Characterization of Novel Insertion Sequences in *bla*$_{IMP-1}$ Integrons Among Gram-Negative Clinical Isolates in São Paulo, Brasil

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AMENDED ABSTRACT

Background: Metallo-ß-lactamases (MßL) are encoded by genes commonly harbored on mobile elements that can spread to different genetic structures and strains. In this study we characterize the genetic context responsible for *bla*$_{IMP-1}$ dissemination among clinical isolates in a Brazilian hospital.

Methods: Primers targeting to the 5'-conserved sequence (5'-CS) and 3'-CS of class 1 integrons were used to amplify the *bla*$_{IMP-1}$–containing region. Upstream sequences (US) were amplified by PCR using a novel degenerate primer approach. Sequencing was performed on both strands using DuPont Automated systems. Southern blot experiments were performed following the manufacturer's instructions and pulsed-field gel electrophoresis (PFGE) according to standard procedures.

Results: *bla*$_{IMP-1}$ was detected in one *P. aeruginosa* (PSA) and seven *Acinetobacter* spp. (ASP) strains. The *bla*$_{IMP-1}$ was harbored in the same integron structure in all 8 strains, with variation only in the downstream region of the integrase. In the PSA strain, the integron showed the expected 25-base pair inverted repeated (25-bp IR$_i$). In the ASP strains, the 25-bp IR$_i$ was replaced by an insertion sequence. The ASP strain 48-501 showed a distinct US, which was closely related to the terminal repeat of IS*26*. The remaining ASP strains possessed identical US, which did not show homology with any sequence in the GenBank. These 5 ASP strains showed 4 PFGE patterns.

Conclusions: The integron found in the PSA was likely to be the progenitor structure responsible for the *bla*$_{IMP-1}$ dissemination. Thus, this genetic structure was later horizontally disseminated among non-clonal related ASP clinical isolates. This report shows the plasticity of these genetic structures and the great capability of resistance markers dissemination.

BACKGROUND

Since the first report of acquired metallo-ß-lactamases (MßL; IMP-1) in Japan, IMP-type enzymes have been reported from many countries worldwide. The genes encoding MßLs can be plasmid- or chromosomally-mediated, and most are associated with integrons. Integrons are able to capture genes that are part of gene cassettes via a specific recombination event between two sites, one in the integron and one in the cassette. Integrons include a receptor site, *attI1*, where captured genes are integrated, together with an adjacent sequence coding for recombinase, IntI1. Uncaptured gene cassettes exist in their free forms as circular molecules consisting only of one open reading frame (ORF) and a 59-base element (59-be) situated downstream. Integration of these gene cassettes involves IntI1-catalysed site-specific recombination between the integron *attI1* site and the 59-be recombination site, associated with the incoming gene cassette. Each gene cassette has a unique 59-be.

Integrons have been primarily found within complete or truncated derivatives of the Mu-like transposon Tn*402*. This transposon resides in broad-host-range plasmids, or within Tn*21* or Tn*21*-like transposons. Variants of Tn*21* may contain different or additional gene cassettes and/or insertion sequences (IS). The high mobility of the gene cassettes conferred by the integrase gene, coupled with the mobility of transposons harboring these genes, facilitates the dissemination of these genes within nosocomial human pathogens.

In this study, we characterized the genetic structures responsible for *bla*$_{IMP-1}$ dissemination in clinical strains of *P. aeruginosa* and *Acinetobacter* spp. collected in a Brazilian hospital participating in the SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial strains. Seven *Acinetobacter* spp. and one *P. aeruginosa* were recovered between June 2001 and September 2002 from patients hospitalized in a tertiary care university hospital located in São Paulo, Brazil, and submitted to the SENTRY Program. These isolates showed resistance to ceftazidime (MIC, ≥ 32 µg/ml), imipenem (MIC, ≥ 16 µg/ml) and meropenem (MIC, ≥ 16 µg/ml) and *bla*$_{IMP-1}$ was detected in all strains by PCR.

PCR and random flanking primer 2 step PCR. In order to further characterize the surrounding *bla*$_{IMP-1}$ genetic context in the studied strains, primers targeting the 5'-conserved sequence (5'-CS) and 3'CS regions of class 1 integrons were used to amplify the *bla*$_{IMP-1}$. Furthermore, DNA sequences upstream of the integrase gene were amplified through a random primer PCR approach (Figure 1). Two nested biotinylated primers designed to anchor within a previously detected DNA sequence downstream from the integrase gene of class 1 integrons were used as reverse primers. Four different random primers designed with a novel sequence tag of 24-bp followed by seven randomly assigned bases were used as forward primers.

Step 1. Consisted of four PCR reactions using a biotinylated primer #1 and one of each of the four random primers. The PCR products were then incubated with Dynal streptavidin linked beads, washed and denaturated with 0.1M NaOH. The beads were then separated using a magnetic rack and the supernatant neutralized with 0.2M HCl and diluted prior to use as template in the second step PCR.

Step 2. Consisted of amplification using primers complementary to the random primers and other biotinylated primer #2 anchored within the known integron DNA sequence. The PCR products from step 2 were run in an electrophoresis gel, purified and then sequenced.

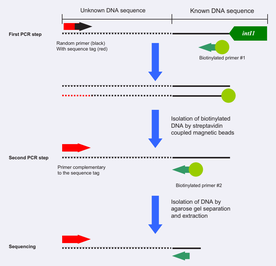
DNA sequencing. PCR products were sequenced on both strands using the Perkin Elmer Systems 377 DNA Sequencer (Advanced Biotechnology Centre, London, UK). The DNA sequences were found to overlap each other, and were assembled to produce the respective contiguous sequences of each strain containing an integron.

Automated ribotyping. MßL–producing isolates were ribotyped using the Riboprinter Microbial Characterizaton System® (Qualicon, Wilmington, Delaware). Overnight cultures were treated with lysis buffer and placed into the automated system. 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.

Pulsed-field gel electrophoresis (PFGE). *bla*$_{IMP-1}$–carrying strains showing the same ribotype were submitted for PFGE analysis. Briefly, genomic DNA was immobilized in agarose blocks and digested with the restriction enzyme *Sma*I (Life Technologies, Gaithersburg, Maryland). Electrophoresis was performed on the CHEF-DR III (BioRad, Richmond, California), under the following conditions: 0.5 x TBE, 1 % agarose, 13°C , 200V, for 23-24 h with the switch time ramped from 5 to 60-90 seconds. Gels were stained with ethidium bromide, distained in distilled water, and photographed under UV light. Isolates with identical profiles were assigned to the same type. Isolates that differed by one to six bands were assigned as subtypes. Strains were considered unique by PFGE if they differed by more than six bands.

Computer sequence analysis. Nucleotide sequence comparisons were performed using software obtained from internet resources (http://www.ebi.ac.uk/fasta33/). Nucleotide sequences and the deduced protein products, alignments and phylogenetic relationships were determined using the Lasergene software package (DNASTAR, Madison, WI, USA).

Figure 1. Strategy used for amplification of unknown DNA sequences upstream of the integrase gene among *bla*$_{IMP-1}$–containing class 1 integron.



RESULTS

- The *bla*$_{IMP-1}$–containing integron showed an identical gene cassette arrangement in all isolates analyzed. This integron consisted of the integrase gene [*intI1*], a cassette integration site [*attI1*], the *bla*$_{IMP-1}$ MßL, an aminoglycoside resistance gene [*aac(6')-31*], an *aadA1*, and the quaternary ammonium resistance gene [*qacE*Δ*1*] in the 3'CS region (Figure 2).
- The integron found in the studied strains showed a complete integrase gene [*intI1*], suggesting that the four gene cassettes found in this integron have been mobilized independently.
- The 25-bp IR$_i$, which is usually found downstream of the integrase in class 1 integrons, was observed in the 48-12346 *P. aeruginosa* strain, but not in the *Acinetobacter* spp. strains (Figure 2).
- The 48-501 *Acinetobacter* spp. strain showed the IS*1006.1* downstream of the integrase gene, which was closely related to the terminal repeat of IS*26*. The *tnp1006* was observed further downstream (Figure 2c).
- The *bla*$_{IMP-1}$–containing integron, found in the remaining six *Acinetobacter* spp. strains, showed identical DNA sequences downstream of the integrase gene (Figure 2b), which did not show homology with any sequence previous available in the GenBank. These six strains showed four distinct PFGE patterns (Table 1).

Figure 2. Schematic representation of the *bla*IMP-1–containing class 1 integron dissemination among *P. aeruginosa* strain and seven *Acinetobacter* spp. strains. The integron found in the 48-12346 *P. aeruginosa* strain (Figure 2a) was likely to be the progenitor genetic structure responsible for the dissemination. Subsequently genetic event inserted a DNA sequence just downstream of the integrase gene in the 48-12346 *P. aeruginosa*, which created the structure found in the *Acinetobacter spp.* strains (Figure 2b). Later, other genetic event inserted the Tn*1006*, and then the structure found in the 48-501 *Acinetobacter* spp. strain was formed (Figure 2c).

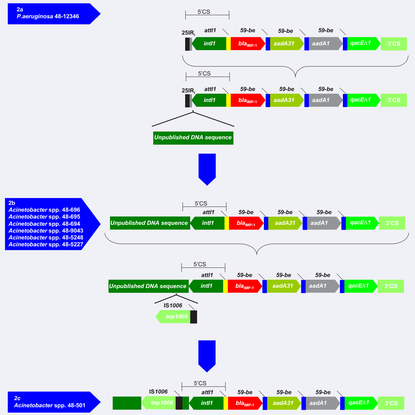


Table 1. Demographics and molecular typing results of *bla*$_{IMP-1}$–producing strains isolated in a Brazilian medical center.

Strains	Strain #	Culture date	Source	Ribotyping	PFGE[a]
A. baumannii	48-694	05/28/02	SSTI[b]	258.105.2	A
A. baumannii	48-696	06/04/02	SSTI[b]	258.105.2	A
A. baumannii	48-9043	06/22/01	BSI[c]	105.815.4	B
A. baumannii	48-695	06/02/02	SSTI[b]	105.815.4	B1
A. baumannii	48-501	05/06/01	BSI[c]	252.43.4	C
Acinetobacter spp.	48-5227	08/14/02	LRTI[d]	258.148.6	D
Acinetobacter spp.	48-5248	09/28/02	LRTI[d]	258.148.2	E
P. aeruginosa	48-12346	07/23/01	BSI[c]	NA[e]	NA[e]

a. PFGE - Pulsed-field gel electrophoresis.
b. Skin and soft tissue infection.
c. Bloodstream infection.
d. Lower respiratory tract infection.
e. NA - Not applicable.

CONCLUSIONS

- The *bla*$_{IMP-1}$–containing integron found in the 48-12346 *P. aeruginosa* strain (Figure 2a) was likely to be the progenitor genetic structure responsible for the dissemination of *bla*$_{IMP-1}$ among clinical strains in this Brazilian hospital.
- A further genetic event inserted a DNA sequence just downstream of the integrase gene in the 48-12346 *P. aeruginosa*. This new structure was later horizontally disseminated among *Acinetobacter* spp. strains with distinct ribotype and PFGE patterns (different clones; Table 1 and Figure 2b).
- The *bla*$_{IMP-1}$–containing integron found among non-clonal related *Acinetobacter* spp. strains (Figure 2b) was possibly located in a large broad-host-range plasmid, which could explain horizontal dissemination.
- A subsequent genetic event inserted the Tn*1006*, which was detected in the 48-501 *Acinetobacter* spp. strain (Figure 2c).
- The results of this study strongly suggest that the *bla*$_{IMP-1}$–containing integron has disseminated among distinct clones of clinical isolates in a single Brazilian hospital. Our results also show the plasticity of these genetic structures and great capability of dissemination of one integron harboring a MßL and aminoglycoside resistance genes.

SELECTED REFERENCES

Bass L, Liebert CA, Lee MD, Summers AO, White DG, Thayer SG, Maurer JJ. (1999). Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance, in avian *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* 43:2925-2929.

Liebert CA, Hall RM, Summers AO. (1999). Transposon Tn*21*, flagship of the floating genome. *Microbiology and Molecular Biology Review* 63:507-522.

Toleman MA, Biedenbach D, Bennett D, Jones RN, Walsh TR. (2003). Genetic characterization of a novel metallo-ß-lactamase gene, *bla*IMP-13, harboured by a novel Tn*5051*-type transposon disseminating carbapenemase genes in Europe: Report from the SENTRY worldwide antimicrobial surveillance programme. *Journal of Antimicrobial Chemotherapy* 52:583-590.

Walsh TR, Toleman MA, Hryniewicz W, Bennett PM, Jones RN. (2003). Evolution of an integron carrying *bla*VIM-2 in Eastern Europe: Report from the SENTRY Antimicrobial Surveillance Program. *Journal of Antimicrobial Chemotherapy* 52:116-119.