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***Research article***

# Spread of VIM-2 metallo-beta-lactamase in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates from Iaşi, Romania

## Răspândirea metalo-beta-lactamazei VIM-2 printre izolate clinice de *Pseudomonas aeruginosa* şi *Acinetobacter baumannii* din Iaşi, Romania

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## Abstract

*Our study investigated the type of acquired metallo-β-lactamases (MBLs) produced by carbapenem-resistant clinical isolates of Pseudomonas aeruginosa and Acinetobacter baumannii from five hospitals in Iasi, Romania and the genetic relatedness of the strains carrying MBL genes. Of 106 carbapenem-resistant strains, 50 were positive for MBL production after screening with Etest MBL. PCR analysis showed that 46 isolates (44 P. aeruginosa and 2 A. baumannii) carried a $bla_{VIM}$ gene. By DNA sequencing we identified two class 1 integrons carrying the $bla_{VIM}$ gene in the P. aeruginosa: IntI1-aacA7-$bla_{VIM-2}$-qacEΔ1 in 43 strains, and IntI1-aacA7-Δ$bla_{VIM}$-ΔcmlA1-qacEΔ1 in one strain. In A. baumannii isolates the $bla_{VIM-2}$ gene was not associated with a class 1 integron. Random amplified polymorphic DNA typing of VIM-2-producing P. aeruginosa strains revealed the presence of a major RAPD type in all five hospitals. Early detection and surveillance of such strains must be accompanied by rigorous infection control measures in order to limit the spread of MBLs in our clinical settings.*

***Key words:*** *VIM-2, metallo-beta-lactamase, Pseudomonas aeruginosa, Acinetobacter baumannii*

## Rezumat

*Studiul nostru a investigat tipul de metalo-β–lactamaze (MBL) produse de Pseudomonas aeruginosa şi Acinetobacter baumannii rezistente la carbapeneme, izolate în cinci spitale din Iaşi, Romania şi inrudirea genetică a acestor tulpini. Din 106 tulpini rezistente la carbapeneme, 50 au dat rezultate pozitive la screening-ul prin Etest pentru producerea de MBL. Analiza PCR a arătat ca 46 dintre izolate (44 P. aeruginosa si 2 A. baumannii) aveau gena $bla_{VIM}$. La tulpinile de P. aeruginosa, secvențierea a demonstrat prezența a doi integroni de clasa 1 care conțin gena $bla_{VIM}$: IntI1-aacA7-$bla_{VIM-2}$-qacEΔ1, la 43 tulpini, respectiv IntI1-aacA7-Δ$bla_{VIM}$-ΔcmlA1-qacEΔ1, la o singură tulpină. La tulpinile de A. baumannii gena $bla_{VIM-2}$ nu a fost detectată într-un integron de*

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*clasa 1. Tiparea prin RAPD (Random amplified polymorphic DNA typing) a tulpinilor de P. aeruginosa producătoare de VIM-2 a evidențiat prezența unui tip RAPD major, în toate cele cinci spitale. Detectarea precoce și supravegherea unor astfel de izolate trebuie însoțită de măsuri riguroase de control al infecției, în vederea limitării răspândirii MBL în unitatile spitalicesti.*

***Cuvinte cheie:*** *VIM-2, metalo-beta-lactamaze, Pseudomonas aeruginosa, Acinetobacter baumannii*

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## Introduction

Carbapenems are the β-lactams with the broadest spectrum, invaluable against multiresistant Gram-negative pathogens, including *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (1). Carbapenem resistance may result from decreased outer membrane permeability, upregulation of the efflux pumps, hyperproduction of a chromosomal AmpC-type cephalosporinase and the production of carbapenemases such as the metallo-β-lactamases (MBLs) (2, 3). Metallo-β-lactamases have emerged as major defense mechanism against carbapenems (1). The ability of MBL to hydrolyze almost all β-lactams, and their association with mobile genetic elements (such as integrons or plasmids) promoting their rapid diffusion, underlines the increasing importance of MBL-producing bacteria in clinical settings. Until now, eleven types of MBLs have been described throughout the world, six of them, IMP, VIM, SPM, GIM, SIM and NDM being reported in both *P. aeruginosa* and *A. baumannii* (4). The dissemination of MBL-producing bacteria was documented in many European countries, such as Greece, Italy, France, Spain and Germany, where VIM-type MBLs are the most prevalent (5, 6, 7, 8). A total of 39 variants of VIM enzymes have been assigned in the β-lactamase database on www.lahey.org/Studies (9). Described for the first time in a *P. aeruginosa* clinical isolate from France in 1996 (10), VIM-2 has become the most important VIM-type MBL in clinical practice, with a worldwide distribution (11) and have been responsible for many outbreaks (12, 13, 14). In Romania IMP-1 and VIM-2 were previously described in *A. baumannii* clinical strains (15) and IMP-13 and VIM-2 in *P. aeruginosa* (16). The present study aimed to investigate the nature and the frequency of MBLs produced by carbapenem-resistant isolates of Gram-negative non-fermenters from hospitals in Iasi, North-East of Romania, and to characterize the detected MBLs at the molecular level.

## Material and methods

### *Bacterial strains*

We tested 106 randomly selected non-duplicate imipenem-resistant clinical isolates of *P. aeruginosa* (90 strains) and *A. baumannii* (16 strains), obtained between 2008 and 2012 in five university hospitals in Iasi. The *P. aeruginosa* strains were isolated from urine (39), pus (31), sputum (10), tracheal aspirate (4), blood (3), CSF (2) and catheter (1). The *A. baumannii* strains were isolated from urine (5), pus (5), sputum (3), tracheal aspirate (1), blood (1) and CSF (1). In addition, two *P. aeruginosa* isolates previously described, Pa16GL and Pa247IS were included in this study as positive controls for $bla_{VIM}$ and $bla_{IMP,}$ respectively (16). Bacterial identification was performed using API 20 NE system (bioMérieux, Marcy-l'Etoile, France). The susceptibility of *P. aeruginosa* strains to ticarcillin (TIC), piperacillin-tazobactam (TZP), ceftazidime (CAZ), cefepime (FEP), aztreonam (ATM), imipenem (IPM), meropenem (MEM), amikacin (AK), gentamicin (CN), tobramycin (TOB), ciprofloxacin (CIP), levofloxacin (LEV) (Oxoid Ltd., Basingstoke, Hampshire, UK) was tested by disk diffusion method according to CLSI guidelines (17). In addition, the *A. baumannii* strains were tested to ampicillin-sulbactam (SAM), doxycycline (DO), trimethoprim-sulfamethoxazole (SXT). *Pseu-*

**Table 1. Primers used in multiplex PCR and PCR mapping in this study**

| Primer | Sequence 5'-3' | Product size | Reference |
|---|---|---|---|
| IMP-DIA-FWD | GGAATAGAGTGGCTTAATTCT | 361 | 18 |
| IMP-DIA-REV | GTGATGCGTCYCCAAYTTCACT | | |
| VIM-DIA-FWD | CAGATTGCCGATGGTGTTTGG | 523 bp | 18 |
| VIM-DIA-REV | AGGTGGGCCATTCAGCCAGA | | |
| NDM-Fm | GGTTTGGCGATCTGGTTTTC | 621 bp | 19 |
| NDM-Rm | CGGAATGGCTCATCACGATC | | |
| 5'CS | CTTCTAGAAAACCGAGGATGC | variable | 18 |
| 3'CS | CTCTCTAGATTTTAATGCGGATG | | |

*domonas aeruginosa* ATCC 27853 was used for quality control. The MICs for IPM, CAZ, colistin (COL) were determined by Etest (bioMérieux, Marcy-l'Etoile, France). Phenotypic screening for MBL producers was performed by EPI microdilution test, with EDTA and 1,10-phenanthroline as chelator agents (18) and Etest MBL strips (bioMérieux, Marcy-l'Etoile, France).

***PCR analysis***

All isolates positive for MBL production were screened for $bla_{IMP}$ and $bla_{VIM}$ -like genes by multiplex PCR with the primers IMP-DIA-FWD, IMP-DIA-REV, VIM-DIA-FWD, VIM-DIA-REV (Eurogentec, Liège, Belgium) (19) and for $bla_{NDM-1}$ with the primers NDM-Fm and NDM-Rm (Eurogentec, Liège, Belgium) (20), listed in *Table 1*. Reactions were carried out using a Palm Cycler Corbett CG1-96 (Qiagen) in the conditions described previously (19, 20). The template DNA was obtained by 15 min boiling of a dense bacterial suspension in 500 µl distilled water. The PCR products were analyzed by electrophoresis in 2% agarose gel in TBE buffer.

***PCR mapping***

The genetic support of the $bla_{VIM}$ genes was investigated using the class 1 integron primers 5'CS Integron and 3'CS Integron (Eurogentec, Liège, Belgium) in combination with VIM-DIA-REV, VIM-DIA-FWD (19). Genomic DNA was extracted with GenElute Bacterial Genomic DNA kits (Sigma-Aldrich, Taufkirchen, Germany). The purified amplicons (Wizard PCR Purification Kit, Promega Corp., Madison WI, USA) were sequenced using the CEQ 8000 Genetic Analysis System (Beckman Coulter, Beverely MA USA) in an external facility.

***Molecular typing***

Molecular typing was carried out by random amplified polymorphic DNA (RAPD) fingerprinting with the primers 272PSE (5'-AGCGGGCCAA-3') and 208PSE (5'-AGCGGGCCAA-3') (Eurogentec, Liège, Belgium), as previously described (21). Amplification products were separated in 1.5% agarose. RAPD patterns were compared as suggested by Campbell et al. (21). For samples with the same banding pattern we assigned a letter, indicating a single RAPD type. Those different by no more than one major band or three minor bands were considered possibly related. Bands were classified as major bands if their intensity was equal to or greater than that of the 1000- bp fragment in the 200-bp ladder (Promega Corp., Madison WI, USA). Minor bands had a lower intensity than that of the 1000- bp fragment.

## Results

***Detection and identification of the MBL genes***

Of 90 carbapenem-resistant *P. aeruginosa* and 16 *A. baumannii* strains, 47 and 3, respectively were positive for MBL production af-

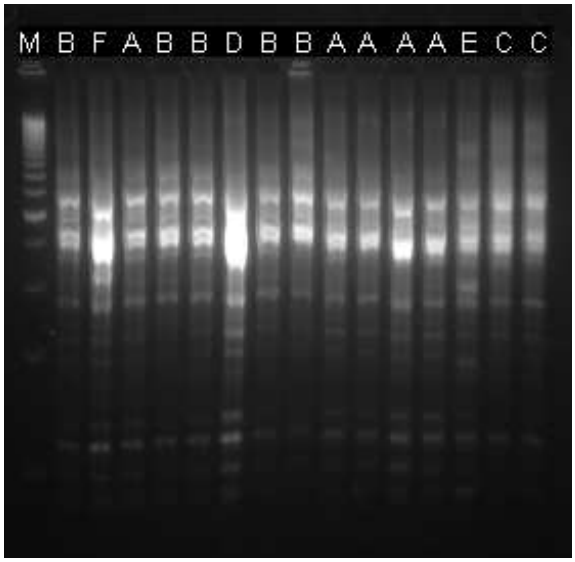


**Figure 1. RAPD patterns of P. aeruginosa isolates carrying blaVIM.** Lane 1, M, molecular weight marker (200 bp DNA Step Ladder; Promega Corp.); lanes 2, 5, 6, 8, 9, RAPD type B, lane 3, RAPD type F, lanes 4, 10, 11, 12, 13 RAPD type A, lane 7, RAPD type D; lanes 15, 16 RAPD type C.

ter screening with Etest MBL strips. The EPI microdilution test exhibited a ≥4 fold reduction of imipenem MICs for 44 *P. aeruginosa* strains. The *A. baumannii* isolates did not grow in the presence of the chelator agents, so we could not interpret the result for the EPI test. The MBL-producing isolates had MICs for IPM of ≥ 128 μg/ml, while the MICs for CAZ ranged between 32 – 512 μg/ml and exhibited a multidrug-resistant phenotype. They were resistant to antipseudomonal penicillins and cephalosporins, carbapenems, aminoglycosides and fluoroquinolones tested. Resistance to ATM was observed in 12 MBL producing *P. aeruginosa* isolates out of 44 (27.27%). No resistance to colistin was detected (*Table 2*). The MBL-producing *A. baumannii* strains were resistant to SAM, TIC, TP, CAZ, FEP, carbapenems, CIP, CN, AK. One was resistant to DO and SXT, as well, and both remained susceptible to TOB and COL. According to the definitions proposed by Magiorakos et al. (22), 31 clinical isolates of *P. aeruginosa* (34.44%) and one of *A. baumannii* were classified as extensively drug-resistant, while the rest were multidrug-resistant. Multiplex PCR with VIM-DIA and IMP-DIA primers yielded a 523 bp amplification product for 44 *P. aeruginosa* (48.88%) strains and 2 *A. baumannii* strains, consistent with the presence of a $bla_{VIM}$-like gene. Sequencing revealed the presence of $bla_{VIM-2}$ in all isolates. $bla_{IMP}$-like and $bla_{NDM}$-like genes were not detected.

***Molecular typing***

RAPD analysis was performed to determine the genetic relatedness of isolates carrying $bla_{VIM-2}$ gene. Distinct RAPD patterns, with sizes from 0.1 kb to 1.4 kb, varying from 8 to 12 bands, were obtained. The 44 strains of *P. aeruginosa* were assigned to 6 RAPD types, as follows: one major type A (27 strains), type B (12 strains) possibly related to type A, type C (2 strains) and types D, E, F, each including only one strain (*Figure 1*). The two *A. baumannii* isolates were not genetically related.

***PCR mapping***

The presence of class 1 integrons was confirmed in all MBL producing isolates. For the *P. aeruginosa* strains, the aproximative sizes of the class 1 integrons carrying the $bla_{VIM}$ gene were 1.5 kb in 43 strains and 2.5 kb in one strain (Pa23). Nucleotide sequencing of the 1.5 kb product showed the following gene cassette array *IntI1-aacA7-*$bla_{VIM-2}$*-qacEΔ1*. The *IntI1*, *aacA7* and *qacEΔ1* genes code for an integrase, an aminoglycoside acetyltransferase, AAC(6')-I1, and resistance to ethidium bromide and quaternary ammonium compounds, respectively. The structure of the 2.5 kb amplicon derived from isolate Pa23 was *IntI1-aacA7-*Δ$bla_{VIM}$-Δ*cmlA1-qacEΔ1* (*Table 2*). In this case, the type of VIM enzyme could not be identified, because sequencing revealed only 454 bp from the 5' end of a $bla_{VIM}$. Downstream this partial fragment followed a sequence of 215 bp, similar to the 3' end of *cmlA1* gene. The 5' end of *cmlA1* (a gene encoding an efflux pump for chloramphenicol) has apparently been truncated by the insertion of

**Table 2. Characteristics of the MBL-producing *P. aeruginosa* clinical isolates**

| Isolate | Specimen[1] | Date | Hospital unit[2] | Suceptible to | RAPD type | Class 1 integron cassettes |
|---|---|---|---|---|---|---|
| Pa36 | U | Jul/08 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa14 | U | Dec/08 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa32 | B | May/09 | ID | COL | A | *aacA7-bla*$_{VIM-2}$ |
| Pa5 | W | Jun/09 | ID | COL, ATM | B | *aacA7-bla*$_{VIM-2}$ |
| Pa10 | U | Sept/09 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa53 | U | Nov/09 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa782 | U | Jan/10 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa1996 | U | May/10 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa1715 | U | May/10 | ID | COL | B | *aacA7-bla*$_{VIM-2}$ |
| Pa1775 | U | May/10 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa54 | U | Jul/10 | ID | COL, ATM | D | *aacA7-bla*$_{VIM-2}$ |
| Pa206 | U | Jul/10 | ID | COL, ATM | B | *aacA7-bla*$_{VIM-2}$ |
| Pa4223 | U | Oct/10 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa4631 | U | Nov/10 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa64 | U | Dec/10 | ID | COL, ATM | B | *aacA7-bla*$_{VIM-2}$ |
| Pa10 | W | Jan/11 | ID | COL, ATM | B | *aacA7-bla*$_{VIM-2}$ |
| Pa813 | U | Feb/11 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa5825 | U | Mar/12 | ID | COL, ATM | B | *aacA7-bla*$_{VIM-2}$ |
| Pa3796 | U | Jul/12 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa507 | CSF | Jul/12 | ID | COL | A | *aacA7-bla*$_{VIM-2}$ |
| Pa4213 | U | Aug/12 | ID | COL, ATM | C | *aacA7-bla*$_{VIM-2}$ |
| Pa205 | SP | Sept/12 | ID | COL | A | *aacA7-bla*$_{VIM-2}$ |
| Pa208 | SP | Sept/12 | ID | COL | A | *aacA7-bla*$_{VIM-2}$ |
| Pa214 | SP | Sept/12 | ID | COL | A | *aacA7-bla*$_{VIM-2}$ |
| Pa222 | W | Nov/12 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa263 | TA | Nov/12 | ID | COL | A | *aacA7-bla*$_{VIM-2}$ |
| Pa221 | P | Nov/12 | ID | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa23 | U | Jan/11 | URO | COL, ATM | B | *aacA7-*Δ*bla*$_{VIM}$ |
| Pa15g | U | Jan/11 | URO | COL, ATM | F | *aacA7-bla*$_{VIM-2}$ |
| Pa146 | TA | Aug/10 | E-ICU | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa69 | W | Aug/10 | E-DER | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa147 | SP | Sept/10 | E-HEM | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa127 | W | Oct/12 | E-ICU | COL | A | *aacA7-bla*$_{VIM-2}$ |
| Pa152 | TA | Feb/11 | E-ICU | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa70 | W | Feb/11 | E-DER | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |
| Pa136 | W | Mar/11 | E-SUR | COL, ATM | E | *aacA7-bla*$_{VIM-2}$ |
| Pa2 | W | Aug/12 | E-HEM | COL, ATM | B | *aacA7-bla*$_{VIM-2}$ |
| Pa338 | W | Oct/12 | E-ORT | COL | A | *aacA7-bla*$_{VIM-2}$ |
| Pa68 | SP | Nov/12 | E- ICU | COL | B | *aacA7-bla*$_{VIM-2}$ |
| Pa300 | W | Nov/12 | E- ICU | COL | B | *aacA7-bla*$_{VIM-2}$ |
| Pa148 | W | Nov/12 | E-D | COL | B | *aacA7-bla*$_{VIM-2}$ |
| Pa11 | W | Nov/12 | E-SUR | COL, ATM | B | *aacA7-bla*$_{VIM-2}$ |
| Pa311 | U | Nov/12 | PED-S | COL, ATM | C | *aacA7-bla*$_{VIM-2}$ |
| Pa925 | W | Sept/09 | CCV | COL, ATM | A | *aacA7-bla*$_{VIM-2}$ |

[1]B, blood culture, CSF, cerebrospinal fluid, SP, sputum, TA, tracheal aspirate, U, urine, W, wound.
[2] CCV, Institute of Cardiovascular Disease (surgery unit); D, Diabetes unit; DER, Dermatology unit; E, Emergency Hospital; HEM, Hematology unit, ICU, Intensive Care Unit; ID, Infectious Disease Hospital, ORT, Orthopaedic unit; Ped-S, Pediatric Hospital; S, Surgery; URO, Urology unit of "Dr. C.I.Parhon" Hospital.

*bla*$_{VIM}$. For *A. baumannii* isolates, a product of approximately 3.0 kb was observed, but the *bla*$_{VIM-2}$ gene was not carried by the class 1 integron, as no amplification products resulted from the combinations of primers 5'CS with VIM-DIA-REV and VIM-DIA-FWD with 3'CS.

## Discussions

The acquisition of metallo-β-lactamases by *P. aeruginosa* and *A. baumannii* is extremely worrisome, since this resistance mechanism reduces the few available therapeutic options. The MBL-producing strains detected in this study exhibited high levels of resistance to imipenem (MIC ≥ 128 μg/ml), consistent with Zhao *et al*., who found a much higher resistance to carbapenems in MBL-producing *P. aeruginosa* than non-MBL-producing strains (23). Co-resistance to broad spectrum β-lactams, aminoglycosides and fluoroquinolones was present in MBL positive strains. Besides colistin (to which all strains were susceptible), aztreonam was the most active drug against MBL producers (72.7% susceptibility), representing a potential therapeutic option. In a Brazilian study, Franco *et al.* published similar results regarding susceptibility to aztreonam among MBL-producing isolates (24). Full susceptibility to colistin and resistance to aminoglycosides were reported by Jovcic *et al*., but contrary to our study, they found levofloxacin and ciprofloxacin as the second most active antimicrobial agents (25). We noticed a good correlation between the results of EPI test and PCR as the "gold standard" for MBL genes detection. Out of 47 strains of *P. aeruginosa* and 3 strains of *A. baumannii* with a positive MBL Etest, 44 and 2, respectively, were confirmed by PCR. False positive results obtained with MBL Etest were previously reported, due to a membrane permeabilizing effect of EDTA (26), especially in settings with low prevalence of MBLs (25, 27). We could not exclude the presence of other types of MBLs, because only IMP, VIM and NDM were searched by PCR. The *bla*$_{VIM-2}$ gene was harbored in a class 1 integron, with only two gene cassettes, downstream of *aacA7* gene cassette encoding an AAC(6′)-I1 aminoglycoside acetyltransferase explaining the aminoglycoside resistance. A similar integron (GenBank accession number EF577406) was detected in a *P. aeruginosa* isolate in Palma de Mallorca, Spain (Juan *et al*., unpublished data), although the sequence downstream *bla*$_{VIM-2}$ gene was not recorded in the database. Integrons with the same gene cassette array were reported in Malaysia (28), Japan (23) and Tunis (29). A class 1 integron carrying *aacA7* and *bla*$_{VIM-2}$ was described in Romania (30), but the downstream region of the *bla*$_{VIM-2}$ gene was different when compared with the sequences from our study. In particular, the former integron had three gene cassettes in the variable region, *aacA7*, *bla*$_{VIM-2}$, *dhfrB*, similar to integrons found in Russia, United States and India (31). Almost all class 1 integrons described in our study had only two cassettes (*aacA7* and *bla*$_{VIM-2}$). In only one strain, Pa23, belonging to RAPD type B, the class 1 integron carried a partial fragment of *bla*$_{VIM}$ of 454 bp, followed by a partial fragment of 215 bp from *cmlA1*, a gene encoding chloramphenicol resistance. Given the fact that the strain had the phenotype of an MBL producer and because we obtained an amplicon of the expected size with the VIM-DIA primers, we suspect that the complete coding sequence of a *bla*$_{VIM}$ gene is harbored somewhere else in the genome. On the basis of the results of RAPD fingerprinting, 27 *P. aeruginosa* clinical isolates were clustered in a major type A, possibly related to the strains from types B, C and F, since they differed by one major band (21). The strains from types D and E yielded distinct patterns. Isolates showing similar patterns (RAPD types A and B) were detected in different units of the same hospital and in more than one hospital, indicating the spread of epidemic clones (*Table 2*). The high frequency of MBLs, 48.8% among *P. aeruginosa*, could be explained by the low number of investigated strains and by the expansion of a clone in a certain geographical area (the hospitals are in the same city).

In this study, we confirmed the presence of *bla*$_{VIM-2}$ in 43 clinical isolates of *P. aeruginosa* and 2 isolates of *A. baumannii* in five university hospitals from Iasi. The type of VIM enzyme could not be identified in the remaining *P. aeruginosa* MBL positive isolate (Pa23), because of the deletion at the 3' end of the *bla*$_{VIM}$ gene. Since a previous study from 2007 (16) in the same hospitals detected only one *P. aeruginosa* carrying MBL gene, *bla*$_{IMP-13}$, among a total of 27 imipenem resistant strains of *P. aeruginosa* and *A. baumannii*, we suggest that the number of MBLs is increasing and VIM-2 emerges as a novel β-lactamase in the region. To the best of our knowledge, this is the first report of VIM-2 metallo-beta-lactamase in imipenem-resistant Gram-negative non-fermenters from Iasi, a city in the North-East Romania. This is also the first report of a new class 1 integron structure harboring *bla*$_{VIM-2}$ in this area. Considering that the majority of the VIM-2 producing strains, isolated in different hospitals and different wards were clonally related, based on RAPD typing, we can conclude that the main mechanism for MBL spreading was clonal expansion. These findings suggest the need for better control measures to reduce cross infection.

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**Disclosure**

The authors have no conflict of interest to declare.

## Abbreviations

AK – amikacin
ATM – aztreonam
CAZ – ceftazidime
CIP – ciprofloxacin
CN - gentamicin
COL – colistin
DO – doxycycline
FEP – cefepime
IPM – imipenem
LEV – levofloxacin
MBL – metallo-β-lactamase
MEM – meropenem
MIC – minimum inhibitory concentration
RAPD - Random Amplified Polymorphic DNA
SAM - ampicillin-sulbactam
SXT - trimethoprim-sulfamethoxazole
TIC – ticarcillin
TOB – tobramycin
TZP - piperacillin-tazobactam

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