MOLECULAR CHARACTERIZATION OF METALLO-β-LACTAMASE (MβL)-PRODUCING *PSEUDOMONAS AERUGINOSA* STRAINS FROM RUSSIA

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INTRODUCTION AND PURPOSE

Carbapenems have potent antibacterial activity and are mainly reserved for the treatment of infections caused by multiresistant nosocomial pathogens, including *Pseudomonas aeruginosa*. However, isolates of this species can develop resistance to carbapenems through alteration of outer membrane permeability, activation of efflux systems or acquisition of carbapenem-hydrolyzing β-lactamases. Production of acquired metallo-β-lactamases (MβLs) is probably the most important mechanism of resistance to carbapenems both in terms of its effectiveness and epidemiological significance. The MβL-producing *P. aeruginosa* strains have been reported worldwide but predominantly in South East Asia and Europe. This study aimed to determine the types of MβLs produced by Russian *P. aeruginosa* strains and to assess their possible genetic relationship.

MATERIALS AND METHODS

Bacterial strains. A total of 48 nosocomial isolates of *P. aeruginosa* that were suspected to produce MβLs based on results of EDTA-double-disk synergy test [1] were included in this study. All the isolates were collected as part of the RESORT national surveillance study in 2002-2004. The putative MBL producers were isolated in 6 hospitals located in 3 geographically distant regions of Russia: Krasnodar (Southern Federal District), Moscow (Central Federal District) and Omsk (Siberian Federal District) (Fig. 1).

Detection of bla_{VIM} and bla_{IMP} genes. The genes for VIM- and IMP-type MβLs were detected by real-time PCRs. The consensus primers used in these PCRs were designed to anneal to the conserved regions of bla_{VIM} and bla_{IMP} genes, respectively (Tab. 1). The 25-µl PCR mixtures contained: 0.5µM of each primer, 200µM of each dNTP, 1.5mM $MgCl_2$, 67mM Tris-HCl (pH=8.3), 17mM $(NH_4)_2SO_4$, 0.1% Tween-20, 0.12mg/ml BSA, 8% Glycerol, 2.5U of DiaTaq polymerase (AmpliSens, Russia), 1µl of SYBR Green I (1:1000 dilution in DMSO, BioGene, UK) and 3µl of template DNA prepared by rapid boiling of 2-3 bacterial colonies in 1mM TE buffer. Amplification was performed in a Rotor-Gene 2000 Real-Time Cycler (Corbett Research, Australia) under the following conditions: 95°C for 2 min, followed by 35 cycles of 95°C for 15 sec, 63°C (for bla_{VIM} gene) or 50°C (for bla_{IMP} gene) for 15 sec, 72°C for 15 sec. The final elongation step was at 72°C for 3 min. Melting of amplification products was performed by increasing the temperature from 72 to 94°C by 1°C every 10 sec. Fluorescence of SYBR Green I was acquired on the "SYBR" channel at the end of each amplification cycle and during the melting analysis.

Figure 1. Geographic locations of the hospitals surveyed in RESORT study. The cities where the MβL-producing strains were isolated are marked by red flags.

DNA sequencing. PCR products corresponding to the internal fragments of bla_{VIM} and bla_{IMP} genes were directly sequenced on an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Stafford, TX). Sequences were identified using the BLAST algorithm available through NCBI. The most closely matching sequences (GenBank Acc. No. AY507153 and AY625689) were then used to design the primers for amplification and sequencing of the entire coding regions of bla_{VIM} and bla_{IMP} genes (Tab. 1).

Table 1. Primers used in this study.

Primer	Sequence, 5'-3'	Application
VIM-F	GGTGTTTGGTCGCATATCGC	Real-time-PCR detection
VIM-R	CGATCGTCATGAAAGTGCGT	Real-time-PCR detection
IMP-F	GCTAAAGATACTGAAAAATTAGT	Real-time-PCR detection
IMP-R	TCATTTGTTAATTCAGATGCATA	Real-time-PCR detection
VIM-BIG-F	TCTATTTGACCGCGTCTATCAT	PCR and sequencing
VIM-BIG-R	AACGACTGAGCGATTTGTGTGC	PCR and sequencing
IMP-BIG-F	CTACCGCAGCAGAGTCTTTGCC	PCR and sequencing
IMP-BIG-R	CGTTTAACCCTTTAACCGCCTG	PCR and sequencing
M13	GAGGGTGGCGGTTCT	RAPD typing
OPB-17	AGGGAACGAG	RAPD typing

Molecular typing. RAPD typing with primers M13 and OPB-17 (Tab. 1) was used to determine clonality between the MβL-producing strains. Amplifications were performed using the Ready-To-Go RAPD Beads (Amersham Biosciences, Piscataway, NJ) as described previously [2]. Cluster analysis of combined RAPD profiles was performed using the GelCompar software v. 4.1 (Applied Maths BVBA, Sint-Martens-Latem, Belgium) with Pearson correlation coefficient and unweighted pair-group method with arithmetic averages (UPGMA) algorithm.

Resistance transfer by conjugation. Broth mating of the representative VIM- and IMP-MβL-producing *P. aeruginosa* isolates (one isolate per hospital, per genotype) with *E. coli* AB1456 (F- Rifr) strain was utilized to analyze transferability of carbapenem-resistance determinants. Mating mixtures (initial donor/recipient ratio = 0.01) were incubated at 30°C for 12 h. Selection of transconjugants was attempted on MH agar containing rifampin (150 µg/ml) and ceftazidime (4 µg/ml) or rifampin and meropenem (0.25 µg/ml).

RESULTS

Positive results of PCR with bla_{VIM} gene-specific primers were obtained for 45 strains expressing the MβL phenotype. The remaining three isolates from a single Moscow hospital gave positive results of amplification with bla_{IMP}-targeting primers (Tab. 2).

Sequencing of the entire bla_{VIM} genes from five representative isolates (one per hospital): 565, 1913, 3389, 2074, 257, revealed their identity to bla_{VIM-2} (GenBank Acc. No. AY507153).

Sequences of the bla_{IMP} genes from isolates 3107, 3110, and 3116 were all identical and differed from bla_{IMP-1} (GenBank Acc. No. AY625689) by a single G/A transition at position 121 of the structural gene, corresponding to E59K amino acid change (BBL numbering). Sequence of the gene for newly identified IMP-1 β-lactamase variant was deposited in GenBank under accession number DQ061191.

Table 2. Characteristics of MβL-positive *P. aeruginosa* strains.

Location/ Hospital	No. of MBL+ strains	MBL type	RAPD type
Omsk	37	VIM-2	A
Moscow 1	5	VIM-2	A
Moscow 2	1	VIM-2	A
Moscow 3	1	VIM-2	D
Krasnodar	1	VIM-2	B
Moscow 4	3	IMP-1 (E59K)	C

Cluster analysis of RAPD profiles (Fig. 2) suggested that the majority of VIM-2 producers, including all isolates from Omsk and 6 isolates from two Moscow hospitals, belonged to a single clonal group (≥81.5% identity of profiles). One VIM-2-producing strain from Krasnodar and, surprisingly, three IMP-producing isolates had the RAPD profiles, designated B and C, similar to those of the major group A (69% of the overall identity). The remaining VIM-2-producing strain from Moscow hospital 3 was apparently unrelated (profile D).

Despite multiple attempts, broth mating of five VIM- (565, 1913, 3389, 2074, 257) and one IMP-positive (3107) isolates with *E. coli* AB1456 recipient produced no transconjugants.

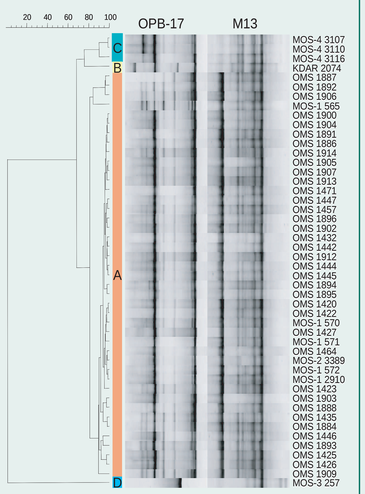

Figure 2. UPGMA clustering of combined RAPD profiles of MβL-producing *P. aeruginosa* isolates.

CONCLUSION

This study for the first time reports the wide geographic dissemination of VIM-2-producing *P. aeruginosa* strains in Russia as well as the identification of a novel IMP-1β-lactamase variant.

The results of molecular typing suggest a possible genetic relatedness of the majority of MβL-producing isolates.

REFERENCES

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