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Emergence of IMP and VIM Metallo-ß-Lactamase Producing *P. aeruginosa* Strains in Mexico: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: Acquired carbapenem (CARB) resistance (R) due to metallo-ß-lactamase (MßL) production is increasing rapidly in Asia, Europe and South America, but has been rarely reported in North America and particularly in Mexico. We report the first discovery of IMP and VIM among *P. aeruginosa* (PSA) clinical strains isolated in Mexican medical centers.
Methods: As part of the SENTRY Program, all isolates are tested for susceptibility (S) by CLSI broth microdilution methods to >30 antimicrobials. Strains R to imipenem (IMP), meropenem and ceftazidime are screened for MßL production by a disk approximation (DA) test. Strains with a positive MßL screen test (enzyme inhibited by EDTA and 2-MPA) were evaluated by PCR for bla_{IMP}, bla_{VIM}, bla_{SPM} and bla_{GIM}, followed by gene sequencing.
Results: 28 PSA strains were collected from a Mexican center (115) in 2004, 4 (14.3%) were R to IMP and 3 of those organisms (75%) showed positive MßL DA test results. All strains had positive PCR results for bla_{IMP} and gene sequence analysis demonstrated greatest homology with IMP-5. The strains showed the same ribotype/PFGE pattern and were isolated in April, July and October, 2004 from different patients with long-term hospitalization and exposure to multiple antimicrobial treatments. A fourth strain with a positive MßL screen test was isolated in January 2005. This strain had a positive PCR result for bla_{VIM}, and negative results for bla_{IMP} and bla_{SPM}. All 4 PSA strains were R to all antimicrobials tested except the polymyxins.
Conclusions: This is the first report of MßL-producing strains (IMP and VIM) in Mexico, and MßL production appears to be the main mechanism of CARB-R among PSA in the index medical center. These results raised the concern of the dissemination of this potent MßL mechanism in this institution, as well as, in the region or nation.

INTRODUCTION

Metallo-beta-lactamase (MßL) production is an emerging mechanism of carbapenem resistance among enteric and non-fermentative Gram-negative bacilli. MßLs of the IMP-family were first reported from Japan and are now prevalent in Asia, Europe and Latin America. Additional families of these enzymes namely VIM (Italy), SPM (Brazil), GIM (Germany) and more recently SIM (Korea) have emerged over time. Certain MßL types, such as SPM have become widely endemic in the geographic regions where they were first identified while others have spread across and between continents (e.g. VIM).

Inspite of increasing incidence of MßLs in various parts of the world, reports of MßL-producing pathogens from North America are a recent event and still relatively rare. There are four reports of VIM-producing *Pseudomonas aeruginosa* (VIM-7 [Texas, two reports] and VIM-2 [Texas and Illinois]) from the USA and one report of an outbreak of IMP-7 producing *P. aeruginosa* from Canada. However, there are no reports of strains producing acquired MßL from Mexico.

In this report, we characterized MßL-producing strains isolated in a Mexican medical center through the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial isolates. In 2004, a medical center in Mexico submitted 28 non-duplicate *P. aeruginosa* isolates to the SENTRY Antimicrobial Surveillance Program. These strains were isolated from bloodstream infections, skin and skin structure infections and pneumonia in hospitalized patients according to protocols used by all SENTRY Program participant centers. Species level identification was confirmed by standard biochemical tests and Vitek cards where necessary.

Susceptibility testing. The isolates were susceptibility tested (minimum inhibitory concentration [MIC]) against >30 antimicrobials by broth microdilution procedure as described by the Clinical and Laboratory Standards Institute (CLSI, formerly the NCCLS) using dry-form panels manufactured by Trek Diagnostics (Cleveland, OH, USA). Interpretations of susceptibility test results were as per CLSI criteria, where available. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were routinely tested in parallel with clinical isolates for quality assurance.

Screening for metallo-ß-lactamases. *P. aeruginosa* isolates resistant to imipenem, meropenem and ceftazidime (MIC >16 µg/ml), were tested for MßL production. Disk approximation screen tests for MßL detection were performed using imipenem, meropenem and ceftazidime as substrates and EDTA as well as 2-mercaptopropionic acid (MPA) as inhibitors. MßL Etest strips with imipenem ± EDTA (AB BIODISK, Solna, Sweden) were used to confirm positive disk approximation test results when needed. Isolates with a positive phenotypic MßL test were screened for bla_{IMP}, bla_{VIM} and bla_{SPM} using PCR primers described elsewhere. Primers derived from conserved sequences of the class 1 integron were used to study genetic context of the MßL genes.

Gene sequencing. PCR reactions were examined for amplification product by electrophoresis on 1% agarose gels. Amplicons cleaned using QIAquick PCR purification kit (QIAGEN GmbH, Germany) were sequenced using Sanger based dideoxy sequencing strategy involving the incorporation of fluorescent-dye-labeled terminators into the sequencing reaction products. Sequences obtained were compared to the available sequences via NCBI BLAST search.

Molecular Typing. Multiple isolates showing a similar antimicrobial susceptibility pattern and a positive disk approximation test for MßL were epidemiology typed using the Riboprinter™ Microbial Characterization system. Isolates with identical ribotypes were further characterized by pulsed-field gel electrophoresis (PFGE).

RESULTS

- Three of the 28 (10.7%) *P. aeruginosa* isolates submitted by the Mexican medical center in 2004 were resistant to most antimicrobials tested, including ß-lactam-ß-lactamase inhibitor combinations, cephalosporins, carbapenems, quinolones and aminoglycosides, but were susceptible to polymyxin B (Table 1).
- All three strains showed positive results for the MßL disk approximation test as well as MßL Etest (Table 2).
- PCR screens for MßLs showed amplification products with bla_{IMP} primers from all three isolates. Sequences obtained from PCR amplicon of index strain (1686D) showed closest similarity with the bla_{IMP-5} sequence.
- IMP-5 shares greatest amino acid identity with IMP-1 and IMP-4 (94.0%) and also with IMP-8 (89.8%). Comparison with these three IMP-types led to the identification of bla_{IMP} from 1686D as IMP-5 (Figure 1).
- A class I integron with an aminoglycoside modifying enzyme gene *AadA6* was identified in 1686D, but the bla_{IMP-5} was not present in this integron.
- Case reports of patients infected with IMP-5-producing strains.
  - Case 1: A 63 year-old Mexican male patient, with no previous hospitalizations and poorly controlled type II diabetes mellitus, was admitted for the treatment of a diabetic foot infection. During the initial evaluation renal failure and chronic obstructive pulmonary disease were diagnosed. Antibiotic treatment for a severe mixed bacterial foot infection, that included MRSA, was initiated with ciprofloxacin and clindamycin and was followed with piperacillin/tazobactam, linezolid, gentamicin, trimethoprim/sulfamethoxazole (TMP/SMX), rifampin and levofloxacin. After 60 days of hospitalization an IMP-5-producing *Pseudomonas aeruginosa* was isolated from the foot wound. *P. aeruginosa* infection was eradicated only after extensive débridement.
  - Case 2: A 22 year-old Mexican male was admitted for the evaluation of acute abdomen. During an exploratory laparatomy a perforated appendix with diffuse peritonitis was found. The patient was started on cefotaxime plus clindamycin and after several days on mechanical ventilation he developed a nosocomial pneumonia. On July 12, 2004 an IMP-5-producing *P. aeruginosa* was isolated from sputum. Antimicrobial therapy was switched to meropenem, amikacin, and ciprofloxacin; sputum cultures subsequently became negative for *P. aeruginosa.*
  - Case 3: A 54 year-old Mexican female with myelocytic leukemia under chemotherapy was admitted for the evaluation of fever. Initial empiric antimicrobial treatment included amoxicillin/clavulanic acid, TMP/SMX, and ciprofloxacin. The patient developed renal failure, *C. difficile* diarrhea and urinary tract infection. An IMP-5 producing *P. aeruginosa* was isolated from the blood on October 26, 2004. Meropenem was then started but after 3 days the patient's clinical condition deteriorated and she expired.

Table 1. Antimicrobial susceptibility of the three IMP-5-producing strains.

	MIC (µg/ml)		
Antimicrobial agent	1686D	2058C	10639A
Imipenem[a]	>256	48	48
Meropenem	>8	>8	>8
Ceftazidime	>16	>16	>16
Cefepime	>16	>16	>16
Piperacillin/tazobactam	>128	128	128
Aztreonam	>16	16	16
Ciprofloxacin	>4	>4	>4
Levofloxacin	>4	>4	>4
Gentamicin	>8	>8	>8
Amikacin	>32	>32	>32
Tobramycin	>16	>16	>16
Polymyxin B	0.5	0.5	0.5

a. Etest results

- All three isolates producing IMP-5 belonged to the same ribogroup and showed similar (<3 bands difference) PFGE patterns. Temporal separation of isolation dates of the three strains (April, July and October, 2004) indicates endemic nature of this cluster at the medical center (Table 1).
- A fourth strain (1112A) with a multidrug-resistance pattern (susceptible only to polymyxin B) was isolated in early 2005. It exhibited a positive MßL disk approximation test with imipenem, meropenem and ceftazidime as substrates and EDTA as enzyme inhibitor. MßL Etest showed marginally positive results (Table 2). PCR screens using bla_{VIM} primers generated a PCR product, but presence of bla_{VIM} could not be confirmed.

Figure 1. Comparison of amino acid sequences of various IMP-types

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IMP-1: MSKLSVFFIFLFCSIATAAESLPDLKIEKLDEGVYVHTSFEEVNGWGVVPKH
IMP-4: MSKLSVFFIFLFCSIATAAEPLPDLKIEKLDEGVYVHTSFEEVNGWGVVPKH
IMP-5: MSKLLVLFMFLLCSITLANSGLPDLKIEKLDEGVYVHTSFEEVNGWGVVLKH
IMP-8: MKKLFVLCVCFLCSITAAGAALPDLKIEKLEEGVYVHTSFEEVNGWGVVSKH

GLVVLVNAEAYLIDTPFTAKDTEKLVTWFVERGYKIKGSISSHFHSDSTGGIEWLNSR
GLVVLVDAEAYLIDTPFTAKDTEKLVTWFVERGYKIKGSISSHFHSDSTGGIEWLNSQ
GLVVLVNTEAYLIDTPFTAKDTEKLVTWFVERGYKIKGSISSHFHSDSTGGIEWLNSN
GLVVLVNTDAYLIDTPFTATDTEKLVNWFVERGYKIKGTISSHFHSDSTGGIEWLNSQ

SIPTYASELTNELLKKDGKVQATNSFSGVNYWLVKNKIEVFYPGPGHTPDNVVVWLPE
SIPTYASELTNELLKKDGKVQAKNSFGGVNYWLVKNKIEVFYPGPGHTPDNLVVWLPE
SIPTYASELTNELLKKDGKVQANNSFSGANYWLVKKKIEVFYPGPGHTTDNVVVWLPE
SIPTYASELTNELLKKDGKVQAKNSFSGVSYWLVKNKIEVFYPGPGHTQDNVVVWLPE

RKILFGGCFIKPYGLGNLGDANIEAWPKSAKLLKSKYGKAKLVVPSHSEVGDASLLKL
RKILFGGCFIKPYGLGNLGDANLEAWPKSAKLLISKYGKAKLVVPSHSEAGDASLLKL
KKILFGGCFVKPDGLGNLGDANLEAWPKSAKILMSKYGKAKLVVSSHSEIGDASLLKR
KKILFGGCFVKPDGLGNLGDANLEAWPKSAKILMSKYGKAKLVVSSHSEIGDASLLKR

TLEQAVKGLNESKKPSKPSN
TLEQAVKGLNESKKPSKLSN
TLEQAVKGLNESKKPSKPSN
TWEQAVKGLNESKKPSQPSN
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Green highlites: Different amino acid in IMP-5 compared to IMP-1 and/or -4
Pink highlites: Different amino acid in IMP-5 compared to IMP-8
Blue highlites: Different amino acid in IMP-5 compared to IMP-1, -4 and -8

Table 2. Summary of tests performed to characterize the MßL-producing *P. aeruginosa* from SENTRY site #115 in Mexico.

	MßL Etest (µg/ml)		MßL disk approximation							
Case #	Isolate #	Imipenem	IMI+EDTA	IMI+EDTA	MER+EDTA	IMI+MPA	MER+MPA	CAZ+MPA	Ribo/PFGE	MßL
1	1686D	>256	8	-	-	+	-	+	258.259.7/A	IMP-5
2	2058C	48	8	-	-	-	-	+	258.259.7/A1	IMP-5
3	10639A	48	8	-	-	-	-	+	258.259.7/A	IMP-5
	1112A	32	4	+	+	-	-	+	ND[a]	-

a. ND = Not done

CONCLUSIONS

- This study constitutes the first report of clonal dissemination of MßL-producing *P. aeruginosa* isolates in a Mexican medical center.
- This is also the first report of a IMP-5 producing *P. aeruginosa*. The only other report of IMP-5 was from an *Acinetobacter baumannii* strain isolated in Portugal.
- Continued surveillance and careful testing of multidrug-resistant *P. aeruginosa* and other common Gram-negative pathogens is imperative to monitor the spread and the epidemiology of this potent resistance mechanism.

SELECTED REFERENCES

Clinical and Laboratory Standards Institute. (2005) *Performance Standards for Antimicrobial Susceptibility Testing. Document M100-S15*. Wayne, PA:CLSI.

DaSilva GJ, Correia M, Vital C, Ribeiro G, Sousa JC, Leitao R, Peixe L, Duarte A (2002) Molecular characterization of bla_{IMP-5}, a new integron-borne metallo-ß-lactamase gene from an *Acinetobacter baumannii* nosocomial isolate in Portugal. *FEMS Microbiology Letters* 215:33-39.

Jones RN, Biedenbach DJ, Sader HS, Fritsche TR, Toleman MA, Walsh TR (2005) Emerging epidemic of metallo-ß-lactamase-mediated resistances. *Diagnostic Microbiology and Infectious Disease* 51:77-84.

National Committee for Clinical Laboratory Standards. (2003) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Document M7-A6.* Wayne, PA:NCCLS.

Senda K, Arakawa Y, Ichiyama S, Nakashima K, Ito H, Oshuka K, Shimokata K, Kato N, Ohta M (1996) PCR detection of metallo-ß-lactamase gene (bla_{IMP}) in gram-negative rods resistant to broad-spectrum ß-lactams, *Journal of Clinical Microbiology* 34:2909-2913.