

# FIRST REPORT OF METALLO-BETA-LACTAMASE PRODUCTION IN *P.AERUGINOSA* STRAINS ISOLATED IN DIFFERENT REGIONS OF RUSSIA

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## REVISED ABSTRACT

**Purpose of the study:** To determine the prevalence of metallo-beta-lactamase (MBL) production in nosocomial strains of *Paeruginosa* from various Russian hospitals.

**Methods:** A total of 992 nonduplicate nosocomial isolates of *Paeruginosa* were collected in ICUs of 31 Russian hospitals during a nationwide survey "RESORT" in 2003-2004. Susceptibilities of these isolates to imipenem (IMI) and meropenem (MEM) were determined using an agar dilution method according to the NCCLS (2004) guidelines. All strains (n=319) resistant to IMI or MEM or both of them (MICs  $\geq 16$  mg/L) were further screened for production of MBLs with an EDTA-double-disk synergy test (EDTA-DDST) on Mueller-Hinton agar. Disks with IMI (10  $\mu$ g) and MEM (10  $\mu$ g) were placed 10 mm (edge to edge) apart from the disk with EDTA (10  $\mu$ l of 0.5M solution, pH=8.0). The enlargement of the inhibition zones between any disk containing carbapenems and disk with EDTA was used as a marker of MBL production.

**Results obtained:** Production of MBL was detected by EDTA-DDST in 48 strains (4.8%). The MBL-producers were found in 6 hospitals located in three geographically distant regions. The majority of MBL-producing isolates (n=37) were obtained in a single hospital in Omsk (Siberia), 10 were isolated in four Moscow hospitals and a single isolate was found in Krasnodar (South Russia). The frequency of isolation of MBL-producing *Paeruginosa* in those hospitals varied from 2.6% (in Krasnodar) to 84.1% (in Omsk). Only one MBL-positive strain had the IMI MIC of 16 mg/L, and the others had the MICs of 32 (n=3), 64 (n=21) and  $\geq 128$  mg/L (n=23). The MEM MICs were 16, 32, 64 and  $\geq 128$  mg/L for 2, 15, 29 and 2 isolates respectively. Two strains with MEM MICs 128 mg/L were positive with IMI-EDTA-DDST, but negative with MEM-EDTA-DDST, while others were positive with both carbapenems.

**Conclusion:** To our knowledge, this is a first time report of the emergence of MBL-producing nosocomial strains of *Paeruginosa* in Russia.

## RESULTS

Production of MBL was detected by EDTA-DDST in 48 strains (4.8% of all collected *Paeruginosa* isolates). The MBL-producers were found in 6 hospitals located in three geographically distant regions (Figure 2). The majority of MBL-producing isolates (n=37)



Figure 2. Russian hospitals where MBL-producing strains were found

were obtained in a single hospital in Omsk (Siberia), 10 were isolated in four Moscow hospitals and a single isolate was found in Krasnodar (South of Russia). The frequency of isolation of MBL-producing *Paeruginosa* in those hospitals varied from 2.6% in Krasnodar to 84.1% in Omsk (Table 1).

Table 1. Frequency of isolation of carbapenem-resistant and MBL-producing *Paeruginosa* strains

Center/ Total no of strains	No (%) of resistant strains		
	IMI	MEM	MBL-positive
Omsk (n=44)	39 (88.6)	39 (88.6)	37 (84.1)
Moscow Hosp #1 (n=32)	10 (31.2)	5 (15.6)	1 (3.1)
Moscow Hosp #2 (n=23)	10 (43.5)	12 (52.2)	5 (21.7)
Moscow Hosp #3 (n=30)	14 (46.7)	14 (46.7)	3 (10)
Moscow Hosp #4 (n=32)	3 (9.4)	1 (3.1)	1 (3.1)
Krasnodar (n=40)	15 (37.5)	14 (35)	1 (2.6)
Other hospitals (n=791)	193 (24.4)	162 (20.5)	0

Distribution of MICs of MBL-producing strains are shown on Figure 3.

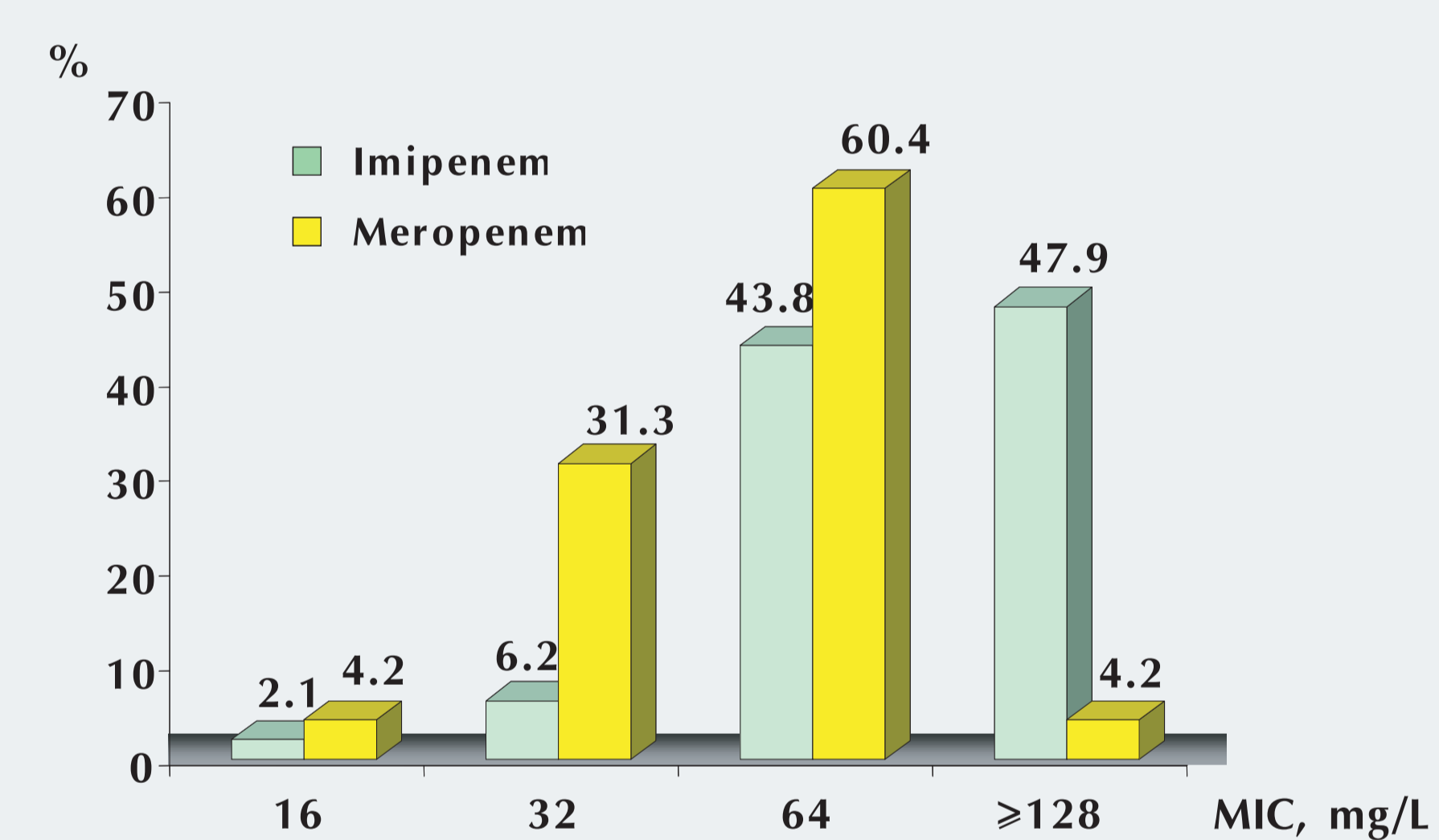


Figure 3. Imipenem MICs distribution of MBL-producing *Paeruginosa* strains

Only one MBL-producing strain had the imipenem MIC of 16 mg/L, and the others had the MICs of 32 (n=3), 64 (n=21) and  $\geq 128$  mg/L (n=23). The meropenem MICs were 16, 32, 64 and  $\geq 128$  mg/L for 2, 15, 29 and 2 isolates, respectively. A synergy between both carbapenems and EDTA was observed for all, but two isolates. The latter gave positive results of EDTA-DDST only with imipenem. Both of these isolates were obtained from Moscow hospital #3 and had meropenem MIC  $\geq 128$  mg/L.

## CONCLUSIONS

- This is the first report of the emergence of MBL-producing nosocomial strains of *Paeruginosa* in Russia.
- Despite the fact that resistance to imipenem (28.9%) and meropenem (25.9%) was common among Russian nosocomial isolates of *Paeruginosa*, the overall incidence of MBL-producing strains was below 5%.
- At the same time, our study has identified a large outbreak of MBL-producing *Paeruginosa* in a single hospital in Omsk.

## References:

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## INTRODUCTION AND PURPOSE

Carbapenem antibiotics have been used as drugs of last resort for the treatment of severe infections caused by multi-drug resistant gram-negative pathogens. Carbapenems are stable to hydrolysis by many beta-lactamases (including ESBL and AmpC), but destroyed by metallo-beta-lactamases (MBLs). Resistance mediated by acquired MBLs is increasingly reported in *Pseudomonas aeruginosa* in Asia, Europe, USA and Brasil. By today four groups of acquired carbapenems-hydrolyzing metallo-enzymes have been described in this species: IMP, VIM, SPM and GIM [1-3].

This is the first study to determine the prevalence of MBL production in nosocomial strains of *Paeruginosa* from various Russian hospitals.

## METHODS

**Bacterial strains:** A total of 992 nonduplicate nosocomial isolates of *Paeruginosa* were collected in ICUs of 31 Russian hospitals during a nationwide survey "RESORT" in 2003-2004. All strains (n=319) resistant either to imipenem or meropenem or both of them (MICs  $\geq 16$  mg/L) were further screened for production of MBLs.

**Susceptibility testing:** Susceptibilities of all isolates to imipenem and meropenem were determined using an agar dilution method according to the NCCLS (2004) guidelines [4]. Antimicrobial agents were obtained from manufactures (imipenem from Merk Sharp & Dohme Idea, Inc, USA and meropenem from AstraZeneca Inc, USA). Quality control was performed by testing *Paeruginosa* ATCC<sup>®</sup> 27853.

**Phenotypic detection of beta-lactamases:** Production of MBLs was screened by EDTA-double-disk synergy test (EDTA-DDST) on Mueller-Hinton agar. Sixty mm agar plate was inoculated with 0.5 McFarland suspension of fresh (18 hours) cultures. Disks with imipenem (10  $\mu$ g) and meropenem (10  $\mu$ g) were placed 10 mm (edge to edge) apart from the disk with EDTA (10  $\mu$ l of 0.5M solution, pH=8.0). The test was interpreted after 18 hours of incubation at 35°C.

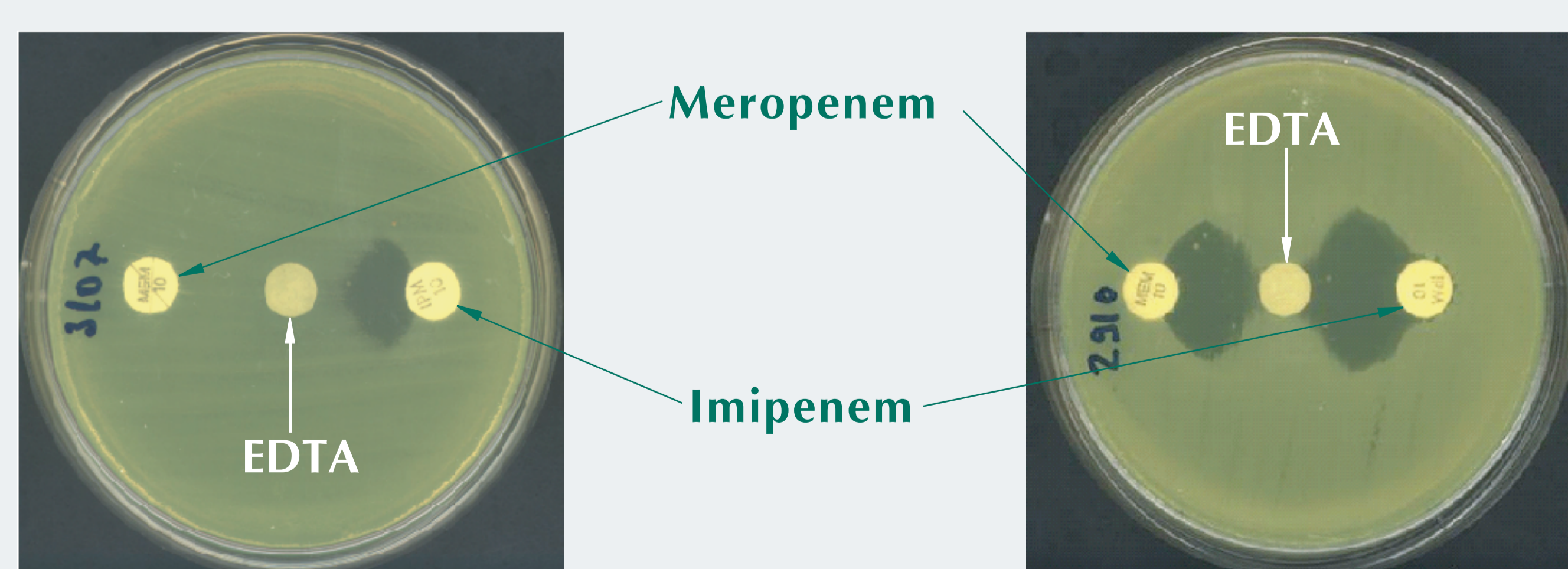


Figure 1. Examples of positive result of EDTA-DDST

The enlargement of the inhibition zones between any disk containing carbapenem and a disk with EDTA was used as a marker of MBL production (Figure 1). *Paeruginosa* ATCC<sup>®</sup> 27853 was used as a negative control and VIM-producing strain *Paeruginosa* 1630 - as a positive control.