bone who had recently completed palliative radiation therapy. He also had a chronic obstructive pulmonary disease. The patient was admitted to the hospital with severe pneumonia and respiratory failure. *P. aeruginosa* strain # 4623 grew from a blood culture. His respiratory status deteriorated very quickly and he expired.

Case two: Strain # 1-1852 was cultured from a 61-year-old female who had a nephrectomy performed in July 2003 for a left adrenal myelolipoma. The patient developed amyloidosis and nephrotic syndrome and was treated with melphalan and an autologus stem cell transplant. She developed prolonged neutropenia, severe mucositis and sepsis. *P. aeruginosa* strain # 1-1852 grew from blood cultures obtained from peripheral sites and a subclavian line. The patient became unresponsive with acute respiratory distress syndrome and rapidly died.

Susceptibility testing**.** Antimicrobial susceptibility testing was performed using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS). Antimicrobial agents were obtained from the respective manufacturers or purchased from Sigma-Aldrich, Inc. (St. Louis, MO). 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 made from fresh cultures. Imipenem, meropenem, and ceftazidime disks were strategically aligned around disks containing either EDTA (750-µg) or thiolactic acid (360-µg). The test was read after 18-20 hours of incubation at 35°C. The appearance of either an “elongated” or a “phantom zone” between the carbapenems and/or ceftazidime and either one of the disks containing an 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.

DNA sequencing: Initially the isolates were screened for VIM-like genes using primers spanning the conserved sequences within *bla*VIM. *bla*VIM-7 and it’s genetic context were studied by sequencing the gene segment amplified using 5’ conserved sequence (CS) and 3’ CS from Class I integron as previously described. Primers used for amplification and sequencing of the *bla*VIM-2 were: VIM-2F (5’-AAAGTTATGCCGCACTCACC-3’) and VIM-2R (5’-TGCAACTTCATGTTATGCCG-3’).

RESULTS

- Both *P. aeruginosa* (strains #4623 and 1-1852) were resistant to all ß-lactams, aminoglycosides and fluoroquinolones tested. The isolates were susceptible only to polymyxin B (data not shown).
- Generic *bla*VIM primers yielded PCR products in both strains. Sequencing results of the complete operon using the integron primers revealed *bla*VIM-7 in strain #4623, the same as the index strain reported in 2001 (strain #7-406). *bla*OXA-45 was identified in the index strain, but not in strain #4623 (Table 1).
- Sequencing results of PCR amplicon obtained from strain #1-1852 revealed *bla*VIM-2 which has many key genetic differences, ruling out the possibility of evolution of the *bla*VIM-7 gene pool that has existed at this large medical center (Figure 1).
- In the previous index strain #7-406, *bla*VIM-7 and *bla*OXA-45 were encoded on separate plasmids while strain #4623 harbors only *bla*VIM-7 and had only one plasmid (Table 1) of the same size as the plasmid in strain #7-406. The more recently isolated strain #1-1852 had no plasmid.
- The VIM-7-producing strain in this study (strain #4623) and the VIM-7-producing strain previously reported in this medical center (strain #7-406) showed distinct ribotype patterns, and both strains were different from the VIM-2-producing strain (#1-1862; Figure 2).

Table 1. Summary of molecular epidemiologic investigation of metallo-ß-lactamase-producing *P. aeruginosa* isolated from an USA medical center.

		Disk approximation results[a]								
Isolate #	Year	IMI+EDTA	MEM+EDTA	IMI+MPA	MEM+MPA	CAZ+EDTA	# plasmids	VIM PCR	Ribotype	Carbapenemases
7-406	2001	+	-	-	-	-	2	+	105.528.6	VIM-7, OXA-45
4623	2003	-	+	-	-	+	1	+	258.151.2	VIM-7
1-1852	2004	+	+	-	+	-	0	+	258.231.1	VIM-2

a. Various substrate inhibitor combination interactions described. IMI = imipenem, MEM = meropenem, CAZ = ceftazidime, MPA = 2-merceaptopropionic/thiolactic acid.

Figure 1. Comparison of amino acid sequences of MßLs VIM-1, VIM-2 and VIM-7. Highlighted sequences indicate discrepancies between VIM-2 and VIM-7.

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VIM-1: MLKVISSLLVYMTASVMAVASPLAHSGEPSGEYPTVNEIPVGEVRLYQIAD
VIM-2: MFKLLSKLLVYLTASIMAIASPLAFSVDSSGEYPTVSEIPVGEVRLYQIAD
VIM-7: MFQIRSFLVGISAFVMAVLGSAAYSAQP-GGEYPTVDDIPVGEVRLYKIGD

GVWSHIATQSFDGAVYPSNGLIVRDGDELLLIDTAWGAKNTAALLAEIEKQIGLPVTR
GVWSHIATQSFDGAVYPSNGLIVRDGDELLLIDTAWGAKNTAALLAEIEKQIGLPVTR
GVWSHIATQKLGDTVYSSNGLIVRDADELLLIDTAWGAKNTVALLAEIEKQIGLPVTR

AVSTHFHDDRVGGVDVLRAAGVATYASPSTRRLAEAEGNEIPTHSLEGLSSSGDAVRF
AVSTHFHDDRVGGVDVLRAAGVATYASPSTRRLAEVEGNEIPTHSLEGLSSSGDAVRF
SISTHFHDDRVGGVDVLRAAGVATYTSPLTRQLAEAAGNEVPAHSLKALSSSGDVVRF

GPVELFYPGAAHSTDNLVVYVPSANVLYGGCAVHELSSTSAGNVADADLAEWPTSVER
GPVELFYPGAAHSTDNLVVYVPSASVLYGGCAIYELSRTSAGNVADADLAEWPTSIER
GPVEVFYPGAAHSGDNLVVYVPAVRVLFGGCAVHEASRESAGNVADANLAEWPATIKR

IQKHYPEAEVVIPGHGLPGGLDLLQHTANVVKAHKNRSVAE
IQQHYPEAQFVIPGHGLPGGLDLLKHTTNVVKAHTNRSVVE
IQQRYPEAEVVIPGHGLPGGLELLQHTTNVVKTHKVRPVAE
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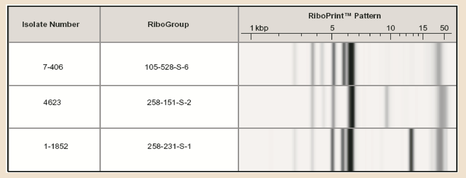
Figure 2. Comparison of riboprint patterns of *bla*VIM-producing *P. aeruginosa* strains isolated at the MD Anderson Cancer Center.



CONCLUSIONS

- The dissemination of the initial *bla*VIM-7-producing strain appears to have been successfully controlled and no other case was detected for nearly two years. However, *bla*VIM-7 re-emerged in a clonally unrelated strain.
- A second distinct MßL, *bla*VIM-2, was detected in the same medical center one year after the second *bla*VIM-7 occurrence.
- These results emphasize the potential for dissemination and the difficulty for complete eradication of these mobile MßL genes in the clinical environment, especially among at-risk patient populations.
- This report also indicates the emergence of MßL-mediated resistance among Gram-negative pathogens in the USA, which has been only described in other parts of the world.

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