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# Phenotypic and genotypic study of carbapenem-resistant *Pseudomonas aeruginosa* strains isolated from hospitalized patients

## Studiu fenotipic și genotipic la tulpinile de *Pseudomonas aeruginosa* izolate de la pacienți spitalizați

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### Abstract

**Introduction:** Nosocomial infections caused by *Pseudomonas aeruginosa* producing carbapenemases represent an important cause of morbidity and mortality among immunosuppressed patients. The aim of our study was to detect the production of metallo-carbapenemases (MBLs) by phenotypic methods and to detect the presence of the MBLs encoding genes (*blaIMP* and *blaVIM*) by PCR in *P. aeruginosa* strains isolated from hospitalized patients to the Regional Institute of Gastroenterology and Hepatology, Cluj-Napoca.

**Material and methods:** Between September 2014-February 2015, we tested thirty-eight *P. aeruginosa* strains resistant to carbapenems according to CLSI 2014 breakpoints, determined by Vitek<sup>®</sup> 2 (BioMérieux), isolated from various clinical specimens. Phenotypic detection of the MBLs production was performed using the KPC/MBL Confirmation kit (ROSCO<sup>®</sup>) and the MBL Etest<sup>®</sup> IP/IPI (BioMérieux). We used the PCR method for detecting MBLs encoding genes: *blaIMP*, *blaVIM*.

**Results:** The strains were obtained from surgery (55.3%), ICU (15.8%) and gastroenterology wards (28.9%), isolated from pus (25.8%), tracheal secretion (22.7%), bile (13.6%), sputum (10.6%), blood (10.6%), other secretions (16.7%). These strains were resistant to multiple classes of antibiotics. By ROSCO<sup>®</sup> method 28/38 strains (73.7%) were positive with imipenem ± dipicolinic acid (DPA) and 22/38 (57.9%) with meropenem ± DPA. Etest<sup>®</sup> was

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positive for the 28/38 strains (73.7%). 11 strains (28.9%) were positive for KPC with the screening method. We identified: 6 blaIMP+ (15.8%), 2 (5.3%) blaVIM+ and 4 blaIMP+/blaVIM+ strains (10.5%).

**Conclusion:** Both genes encoding MBL were found, alone or in combination. The increasing level of carbapenem resistance of these strains impose their routine testing to detect MBL.

**Keywords:** double-disk synergy; E-test; metallo- $\beta$ -lactamase; PCR; *Pseudomonas aeruginosa*.

## Rezumat

**Introducere:** Infecțiile nosocomiale determinate de tulpinile de *Pseudomonas aeruginosa* producătoare de carbapenemaze reprezintă o importantă cauză de morbiditate și mortalitate la pacienții imunosupresați. Acest studiu și-a propus să detecteze prin metode fenotipice de screening producerea de metalo- $\beta$ -lactamaze (MBL) și să detecteze genele cele mai frecvente, răspunzătoare de secreția lor (genele blaIMP și blaVIM) prin metoda PCR la tulpini de *P. aeruginosa* izolate la pacienți spitalizați în Institutul Regional de Gastroenterologie și Hepatologie din Cluj-Napoca.

**Material și metodă:** În perioada septembrie 2014-februarie 2015, au fost testate 38 de tulpini de *P. aeruginosa* rezistente la carbapenemi, interpretate cu sistemul Vitek®2 Compact (BioMérieux) conform CLSI 2014, izolate din produse patologice diverse. Detecția fenotipică a MBL s-a realizat cu ajutorul kit-urilor KPC/MBL Confirmation kit (ROSCO®) și MBL Etest®IP/IPI (BioMérieux). Pentru detecția genelor blaIMP, blaVIM s-a utilizat metoda PCR, folosind primeri specifici.

**Rezultate:** Tulpinile testate au provenit din secțiile de chirurgie (55.3%), terapie intensivă (15.8%) și gastroenterologie (28.9%), fiind izolate din: puroi (25.8%), secreții traheale (22.7%), bilă (13.6%), spută (10.6%), hemoculturi (10.6%), alte secreții (16.7%). Aceste tulpini au prezentat un profil de rezistență multiplă la antibiotice. Prin metoda ROSCO®, 28/38 de tulpini (73.7%) au fost pozitive, observându-se sinergie între imipenem și dipicolinic acid (DPA), iar la 22/38 (57.9%) s-a observat sinergie între meropenem și DPA. 11 tulpini (28.9%) au fost pozitive pentru KPC prin metoda screening. Testul MBL Etest®IP/IPI a detectat 28/38 tulpini MBL+ (73.7%). Au fost identificate prin PCR: 6 tulpini blaIMP+ (15.8%), 2 blaVIM+ (5.3%) și 4 blaIMP+/blaVIM+ (10.5%).

**Concluzii:** Au fost identificate ambele gene blaIMP și blaVIM atât singure cât și în combinație. Creșterea prevalenței infecțiilor determinate de tulpinile rezistente la carbapenemi impune testarea lor de rutină pentru detecția MBL.

**Cuvinte cheie:** dublu-disc sinergie; E-test; metalo-beta-lactamaza; PCR; *Pseudomonas aeruginosa*.

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## Introduction

Nosocomial infections caused by *Pseudomonas aeruginosa* producing carbapenemases are being reported ever more frequently worldwide, and they represent an important cause of morbidity and mortality among immunosuppressed patients (1–3). Most carbapenem resistance is due to impermeability by the loss of the *OprD* porin, but carbapenem-hydrolyzing metallo- $\beta$ -lactamases (MBLs) are increasingly reported (4). The  $\beta$ -lactamases are divided into four Ambler molecular classes (A, B, C, D), considering their amino-acid sequences and into 2 functional groups, such as serine  $\beta$ -lactamases (A, C, D) and MBLs (B), according to their catalytic ac-

tion. MBLs are metallo-enzymes that use bivalent metal ions ( $Zn^{2+}$ ,  $Cd^{2+}$ ) as co-factors in their catalytic action on the  $\beta$ -lactam ring of carbapenems (3,5–7). Group A includes penicillinases, extended-spectrum beta-lactamases (ESBL) and class A carbapenemases. *Klebsiella pneumoniae* carbapenemases (KPCs) belongs to this group, which hydrolyze penicillins, cephalosporins and carbapenems. These enzymes are inhibited or partially inhibited by clavulanate or tazobactam (5). KPC-producing *P. aeruginosa* isolates were first reported in 2006 from Colombia and subsequently in Puerto Rico, in Trinidad and Tobago, in the southern part of the United States, and in China (8). Group C includes *AmpC* cephalosporinases, which exhibits a greater hydrolysis

for cephalosporins in comparison to benzylpenicillin (5,6). Group D includes oxacillinases (OXAs), enzymes able to hydrolyze cloxacillin or oxacillin. Some of them can hydrolyse carbapenems, such as OXA-48 or OXA-23 (5,9). MBLs are the most important carbapenemases class both due to their ability to hydrolyse all  $\beta$ -lactams drugs, with the exception of monobactams (3,4), and also due to their resistance to inhibitors (10). At present, group B of MBLs are divided into three subclasses (B1, B2 and B3) (11). The most common MBLs reported worldwide belong to the subclass B1, being represented by different families: IMP, VIM, SPM, SIM, GIM, NDM, DIM (11). The most prevalent families of MBLs are the IMP and VIM enzymes; 53 IMP subtypes and 46 VIM variants are known, grouped into 3 clusters (11–13). IMP-type MBL determinants are mostly found in *P. aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae* strains (12). VIM enzymes are prevalent in Europe, although they are currently widespread on all 5 continents, especially VIM-2 (14). VIM-2 has been shown to be associated with a gene cassette, which encodes enzymes that inactivate aminoglycosides (15).

In 2014, the level of carbapenem resistance in *P. aeruginosa* strains isolated from our hospital reached an average value of 66.9%, which led us to initiate this study for MBLs detection.

The aim of the present study was to detect the production of carbapenemases in *P. aeruginosa* strains by phenotypic methods (double disk synergy test, E-test) and the presence of the most common MBLs encoding genes ( $bla_{VIM}$ ,  $bla_{IMP}$ ) by polymerization chain reaction (PCR).

## Materials and methods

We conducted a prospective study between September 2014 - February 2015 in the micro-

biology department of the Regional Institute of Gastroenterology and Hepatology (RIGH), Cluj-Napoca, Romania. In this study, a total of 38 non-repeat *P. aeruginosa* strains, with resistance to carbapenems isolated from the hospitalized patients during this period, were included. We obtained the agreement of the Ethics Committee for this study.

The *P. aeruginosa* strains were identified using the VITEK®2 GN card (BioMérieux, Marcy-l'Étoile, France).

Antibacterial susceptibility testing was performed with the VITEK®2 Compact automated system using N222 and XN05 cards. The results were interpreted according to Clinical and Laboratory Standards Institute 2014 (CLSI) breakpoints (16). The resistance phenotypes were also analyzed by VITEK®2 Compact system. Internal quality control was performed with the control strains: *P. aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218 and *K. pneumoniae* ATCC 700603.

Further studies were performed on all 38 non-susceptible of *P. aeruginosa* strains to carbapenems (MICimipenem  $\geq 16$   $\mu\text{g/ml}$ , MICmeropenem  $\geq 16$   $\mu\text{g/ml}$ ). The carbapenemase production was confirmed by plating the strains on chromID® CARBA (BioMérieux, France). For all carbapenemase-producing strains, the antimicrobial susceptibility to aztreonam (ATM) was tested by Kirby-Bauer method with ATM 30  $\mu\text{g}$  disk (Oxoid, UK). The interpretation was performed according to CLSI 2014 standards (16).

I. The phenotypic detection of MBLs production was done by:

*I.a. double disk synergy test (DDST)* using the KPC/MBL in *P. aeruginosa*/*Acinetobacter baumannii* confirmation kit (ROSCO® Diagnostics, Taastrup, Denmark). An overnight broth culture from the tested strains (turbidity adjust-

ed to 0.5 McFarland standard) was inoculated on a Mac Conkey agar plate in accordance to the manufacturer's rules. Then, five disks: IMI-10 µg (imipenem), MRP-10 µg (meropenem), DPA (dipicolinic acid), MRPBO (meropenem combined with phenylboronic acid - PBO) and MPCXH (meropenem combined with high concentration of cloxacillin - CXH) were applied on the agar plate. The plate was incubated at 37°C for 18±2 hours (overnight) (17). Internal quality control was performed with the control strains: *P. aeruginosa* ATTC 10145/CCUG 59626, MBL positive and *K. pneumoniae* CCUG 56233, KPC positive (Table 1).

*I.b. MBL E-test (Epsilon meter - test)* was performed with the Etest® MBL IP/IPI 256/64 (BioMérieux, Marcy-l'Étoile, France), IP (imipenem) (4-256 µg/ml) / IPI (imipenem: 1-64 µg/ml

+ ethylenediaminetetraacetic acid –EDTA - at a constant level) (18).

Interpretation: MBL positive: a reduction in the minimum inhibitory concentration (MIC) in the presence of EDTA greater than or equal to eight (IP/IPI ≥8); the deformation of the ellipse or the presence of a phantom-zone (18).

II. The genotypic detection of MBL production was done by *PCR method for genes: blaIMP, blaVIM*.

Fresh cultures were harvested and boiled in buffer TBE (Tris Borat EDTA) for 15 minutes, and briefly centrifuged (13,000 rpm, 2 min). Supernatants were used as PCR templates. The following primers were synthesized (at Eurogentec, Belgium) and used. The selected primers for the genes that codify the MBLs had been chosen as general primers for the IMP and VIM

**Table 1. Interpretation of the ROSCO® method**

	Mac Conkey agar		MRPBO	MPCXH	DPA	Reference
<b>No carbapenemases</b>	<i>P. aeruginosa</i>	MRP 10	-	≥ 5 mm.	-	(17)
<b>KPC</b>	<i>P. aeruginosa</i>	MRP 10	≥ 4 mm.	< 3 mm.	-	(17)
		MPCXH	≥ 4 mm.	-	-	(17)
<b>MBLs</b>	<i>P. aeruginosa</i>	IMI 10	-	-	synergism	(17)

MRP 10 - meropenem disk; IMI 10 - imipenem disk; MRPBO - meropenem disk combined with phenylboronic acid; MPCXH - meropenem disk combined with cloxacillin high; DPA - dipicolinic acid; KPC - *Klebsiella pneumoniae* carbapenemase; MBLs - metallo-β-lactamases.

**Table 2. PCR primers used for detection of carbapenemase resistance genes in *P. aeruginosa***

Gene	Primers	References	Product size	Anneling temperature	Extension
BlaIMP-F	5'- GAA GGC GTT TAT GTT CAT AC - 3' (Eurogentec, Belgium)	(19)	578 bp	51°C	50 sec
BlaIMP-R	5'- GTA TGT TTC AAG AGT GAT GC - 3' (Eurogentec, Belgium)				
BlaVIM-F	5'- GTT TGG TCG CAT ATC GCA AC - 3' (Eurogentec, Belgium)		382 bp	55°C	45 sec
BlaVIM-R	5'- AAT GCG CAG CAC CAG GAT AG - 3' (Eurogentec, Belgium)				

genes (19). See table 2. Reactions were carried out using Promega DNA Taq-polymerase, with 1.6 mM MgCl<sub>2</sub> on a Seegene™ thermal cycler with the following protocols: 8 min. initial denaturation at 95°C, 40 cycles of 30'' 95°C, 35'' T<sub>ann</sub>, 72°C extension; 10 min. final extension at 72°C. Electrophoresis was carried out on 1.8% agarose gel. The molecular tests for *bla*IMP and *bla*VIM were separately done (mono PCR). The tests were performed in duplicate and the results were similar. We used two strains as internal positive control, one positive for *bla*IMP, and the other one, positive for *bla*VIM, confirmed in another center (Table 2).

### Statistical analysis

The analysis of concordance between phenotypic screening methods (ROSCO® and E-test) and PCR, considered the reference method, we applied the Mc'Nemar test, Cohen's Kappa coefficient. Fisher's exact test was used for testing the association between the ATM variable and studied tests (ROSCO® and E-test). For all two-sided tests, we considered that statistical significance was achieved when the estimated *p*-value was <0.05. Data were analyzed using IBM SPSS Statistics v.20 (Armonk, NY: IBM Corp.).

### Results

The thirty-eight tested strains were isolated from surgery (n=21; 55.3%), intensive care unit - ICU (n=6; 15.8%), and gastroenterology wards (n=11; 28.9%). These strains were isolated from various clinical specimens: pus (n=9; 23.7%), tracheal secretion (n=5; 13.2%), bile (n=7; 18.4%), sputum (n=6; 15.8%), blood (n=3; 7.9%), central venous catheters (n=2; 5.3%), urine (n=2; 5.3%), nasal secretion (n=1; 2.6%), stool (n=1; 2.6%) and other secretions (n=2; 5.3%).

The tested *P. aeruginosa* strains had significant resistance to multiple classes of antibiotics (anti-pseudomonal β-lactams, aminoglycosides,

fluoroquinolones). See figure 1. Only one strain (2.6%) showed resistance to polypeptides (MIC-colistin: 4 mg/l). To ATM, 18/38 strains (56.5%) were susceptible, 8/38 intermediate (21.1%), and 12/38 (31.6%) were resistant. All these strains grew on chromID® CARBA (Figure 1).

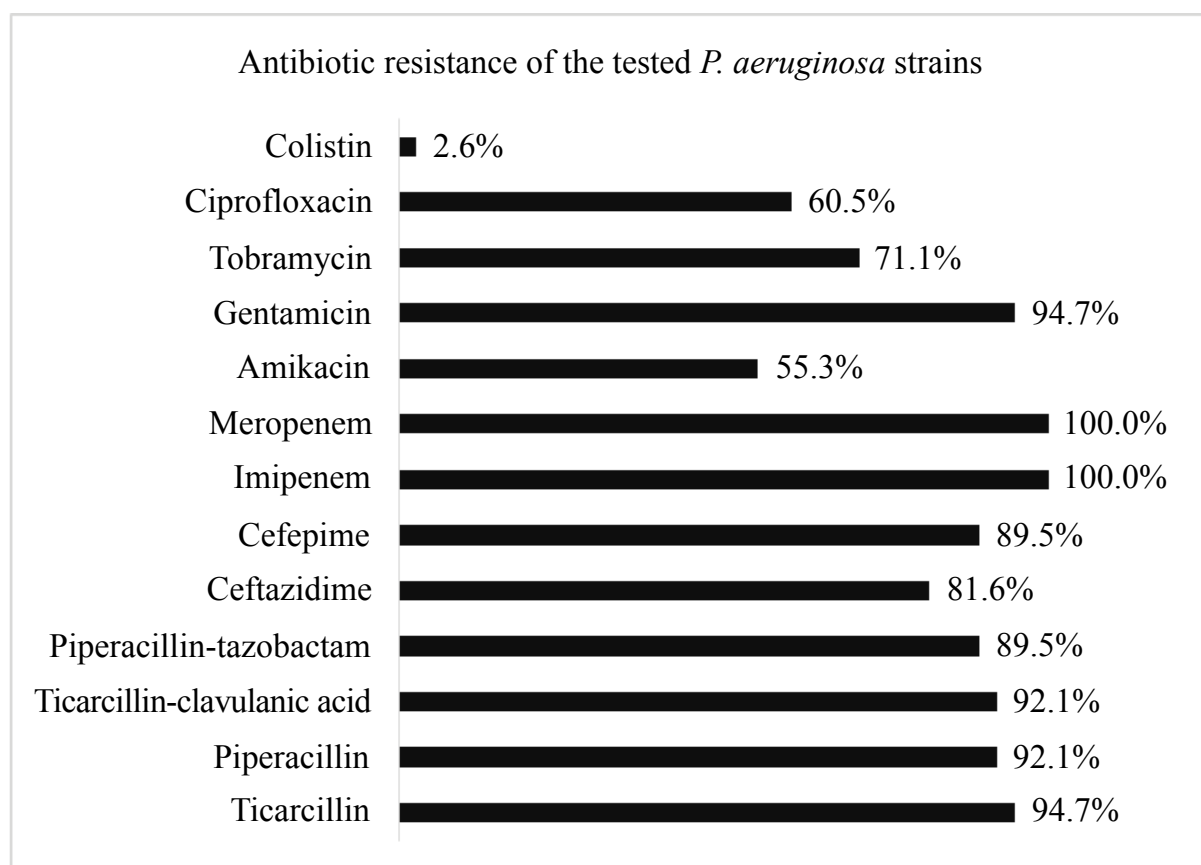
*The phenotypic detection of carbapenemases production:*

*I.a. Phenotypic detection of MBL with the ROSCO® method:* The synergy area was observed between IMI ± DPA disks in twenty-eight strains (73.7%), ten strains (26.3%) were MBL negative. For MRP, the synergy area was obvious in twenty-two strains (57.9%) and absent in sixteen strains (42.1%). Eleven strains were positive for KPC (28.9%), twenty-seven strains were negative for KPC (71.1%).

*I.b. MBL IP/IPI E-test:* Twenty-eight strains (73.7%) were MBL+ for a ratio IP/IPI ≥ 8 and the rest were negative.

*II. Genotypic detection of genes encoding MBLs (blaIMP, blaVIM) by PCR method:* 12 strains out of 38 tested strains (34.2%) carried one or both genes - 6 (15.8%) strains were *bla*IMP+, 2 (5.3%) strains were *bla*VIM+ and 4 (10.5%) strains were *bla*IMP+/+*bla*VIM+. Two *bla*IMP+ and one *bla*VIM+ strains were isolated from ICU, 2 *bla*IMP+, one *bla*VIM+ and 2 *bla*IMP+/+*bla*VIM+ strains were isolated from surgery. Two *bla*IMP+, one *bla*VIM+ strain and one *bla*IMP+/+*bla*VIM+ were isolated from gastroenterology wards.

The *bla*IMP+ strains were isolated from sputum, tracheal secretion, nasal secretion, pus, blood, and central venous catheters, the *bla*VIM+ strains from urine and sputum, and the *bla*IMP+/+*bla*VIM+ strains from tracheal secretion, pus and other secretions. These pathological specimens were related to several medical devices (intravenous or urinary catheters, tubes, cannulae, assisted ventilation appliances), on which *P. aeruginosa* has the ability to attach and multiply, forming an impossible to remove bio-film.



**Figure 1. Antimicrobial resistance of the carbapenems-resistant *P. aeruginosa* strains**

These strains showed a very high level of resistance to all anti-pseudomonal  $\beta$ -lactams. All the *bla*IMP and *bla*VIM positive strains (12/38) were resistant to ticarcillin (TIC), ticarcillin combined with clavulanat (TIM), piperacillin (PIP) and piperacillin combined with tazobactam (TPZ), ceftazidime (CAZ) and cefepime (FEP). (**Table 3**).

Regarding the association between the two phenotypic tests, there was concordance between their results. The proportion of positive and negative results of the ROSCO® method was similar to those of the E-test method for the strains with positive PCR (Mc’Nemar test,  $p=0.625$ ) and for those with negative PCR (Mc’Nemar test,  $p=0.754$ ).

Susceptibilities to ATM were similar for strains with positive and negative ROSCO test (50% vs 40 % at the sensitive strains, 21.4% vs 20% at the intermediate strains or 28.6% vs 40% at the resistant strains) and E-test (17.9% vs 40% at the sensitive strains, 17.9% vs 20% at the intermediate strains or 64.3% vs 40% at the resistant strains) (Fisher’s exact test,  $p=0.888$ ).

## Discussions

*P. aeruginosa* is one of the main opportunistic pathogens in the hospital environment. By its important intrinsic resistance to many antibacterials and by its great adaptability and ability to acquire many resistance mechanisms,

**Table 3. Antibiotic resistance profile of the *P. aeruginosa* strains tested by PCR**

Test PCR	MBLs genes	Antibiotic resistance profile	Number of strains
Positive	<i>blaIMP</i> +	R: TIC, TIM, PIP, TPZ, CAZ, FEP, ATM, GEN	2
		R: TIC, TIM, PIP, TPZ, CAZ, FEP, ATM, GEN, TOB	1
		R: TIC, TIM, PIP, TPZ, CAZ, FEP, ATM, GEN, TOB, CIP	2
		R: TIM, PIP, FEP, ATM, LVX	1
		<b>Total</b>	<b>6</b>
	<i>blaVIM</i> +	R: TIC, TIM, PIP, TPZ, CAZ, FEP, ATM, GEN, TOB, AK, CIP	2
		<b>Total</b>	<b>2</b>
	<i>blaIMP</i> +/ <i>blaVIM</i> +	R: TIC, TIM, PIP, TPZ, CAZ, FEP, ATM, GEN, TOB, AK, CIP	3
		R: TIM, PIP, FEP, ATM, LVX	1
		<b>Total</b>	<b>4</b>
Negative	<i>blaIMP</i> -/ <i>blaVIM</i> -	R: TIC, TIM, PIP, TPZ, CAZ, FEP, GEN, TOB	9
		R: TIC, TIM, PIP, TPZ, CAZ, FEP, GEN, TOB, CIP	5
		R: TIC, TIM, PIP, TPZ, CAZ, FEP, GEN, TOB, AK	1
		R: TIC, TIM, PIP, TPZ, CAZ, FEP, GEN, TOB, AK, CIP	5
		R: TIC, TIM, PIP, TPZ, FEP, GEN, TOB, AK, CIP	1
		R: TIM, PIP, FEP	1
		R: TIM, PIP, FEP, LVX	3
		R: TIM, PIP, FEP, LVX, CT	1
		<b>Total</b>	<b>26</b>

TIC - ticarcillin; TIM - ticarcillin combined with clavulanat; PIP - piperacillin; TPZ - piperacillin combined with tazobactam; CAZ - ceftazidime; FEP - cefepime; ATM - aztreonam; GEN - gentamicin; TOB - tobramycin; AK - amikacin; CIP - ciprofloxacin; LVX - levofloxacin; CT - colistin.

*P. aeruginosa* became a fearsome bacterium, called deadly superbug, for which the treatment options are extremely limited. The *blaIMP* and *blaVIM* negative strains which were resistant to TIC, TIM, PIP, TPZ, CAZ, FEP could suffer structural modifications of *AmpC* gene (20) or could be carriers for mobile genetic elements (plasmids, integrons) that encode other types of MBLs or OXAs (21). In a study performed in Spanish hospitals in 2008, Cabot et al. showed a correlation of 24.2% between the resistance to IMI, MRP, CAZ, FEP, TPZ and the *AmpC* over-expression among *P. aeruginosa* strains isolat-

ed from bloodstream infections (22). One strain PCR/ROSCO®/E-test negative which was susceptible to CAZ, but resistant to TIC, TIM, TPZ, FEP could suffer a mutation that causes *MexEF-OprN* hyperproduction combined with *OprD* downregulation, a mechanism more rarely seen, which primarily affects fluoroquinolones (20). MBLs hydrolyse all  $\beta$ -lactams, except monobactams, whose representative is ATM (23). ATM is considered 2nd therapeutic option (after colistin) (23) among active agents against MBLs+ *P. aeruginosa* strains. All the *blaIMP*+ and *blaVIM*+ strains were susceptible to ATM. We

have not found significant statistical association between sensitivity to ATM and the ROSCO® method. Likewise we have not found significant statistical association between sensitivity to ATM and the E-test. This could be explained by the existence of other MBLs (unexplored by us), or association with other mechanisms: production of ESBL or KPCs (23), hyper-production of a chromosomal *AmpC* (4), or the synthesis of carbapenemases class D (OXA-23, OXA-24, OXA-58, OXA-48, OXA-181) (21). In our study, the *blaIMP*<sup>+</sup> strains were sensitive to amikacin, resistant to gentamicin and had variable sensitivity to tobramycin while all the *blaVIM*<sup>+</sup> strains were resistant to aminoglycosides. It is known that the *blaVIM* gene is associated to class 1 integrons with one or more genes for resistance to aminoglycosides, being able to inactivate antipseudomonal aminoglycosides (gentamicin, tobramycin, amikacin) (24). All the *blaVIM*<sup>+</sup> strains were resistant to ciprofloxacin while 2 out of 4 *blaIMP*<sup>+</sup> strains were susceptible to ciprofloxacin. It could be explained by presence of multiple mechanisms of resistance, such as the MBL synthesis associated with the expression of efflux pumps *MexAB-OprM* or *MexXY*, responsible for associated resistance to fluoroquinolones (20). It is possible that a strain produces more types of carbapenemases. We have detected four positive strains for *blaIMP* and *blaVIM* genes. M. Wang et al. reported a strain of *P. aeruginosa* producing multiple carbapenemases: NDM-1, VIM-5, VIM-6 (25).

There were important differences between the molecular and phenotypic tests. These could be explained by the secretion of other MBLs, which were not tested in our study. Several Romanian studies regarding MBLs in *P. aeruginosa* strains were made in the last years. In a study conducted in Cluj-Napoca, between 2008-2009, Craciunas et al have not detected *blaIMP* and *blaVIM* genes in *P.*

*aeruginosa* strains but OXA-50 and OXA-2 genes, responsible for multidrug resistance of tested strains, were detected (28). The first report of *blaVIM* gene in the eastern part of Romania was made between 2007-2011, by Mereuta et al. Of the tested strains, 41.5% were secreting VIM enzyme but the *blaIMP* gene was not detected (29). In another study performed in Iasi between 2008-2012 presence of *blaVIM*-2 gene was confirmed in clinical isolates of *P. aeruginosa* (30). Gheorghe et al. reported the presence of *blaVIM*-4 gene in isolates recovered from the southern part of Romania in the period between 2011-2012. In their study, the most frequent genes found in *P. aeruginosa* strains resistant to carbapenems were *blaOXA*-23, *blaGES*-like and *blaVIM*-4 (31).

## Conclusions

This was the first study performed in our hospital regarding testing *blaIMP* and *blaVIM* genes to carbapenem-resistant strains of *P. aeruginosa*. 6 *blaIMP*<sup>+</sup>, 2 *blaVIM*<sup>+</sup> were detected and 4 strains were secreted both MBLs *blaIMP*<sup>+</sup>/*blaVIM*<sup>+</sup> strains. We showed the presence of *blaIMP* gene in this part of Romania. The phenotypic methods for screening MBLs were useful for diagnosis. In further studies, we intend to investigate a wider field of genes to follow the genetic resistance profile of the circulating *P. aeruginosa* strains in our hospital.

## Abbreviations

<i>P. aeruginosa</i>	- <i>Pseudomonas aeruginosa</i>
MBLs	- Metallo-carbapenemases
ESBL	- Extended-spectrum $\beta$ -lactamase
OXAs	- Oxacillinases
IMP	- Imipenemase



VIM	- Verona imipenemase
NDM	- New Delhi metallo- $\beta$ -lactamase
SPM	- Sao Paulo imipenemase
SIM	- Seul imipenemase
GIM	- German imipenemase
DIM	- Dutch imipenemase
ICU	- Intensive Care Unit
DDST	- The double-disk synergy test
DPA	- Dipicolinic acid
KPCs	- <i>Klebsiella pneumoniae</i> carbapenemases
PCR	- Polymerization chain reaction
RIGH	- Regional Institute of Gastroenterology and Hepatology
CLSI	- Clinical and Laboratory Standards Institute
MIC	- Minimum inhibitory concentration
IMI-10 $\mu$ g	- Imipenem
MRP-10 $\mu$ g	- Meropenem
MRPBO	- Meropenem combined with phenylboronic acid
PBO	- Phenylboronic acid
MPCXH	- Meropenem combined with high concentration of cloxacillin
CX	- Cloxacillin
EDTA	- Ethylenediaminetetraacetic acid
IP/IPI	- Imipenem/imipenem+EDTA
buffer TBE	- Buffer Tris Borat EDTA
ATM	- Aztreonam
TIC	- Ticarcillin
TIM	- Ticarcillin combined with clavulanat
PIP	- Piperacillin
TPZ	- Piperacillin combined with tazobactam
CAZ	- Ceftazidime
FEP	- Cefepime
T <sub>ann</sub>	- Annealing time
CIs	- Confidence intervals
GES	- Guiana extended-spectrum $\beta$ -lactamase

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## Conflict of interest

The authors declare that there are no conflicts of interest.

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