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Characterization of Mobile Elements Carrying Metallo-ß-Lactamase Genes, *bla*$_{IMP-1}$, *bla*$_{IMP-16}$, *bla*$_{SPM-1}$, *bla*$_{VIM-2}$ from Latin American Medical Centres: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: *bla*$_{SPM-1}$ and *bla*$_{IMP-1}$-like metallo-ß-lactamase (MßL) genes have been reported from Latin America (LA) but limited information has been generated on the dissemination of these genes. As part of the SENTRY Program, *Pseudomonas* spp. (PSP) and *Acinetobacter* spp. (AC) were analyzed for MßL genes and their mobile elements.

Methods: Isolates from Brazil (57 PSP and 7 AC), Venezuela (4 PSP and 1 AC), Chile (1 PSP), Mexico (4 PSP) and Argentina (17 AC) were biochemically analyzed by imipenem (IMP) hydrolysis ± EDTA (20mM). All isolates were screened for *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{SPM}$ using generic primers. Isolates carrying MßL genes had their gene context analyzed by PCR of the integrase gene and *sul/qacE*Δ1, as well as, other MßL gene cassettes (*accA4* and *aadA1* genes).

Results: Four isolates, one from Chile and three from Venezuela, carried *bla*$_{VIM-2}$. Eight isolates carried *bla*$_{IMP-1}$ (7 AC and 1 PSP from Brazil), one *bla*$_{IMP-16}$ (*P. aeruginosa* [PSA] from Brazil) and 10 *bla*$_{SPM-1}$ (PSA from Brazil). All *bla*$_{IMP-1}$ isolates contained *aadA1* as part of the Class 1 cassette. The *bla*$_{IMP-1}$ carrying class 1 integrons showed identical gene cassette arrangement. An ORF with 696-bp long was found downstream the *bla*$_{IMP-1}$ and the predictive protein showed 83.6% identity with AAC(6')-Ib. The genetic context of *bla*$_{SPM-1}$ showed that in 2 of 10 isolates *bla*$_{SPM-1}$ was in a different genetic context compared to that previously reported (ICAAC, 2002).

Conclusions: These data suggests MßLs are likely to be a significant clinical problem in LA, particularly in Brazil.

BACKGROUND

Carbapenems, mainly imipenem and meropenem, are potent agents for the treatment of infections due to multiresistant *Pseudomonas* spp. and *Acinetobacter* spp. These drugs have considerable ß-lactamase stability and overall have the broadest spectrum of activity compared with other ß-lactams. Resistance to imipenem in *P. aeruginosa* is usually low level (MIC, 8 to 32 μg/ml) and due to OprD porin loss. Resistance by this mechanism depends on continued expression of the chromosomal Amp C ß-lactamase. Low level resistance to both imipenem and meropenem can also arise via overexpression of the efflux pumps. High-level resistance to these compounds (MIC, >32 μg/ml) is still uncommon in *P. aeruginosa* and *Acinetobacter* spp., but can be caused by the production of class B ß-lactamases.

Metallo-ß-lactamases (MßLs) are usually zinc dependent. These MßL's plus metal ions co-ordinate water molecules that serve as nucleophiles, which attack and break the cyclic amide bond of the ß-lactam ring, rendering the ß-lactam compound biologically inactive. The enzyme types are IMP, VIM and the recently described SPM ß-lactamase. As part of the SENTRY Program, *Pseudomonas aeruginosa* and *Acinetobacter* spp. were analyzed for MßL genes and their mobile elements.

MATERIAL & METHODS

Study design. The SENTRY Program monitors pathogen frequency and antimicrobial resistance patterns of nosocomial and community-acquired infections through > 90 sentinel hospitals worldwide. In Latin America, 10 medical centers were distributed throughout six countries: Brazil (Sao Paulo, Florianopolis, Porto Alegre, and Brasilia); Argentina (Buenos Aires and Rosario); Chile (Santiago, two centers); Mexico (Mexico City); and Venezuela (Caracas). Among other selected pathogens, *Pseudomonas* spp. and *Acinetobacter* spp. strains resistant to imipenem (MIC, ≥ 16 μg/ml), meropenem (MIC, ≥ 16 μg/ml) and ceftazidime (MIC, ≥ 32 μg/ml) have been routinely examined for antimicrobial resistant genes through the amplification and sequencing of the variable region of class 1 integrons.

Susceptibility testing. All isolates collected in the SENTRY Program were susceptibility tested by the reference broth microdilution method as described by the NCCLS. Antimicrobial agents were obtained from the respective manufacturers and quality control was performed by concurrent testing of *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 29213, and *E. faecalis* ATCC 29212.

Automated ribotyping. MßL producing isolates were ribotyped using the Riboprinter Microbial Characterization System® (Qualicon, Wilmington, Delaware). In brief, this automated process includes bacterial cell lysis, cleavage of DNA using the restriction enzime *Eco*RI, size separation using gel electrophoresis and modified Southern blotting. Results were analyzed by the Riboprinter and isolates were considered to have the same ribotype if the similarity coefficient was ≥ 0.93.

MATERIALS AND METHODS (Continued)

Phenotypic detection of ß-lactamases. Production of MßL was screened by the disk approximation test. A 100mm Mueller-Hinton agar plate was inoculated using a 0.5 McFarland suspension from fresh cultures. Imipenem, meropenem, and ceftazidime disks were aligned around disks contained either EDTA (750 μg) or 2-MPA (360 μg). The appearance of either an enhanced or a phantom zone between the carbapenems and/or ceftazidime and either one of the disks containing a MßL inhibitor 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.

Antimicrobial resistance gene screening. Among other selected pathogens, *Pseudomonas* spp. and *Acinetobacter* spp. strains having positive MßL screens have been routinely examined for antimicrobial resistant genes through the amplification and sequencing of the variable region of class 1 integron. Oligonucleotide primers targeting to conserved regions of *bla*$_{VIM}$, *bla*$_{IMP}$ and *bla*$_{SPM}$ genes were initially used to determine the genetic basis of the resistance. Additional primers designed for the 5'- conserved segment (CS) and 3'CS regions of class 1 integrons were used to amplify the integron resident in those strains. Primers for 5'CS and 3'CS of class 1 integron, as well as primers for the gene cassette yielded PCR products that were sequenced on both strands using DuPont Automated systems. Nucleotides sequences and their deduced protein products, alignments and phylogenetic relationships were determined using the Lasergene software package (DNASTAR, Madison, WI).

COMMENTS

- A total of 1,408 strains of *Pseudomonas* spp. and *Acinetobacter* spp. from Latin American medical sites were submitted to the SENTRY Program in 2002. Among those, 263 (18.7%) strains were resistant to ceftazidime, imipenem and meropenem (CAZ-IMI-MER-resistant strains), and therefore, selected for antimicrobial resistance gene analysis.
- The medical center 048 (Sao Paulo, Brazil) showed the highest prevalence of CAZ-IMI-MER-resistant strains (49.3%), followed by medical center 039 located in Buenos Aires, Argentina (24.3%) (Table 1).
- Among the CAZ-IMI-MER-resistant strains, 91 (34.6%) showed positive results for MßL phenotypic tests. The presence of MßL genes was confirmed by PCR and sequence analysis in 22 of those strains (24.2%; Figure 1).
- Four MßL types were detected in Latin America: SPM-1 (41%); IMP-1 (36%); VIM-2 (18%) and IMP-16 (5%; see Figure 1). However, 77% of the MßL-producing isolates were found in one medical center and all isolates carrying *bla*$_{IMP-1}$ (8/22) and *bla*$_{SPM-1}$ (9/22) were found in this hospital.
- VIM-2 producing isolates were observed in Santiago, Chile (one strain) and Caracas, Venezuela (three strains). Additionally, one isolate harboring a new bla$_{IMP}$-variant gene, namely *bla*$_{IMP-16}$, was detected in Brasilia, Brazil.
- *bla*$_{IMP-16}$, and *bla*$_{SPM-1}$ were found only in *P. aeruginosa* isolates. *bla*$_{VIM-2}$ was identified in *P. aeruginosa* and *P. fluorescens*. *bla*$_{IMP-1}$ was detected in both *Acinetobacter* and *P. fluorescens* isolates.
- Molecular typing results showed that *P. aeruginosa* strains carrying *bla*$_{SPM-1}$ had an identical ribogroup (data not showed); meanwhile, five ribotype patterns were detected among 7 *Acinetobacter* spp. isolates carrying *bla*$_{IMP-1}$ (Figure 2).
- All the Latin America MßL genes were harbored in class 1 integrons and all *bla*$_{IMP-1}$ carrying integrons showed an identical gene cassette arrangement. The *bla*$_{VIM-2}$ containing integrons detected in isolates from Chile and Venezuela also showed identical gene cassettes (Figure 3).
- The *bla*$_{IMP-1}$ integron contained a new ORF which was 696-bp long. Its putative protein contains 184 amino acids and showed greatest identity (83.6%) with the previously described AAC(6')-Ib. This new ORF was followed by the *aadA1* gene cassette, which was similar to those previously described. The integron also contained the normal 3'-CS.

RESULTS

Table 1. *P. aeruginosa* and *Acinetobacter* spp. resistance rates for imipenem, meropenem, and ceftazidime listed by country and medical center (SENTRY Program, Latin America, 2002).

Country	% of resistant strains[a]	Medical center	% (no. resistant strains[a]/total)
Argentina	16.1	039	24.8% (36/145)
		040	6.2% (8/129)
Brazil	25.4	046	10.2% (18/177)
		048	49.3% (150/304)
		057	0.8% (1/121)
		101	14.3% (21/147)
Chile	1.9	042	0.0% (0/66)
		043	2.7% (4/148)
Colombia	10.0	044	10.0% (1/10)
Mexico	17.4	045	17.4% (12/69)
Venezuela	13.0	049	13.0% (12/92)

a. Number of strains resistant to imipenem (MIC, ≥ 16 μg/ml), meropenem (MIC, ≥ 16 μg/ml) and ceftazidime (MIC, ≥ 32 μg/ml).

Figure 1: Schematic representation of the MßL-producing strains found in Latin America.

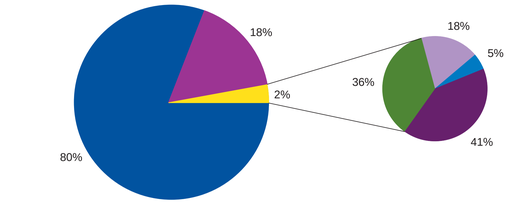
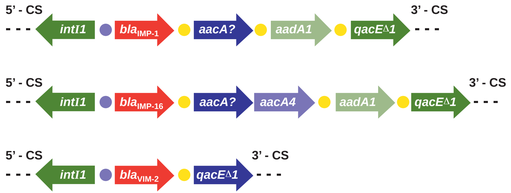


Figure 2: Ribotyping of seven *Acinetobacter* spp. isolates carrying *bla*$_{IMP-1}$ from Sao Paulo, Brazil. These strains were clustered in five ribogroups. Two pairs of isolates shared the same ribogroup: isolates B9043 and B695 (ribogroup 105-815-S-4) and isolates B694 and B696 (ribogroup 258-105-S-2).

Figure 3: Schematic representation of the class 1 integron containing *bla*$_{IMP-1}$, showing the same gene cassette arrangements in *P. fluorescens* and *Acinetobacter* spp. isolated in Sao Paulo, Brazil. The class 1 integron containing *bla*$_{IMP-16}$ (Brasilia) and *bla*$_{VIM-2}$ (Chile and Venezuela) are also displayed. The horizontal arrows indicate the gene cassettes and their respective translation orientation. Blue and yellow circles represent the *attI1* site and the 59-be, respectively.



CONCLUSIONS

- Four different MßLs (SPM-1, VIM-2, IMP-1, IMP-16) were detected and characterized by the SENTRY Program among *Pseudomonas* spp. and *Acinetobacter* spp. strains isolated from Latin American medical centers.
- The prevalence of MßL-producing strains is extremely high in some of the medical centers evaluated by the SENTRY Program.
- The *bla*$_{IMP-1}$ containing integron was found in *Acinetobacter* spp. strains with five distinct ribotyping patterns and in one *P. fluorescens* strain, which demonstrates the mobility of this integron.
- The presence of mobile elements simultaneously carrying MßL and aminoglycoside resistance genes raises the question of whether the clinical use of broad-spectrum antimicrobial agents may increase the selective pressure for such multi-resistant isolates, and also the transfer of these integrons into susceptible hosts. These events may be enhanced by compromised infection control practices in some locations.

SENTRY PARTICIPANT GROUP LATIN AMERICA - 2002

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