CHARACTERIZATION OF CLASS 1 INTEGRONS CARRYING THE GENES FOR VIM- AND IMP-TYPE METALLO-β-LACTAMASES IN *PSEUDOMONAS AERUGINOSA* STRAINS FROM RUSSIA

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INTRODUCTION AND PURPOSE

The global spread of acquired metallo-β-lactamases (MβLs) has raised serious concern because of their ability to confer resistance of gram-negative bacteria to a broad spectrum of β-lactam antibiotics, including carbapenems. Generally, the MβL genes are carried as parts of the mobile gene cassettes inserted in plasmid- or chromosome-borne integrons. Most of these integrons belong to class 1, but their structures may vary among strains, largely due to rearrangements in the cassette arrays (i.e. insertion or loss of gene cassettes). This study aimed to investigate the genetic context of the MβL-coding genes in Pseudomonas aeruginosa strains from Russia.

MATERIALS AND METHODS

Bacterial strains. For this study, six strains were selected from a group of 45 VIM- and 3 IMP-MβL-producing *P. aeruginosa* isolates collected as part of the National Survey of Resistance of Nosocomial Pahogens in Russian ICUs (RESORT) in 2002-2004. The selected strains were representatives of the 4 clonal groups and were isolated, respectively, in 6 hospitals of 3 geographically distant regions of Russia [4].

PCR for class 1 integrons. The presence of class 1 integrons in each strain was assessed using the PCR with primers specific for the intI1 and *qacEΔ1* genes, which are located, respectively, on the 5' and 3' conserved parts of these integrons, as previously described [2].

Detection of location of MβL genes within the integrons. The PCRs with primer INT/5CS that anneals to the intI1 [3] and reversed primers to the bla_{VIM} or bla_{IMP} genes [4] were used to confirm their linkage within the class 1 integrons.

Determination of genetic structures of MβL-gene-containing integrons. The structures of variable regions of MβL-gene-containing integrons were deduced by amplifying the two overlapping sections of each cassette array separately using the following sets of primers:

- INT/5CS and VIM-BIG-R [4] - for the 5' sections upstream of the bla_{VIM-2};
- VIM-F [4] and INT/3CS [3] - for the 3' sections downstream of the bla_{VIM-2} in the strains 257, 2074 and 3389;
- VIM-F and TniC-rev (5'-GTGGGCGATCTCTGCGAAG-3') - for the 3' sections downstream of the bla_{VIM-2} in the strains 565 and 1913;
- INT/5CS and IMP-BIG-R [4] - for the 5' sections upstream of the bla_{IMP};
- INT/5CS and IMP-BIG-R [4] - for the 5' sections upstream of the bla_{IMP};

The two sections of each integron were then sequenced using the custom designed primers and aligned together to produce a single contig of each MβL-containing integron. Sequencing was performed using an ABI PRISM Big Dye Terminator V3.1 cycle sequencing kit and an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Stafford, TX). The nucleotide sequences were identified by comparison with the sequences of known gene cassettes using the BLAST program available through NCBI (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS AND DISCUSSION

A PCR with primers to 5' and 3' conserved sequences (CSs) of class 1 integrons yielded multiple DNA fragments of >1.4 kb probably reflecting the presence of multiple integrons in each of the strains studied (Fig. 1a). At the same time, PCR using a combination of *intI1*-specific primer with bla_{IMP}- or bla_{VIM}-specific primers gave rise to a single amplification product per strain (Fig. 1b) suggesting that the MβL genes were associated with only one of the integrons carried by each strain.

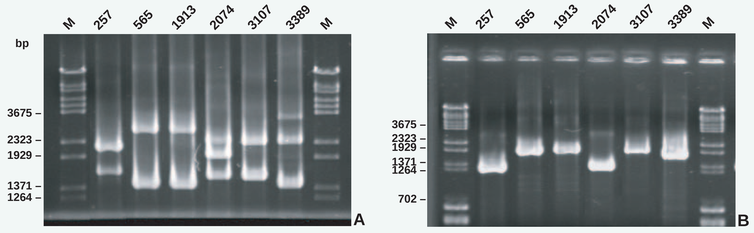

Figure 1. Amplification products obtained with the primers to the 5′ and 3′ CSs of class 1 integrons (A) and primer to the 5′ CS in combination with MβL-gene-specific primers (B)

CONCLUSIONS

In Russian *P. aeruginosa* isolates, the bla_{VIM} and bla_{IMP} gene cassettes are located in the class 1 integrons.

The four new integrons with unique combinations of cassette arrays were described in this study.

The complete identity of integron structures identified in the Russian outbreak strains from this study and the USA outbreak strain 7052 probably suggests the common origin of the bla_{VIM} genes in these strains.

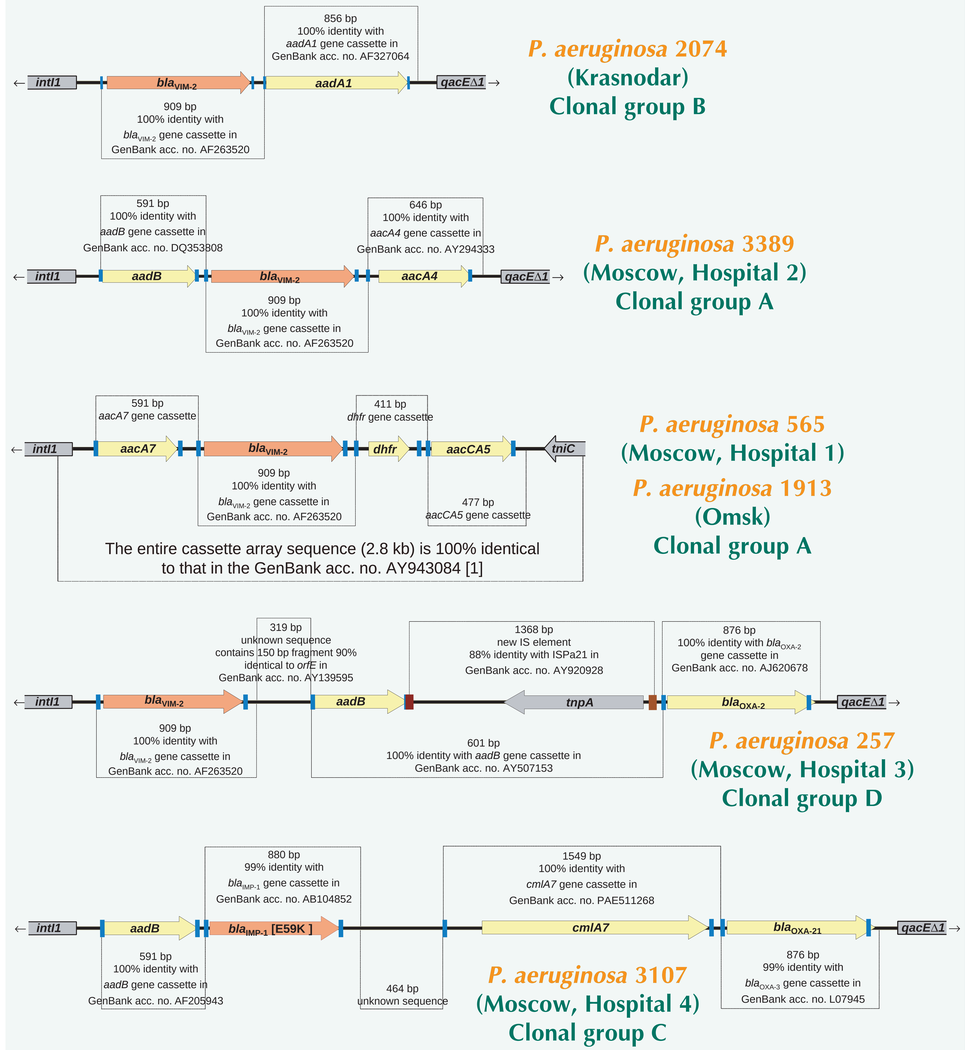

Figure 2. Genetic structures of class 1 integrons carrying the MβL genes.
(▌) symbol indicates the positions of core sites and inverse core sites;
(▮) symbol indicates the positions of IS terminal repeats

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