# EPIDEMIC POPULATION STRUCTURE OF MBL-PRODUCING *Pseudomonas aeruginosa* IN RUSSIA

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## INTRODUCTION

Previously, we described appearance of MBL-producing *P. aeruginosa* in Russia [1] and characterized genetic context of MBL-coding genes among these strains [2]. In this study, we further investigated the genetic relatedness between recent and previously isolated MBL-producing strains of *P. aeruginosa* in Russia.

## MATERIALS AND METHODS

- A total of 103 MBL-producing *P. aeruginosa* strains were studied:
  - Isolates were collected in 17 hospitals of 8 geographically distant Russian cities as part of the prospective national surveillance study "METALL" in 2005-2007 (Figure 2).
  - MBL phenotype was determined by EDTA double-disk synergy test [3].
- The presence of $bla_{IMP}$ and $bla_{VIM}$ genes was investigated by multiplex real-time PCR with consensus primers (Table 1).
  - 25-µl PCR mixtures contained: 0.5 µM of each primer, 200µM of each dNTP, 1.5 mM $MgCl_2$, 1.5U of Taq-F DNA-polymerase (AmpliSens, Russia), 1 µl of SYBR Green I (SBG I; 1:1000 dilution in DMSO) and 3 µl of template DNA prepared by boiling of 3-5 colonies in TE buffer.
  - Thermal cycling was performed on a Rotor-Gene 2000 System (Corbett Research, Australia) and included initial 15-min denaturation at 95ºC and 30 cycles of 15-sec denaturation at 95ºC, 15-sec annealing at 50ºC, and 15-sec elongation at 72ºC. The amplification was monitored by recording the fluorescence of SBG I at the end of each cycle. The postamplification melting curve analysis was used to distinguish the PCR products of $bla_{IMP}$ and $bla_{VIM}$ genes according to their melting temperatures (~81ºC and ~84ºC, respectively).
- Isolates were typed by:
  - RAPD with primers M13 and OPB-17 [1];
  - MLST [4, http://pubmlst.org/paeruginosa/].

Cluster analysis of combined RAPD profiles was performed using the GelCompar software v.4.1 (Applied Maths, Belgium) with Pearson coefficient and UPGMA algorithm. Clustering of MLST profiles was performed using the online Minimum-spanning tree software (http://pubmlst.org/analysis/).

- Structures of MBL-encoding integrons were analyzed by PCR-RFLP and compared to those determined previously [2]. Integrons were amplified in two parts using the primers for internal $bla_{VIM}$ sequences and conserved sequences at the 5' (*intI1*) and 3' (*qacEdelat*1 or *tni*C) ends (Table 1) and were digested with *Taq*I restriction endonuclease.

**Table 1.** Primers used in this study*

| SPrimer | Target | Sequence, 5'-3' | Application |
|---|---|---|---|
| VIM-Fa | $bla_{VIM}$ | GTTTGGTCGCATATCGC | Real-time PCR detection |
| VIM-Ra | $bla_{VIM}$ | TCGTCATGAAAGTGCGT | Real-time PCR detection |
| IMP-F | $bla_{IMP}$ | GCTAAAGATACTGAAAAATTAGT | Real-time PCR detection |
| IMP-R | $bla_{IMP}$ | TCATTTGTTAATTCAGATGCATA | Real-time PCR detection |
| M13 | | GAGGGTGGCGGTTCT | RAPD typing |
| OPB-17 | | AGGGAACGAG | RAPD typing |
| INT/5CS | *intI*1 | CTTCTAGAAAACCGAGGATGC | PCR-RFLP of integrons |
| 3-CS QAC-EXT | *qacE*Δ1 | AATGCGGATGTTGCGATTAC | PCR-RFLP of integrons |
| TniC-rev | *tniC* | GTGGGCGATCTCTGCGAAG | PCR-RFLP of integrons |
| VIM-OUT-F | $bla_{VIM}$ | CTACCCGGAAGCACAGTTCGTC | PCR-RFLP of integrons |
| VIM-OUT-R | $bla_{VIM}$ | ACCGGAATTTCGCTGACTGTCG | PCR-RFLP of integrons |

* Primers used for MLST are not shown.

## RESULTS AND DISCUSSION

All the isolates collected in 2005-2007 were found to carry the $bla_{VIM}$ genes.

According to cluster analysis of RAPD profiles, the strains were divided into two clonal groups: one (group A) comprised of 93 isolates from the regions of Lipetsk, N.-Novgorod, Novosibirsk, Smolensk, and Tyumen cities and another (group B) comprised of the remaining 10 isolates from the regions of Smolensk and Voronezh cities (Figure 1).

PCR-RFLP analysis showed that all group A isolates harbored the integron identical to that of the *P. aeruginosa* strains from Russia [DQ522233], USA [5, AY943084] and Norway [6]. Isolates of the group B contained the integron identical to that of another Russian strain reported earlier [2, DQ522236] (Figure 3).

Nine representative strains (one per city per RAPD group) were analyzed by MLST to confirm their common genetic origin. The strains of clonal group A belonged to ST235 and the strains of cluster B belonged to ST234. The MBL-producing strains of these STs were identified in Russia since 2003 [7].

Interestingly, phylogenetic analysis of MLST data revealed a striking genetic relatedness among the Russian *P. aeruginosa* strains of major cluster A (ST235) and some of the MBL-producing strains reported from Italy, Greece and Sweden [8]. Despite the apparent diversity in the MBL and integron contents, the latter strains differ from Russian ST235 isolates by no more than one allele in their MLST profiles (Figure 4).

## CONCLUSIONS

- The results of our study reveal an epidemic population structure of MBL-producing *P. aeruginosa* in Russia with two clones spreading in different hospitals across the country.
- Notably, the MLST data analysis suggested a phylogenetic relationship between the Russian and European *P. aeruginosa* strains that contain different MBL genes as parts of different class I integrons which in turn indicate their independent acquisition by isolates of a single genetic lineage.

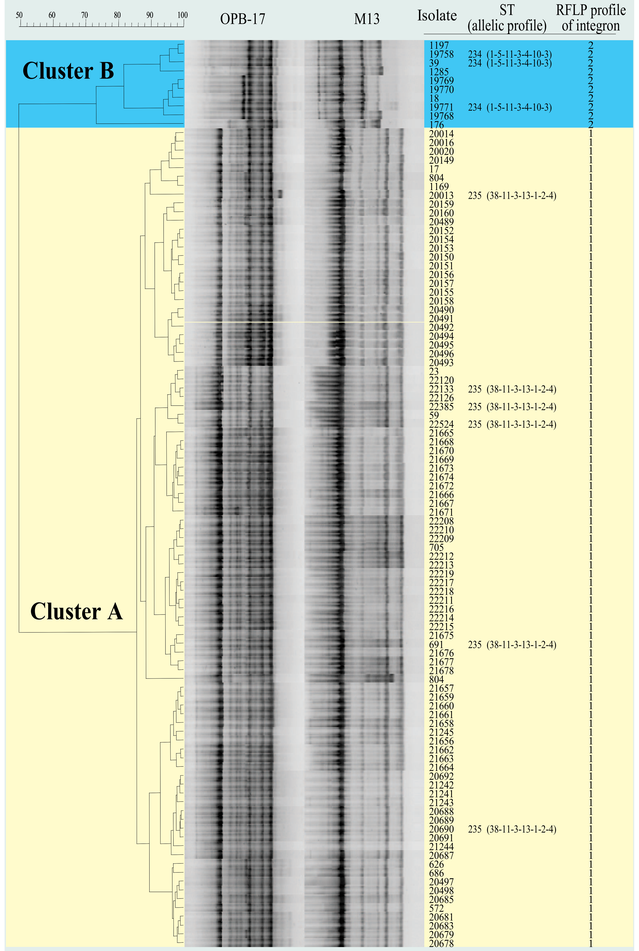


**Figure 1.** UPGMA clustering of combined RAPD profiles and genetic characteristics of MBL- producing *P. aeruginosa* isolates collected in 2005-2007

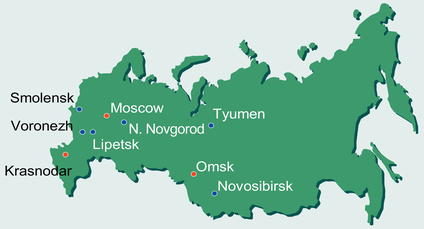


**Figure 2.** Geographic location of the hospitals where the MBL-producing strains were isolated
- hospitals surveyed in 2002-2004 (RESORT study)
- hospitals surveyed in 2005-2007 (present study)

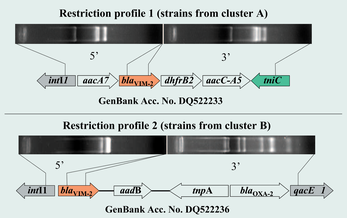


**Figure 3.** Restriction profiles and corresponding structures of variable parts of MBL-coding integrons

| Sequence type | Isolate | Year, place of isolation | Allelic profile* | MBL type | Integron structure |
|---|---|---|---|---|---|
| ST227 | VR143** | 1997, Verona, Italy | 38-11-3-**9**-1-2-4 | VIM-1 | intI1 $bla_{VIM}$ aacA4 aphA15 aadA1 qacEΔ1 |
| ST235 | Mos-3107 | 2004, Moscow, Russia | 38-11-3-13-1-2-4 | IMP-1$^{E59K}$ | intI1 aadB $bla_{IMP}$ cmlA7 $bla_{OXA-2}$ qacEΔ1 |
| | Kda-2074 | 2003, Krasnodar, Russia | 38-11-3-13-1-2-4 | VIM-2 | intI1 $bla_{VIM}$ aadA1 qacEΔ1 |
| | Mos-3389 | 2003, Moscow, Russia | 38-11-3-13-1-2-4 | VIM-2 | intI1 aadB $bla_{VIM}$ aacA4 qacEΔ1 |
| | Mos-565 | 2003, Moscow, Russia | 38-11-3-13-1-2-4 | VIM-2 | intI1 aacA7 $bla_{VIM}$ dhfrB2 aacC-A5 tniC |
| | Tmn-20013 | 2005, Tyumen, Russia | 38-11-3-13-1-2-4 | VIM-2 | intI1 aacA7 $bla_{VIM}$ dhfrB2 aacC-A5 tniC |
| ST230 | Ps100** | 2002, Heraklion, Greece | 38-11-**5**-13-1-2-4 | VIM-4 | intI1 $bla_{VIM}$ aadA6 qacEΔ1 |
| | PA66** | 2001, Stockholm, Sweden | 38-11-**5**-13-1-2-4 | VIM-4 | intI1 $bla_{VIM}$ aacA4 $bla_{OXA-2}$ qacEΔ1 |

**Figure 4.** Minimum-spanning tree showing the close relationship between the three sequence types comprised by MBL-producing strains from Russia and three other European countries

* Alleles that distinguish the ST227 and ST230 from ST235 are shown in bold.
** Data from Giske C.G. et al. [8].

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