Dissemination of IMP-Type Metallo-ß-lactamases among *P. aeruginosa* and Emergence of VIM-2 in *Klebsiella oxytoca* in Mexico: Report from the SENTRY Antimicrobial Surveillance Program

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ABSTRACT

Background:
Metallo-ß-lactamase (MBL) producing pathogens are relatively rare in North America (NA); however, IMP-15 & -18 and VIM-2, & -7 producing *P. aeruginosa* (PSA) have been described. We characterized MBL-producing strains isolated in a Mexican hospital.

Materials and Methods:
A total of 28 *P. aeruginosa* (PSA) and 6 *Klebsiella* spp. strains resistant (R) to carbapenems were screened for MBL production by Etest and disk approximation test (DA), and further characterized by PCR and sequencing. MBL-producing isolates were evaluated for clonality by ribotyping/PFGE. One strain of each molecular type had integrons content sequenced.

Results:
20 PSA and one *K. oxytoca* (KOX) isolate were MBL screen-positive. All PSA demonstrated a multidrug-R phenotype (S only to polymyxins) and were bla_{IMP} positive by PCR; but KOX yielded a bla_{VIM} product. PSA strains were grouped into 5 epidemic clusters, 3 clusters harbored bla_{IMP-15}, while strains from the other 2 clusters carried bla_{IMP-18}. bla_{IMP-15} was associated with *qac*H, *aac*A4, *aad*A1 and bla_{OXA-2} on a class 1 integron, while bla_{IMP-18} integron harbored *aac*A1. bla_{VIM-2} along with *aac*A7 were present on a class 1 integron in KOX.

Organism	MBL	Ribotype/PFGE (no. of strains)	Year of isolation
PSA	IMP-15	259.7/A (3)	2004
		1421.2/C (6)	2005, 2006
		1421.2/F (1)	2006
	IMP-18	566.6/D (8)	2004,2006
		566.6/E (2)	2004
KOX	VIM-2	Not done	2006

Conclusions:
IMP-15 and IMP-18-producing PSA have become endemic and MBL-producing ENT has emerged in this hospital in Mexico. Our results highlight the importance of screening for MBL-producing strains in USA hospitals since IMP-15 and -18 producing PSA with origins in Mexico have already been imported into the USA.

INTRODUCTION

Carbapenems remain the cornerstone of antimicrobial therapy for serious nosocomial infections caused by many antimicrobial-resistant Gram-negative pathogens. However, in the recent years resistance to carbapenems has emerged due to several mechanisms. *Pseudomonas aeruginosa* can become resistant to imipenem or meropenem by over-production of AmpC ß-lactamases associated with loss of outer membrane porin proteins and/or up-regulation of efflux pumps. The production of carbapenemases (serine or metallo--lactamases [MßL]), can also confer high-level resistance to carbapenems.

Acquired carbapenem resistance due to MßL production has increased rapidly in Asia, Europe and Latin America, but remains relatively rare in North America and particularly in the United States (USA). Only five MßL-types have been reported from the USA (VIM-2, -7 and IMP-15, -18 in *P. aeruginosa* and a VIM-like enzyme in *Klebsiella pneumoniae*). Interestingly, both IMP-type producing strains were isolated from patients with treatment history or recent travels in Mexico.

We describe here the characterization of IMP- and VIM-type MßL producing strains isolated in a Mexican hospital obtained through the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial isolates: In the years 2004-2006, the SENTRY Program received 128 *P. aeruginosa* and 167 *Klebsiella* spp. consecutively collected (prevalence mode) isolates and 49 carbapenem-resistant referral *P. aeruginosa* isolates as a participant medical center in Mexico. These SENTRY Program isolates were collected from bloodstream, respiratory tract, 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 Vitek Systems (bioMérieux; Hazelwood, Missouri, USA), where necessary.

Susceptibility testing: All isolates were susceptibility tested against more than 25 antimicrobials by broth microdilution procedure described by the Clinical and Laboratory Standards Institute (CLSI; 2006) using validated panels manufactured by TREK Diagnostics (Cleveland, Ohio, USA). Interpretations of susceptibility testing results were by CLSI (2007) criteria. *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were concurrently tested for quality assurance.

Screening for carbapenemases: All *P. aeruginosa* isolates resistant to imipenem or meropenem (MIC, ≥16 µg/ml) and ceftazidime (MIC, ≥32 µg/ml), and Enterobacteriaceae isolates with reduced susceptibility to imipenem and meropenem (MIC, ≥2 µg/ml) were screened for production of carbapenemases by MßL Etest strips (*P. aeruginosa*) (AB BIODISK, Solna, Sweden) or disk approximation techniques (Enterobacteriaceae). Disk approximation screen tests for MßL detection were performed using imipenem, meropenem and ceftazidime as substrates and EDTA as well as 2-mercaptopropionic acid as inhibitors. Isolates exhibiting positive MßL-screen test results were evaluated for the presence of bla_{IMP}, bla_{VIM} and bla_{SPM-1} using PCR primers as previously described. Class 1 Integrons carrying MßL genes were amplified to study their genetic context.

Gene sequencing: PCR products for the MßL genes and integrons were sequenced using a Sanger-based dideoxy sequencing strategy involving the incorporation of fluorescent-dye-labeled terminators into the sequencing reaction products. Sequences obtained were analyzed using Lasergene® software (DNAStar, Madison, Wisconsin) and compared to available sequences via NCBI BLAST search to determine the enzyme type.

Molecular typing: MßL-producing *P. aeruginosa* isolates were typed using the Riboprinter™ Microbial Characterization System (Qualicon Inc., Wilmington, Delaware, USA) followed by pulsed-field gel electrophoresis (PFGE) using a CHEF DRII apparatus (Bio-Rad, Hercules, California, USA) to evaluate clonality.

RESULTS

- Among isolates tested for MßL production (28 *P. aeruginosa* and 6 *Klebsiella* spp.), 20 *P. aeruginosa* isolates yielded products with generic bla_{IMP} primers; while one *K. oxytoca* isolate yielded a bla_{VIM} product.
- All *P. aeruginosa* isolates with bla_{IMP} demonstrated a multidrug-resistant phenotype (susceptible only to polymyxins). The *K. oxytoca* isolate was susceptible to cefepime, aztreonam, ciprofloxacin, tigecycline, aminoglycosides, trimethoprim/sulfamethoxazole, and the polymyxins (Table 1).

Table 1. Antibiotic susceptibilities of metallo-ß-lactamase producing isolates from the medical center in Mexico and Lexington, Kentucky.

	Organism (no. isolates, MIC range µg/ml)				
	P. aeruginosa		*K. oxytoca* (1)	*E. cloacae*	
Antimicrobial agent	Mexico (20)	Kentucky (1)		3876A	1068D
Imipenem	>8	>8	>8	4	8
Meropenem	>8	>8	>8	1	0.25
Piperacillin/tazobactam	32->64	>64	32	64	64
Aztreonam	≥16	16	0.5	≤0.12	>16
Cefepime	>16	>16	4	2	2
Gentamicin	8->8	>8	≤2	≤2	≤2
Amikacin	>32	>32	≤4	4	8
Tobramycin	>16	>16	4	8	16
Ciprofloxacin	>4	>4	1	≤0.03	0.5
Trimethoprim/sulfamethoxazole	>2	>2	≤0.5	1	>2
Polymyxin B	≤0.5-1	≤0.5	≤0.5	>4	≤0.5
Colistin	≤0.5-2	≤0.5	≤0.5	>4	≤0.5
Tetracycline	>8	>8	4	≤2	>8
Tigecycline	>4	>4	0.25	0.5	0.5

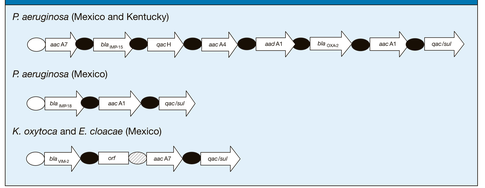
Table 2. Characterization of metallo-ß-lactamase producing strains from a medical center in Mexico.

Organism		Ribotype/PFGE	MßL screen		
(no. of isolates)	MßL	(no. of strains)	Positive	Negative[a]	Year(s) of isolation
P. aeruginosa (20)	IMP-15	259.7/A (3)	3	0	2004
		1421.2/C (6)	5	1	2005, 2006
		1421.2/F (1)[b]	1	0	2006
	IMP-18	566.6/D (8)	7	1	2004,2006
		566.6/E (2)	0	2	2004
K. oxytoca (1)	VIM-2	Not done	1	0	2006
E. cloacae (2)[c]	VIM-2	A (1)	1	0	2005
		B (1)	1	0	2006

a. Includes non-interpretable results; no true negative tests were observed.
b. Ribotype and PFGE pattern identical to that of the IMP-15-producing *P. aeruginosa* strain reported in Lexington, Kentucky (Morita et al., 2006).
c. Isolates identified after the submission of the abstract.

- IMP-producing *P. aeruginosa* strains were grouped into 5 epidemic clusters based on molecular typing results. Three clusters harbored bla_{IMP-15}, while bla_{IMP-18} was identified in strains belonging to the other two clusters (Table 2).
- Integrons of isolates with the same bla_{IMP}-type and distinct molecular typing patterns were identical, indicating polyclonal dissemination of the bla_{IMP}-integrons (Figure 1).
- bla_{IMP-15} was associated with *qac*H, *aac*A4, *aad*A1, *aad*A1, bla_{OXA-2} and *aac*A1 genes on a class 1 integron, identical to In95 (GenBank #EF184216). Integron carrying bla_{IMP-18} harbored *aac*A1 and was identical to the published In96 sequence (GenBank #EF184215; Figure 1).
- A *P. aeruginosa* strain with a ribotype identical to strains evaluated in the present study (1421.2) and the integron structure described here was previously isolated in Lexington, Kentucky in late 2005 (Table 2; see references).
- bla_{VIM-2} was located in the first position of a class 1 integron in the *K. oxytoca* strain. bla_{VIM-2} was followed by a 400bp DNA fragment designated *orf* (Figure 1). This fragment, which has no homology with previously described DNA sequences, was embedded in a gene cassette and followed by *aac*A7.
- After submission of the abstract, we identified two additional *Enterobacter cloacae* strains with distinct molecular typing patterns and harboring bla_{VIM-2} in the same medical center (Tables 1 and 2). Integron analysis revealed identical structure to that of the class 1 integron found in *K. oxytoca* isolate producing VIM-2.

Figure 1. Schematic representation of the structures of the class 1 integron harboring IMP-15, IMP-18 and VIM-2 metallo-ß-lactamases isolated in Guadalajara, Mexico and Lexington, Kentucky. Genes are represented as open arrows, direction of the arrows indicate the direction of transcription of the resistance markers. Filled ellipses represent 59 base elements. Open ellipses represent arrl1 sites, striped ellipse indicates attC site.



CONCLUSIONS

- Multiple IMP-15 and IMP-18-producing *P. aeruginosa* clusters have become endemic in this Mexican hospital.
- Our results indicate that bla_{VIM-2} has also emerged and disseminated among Enterobacteriaceae species in this institution.
- This investigation highlights the importance of screening for MßL-producing strains in USA hospitals since IMP-15 and -18 producing *P. aeruginosa* with origins in Mexico have already been imported into the USA (Kentucky).
- Worldwide antimicrobial resistance surveillance programs, such as the SENTRY Program, play an important role in identifying the emergence and tracking the movement of resistance mechanisms around the world. This information should help guide therapeutic decisions and modify infection control practices.

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