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Case Report

First VIM-Producing Representative of *Pseudomonas Putida* Group from the Largest Bulgarian Hospital

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Abstract

Here we describe the first detected VIM-2-producing representative of *Pseudomonas putida* group – *Pseudomonas kurunegalensis* from the largest Bulgarian hospital – St George University Hospital in Plovdiv.

A 59-year-old female patient with right-sided lung abscess was hospitalized in the Second Clinic of Thoracoabdominal Surgery. She was repeatedly treated for pulmonary infections. Punctate from the abscess cavity was taken for microbiological investigation. Identification process and antimicrobial susceptibility were performed by Vitek 2. The species group *P. putida* was confirmed with MALDI-TOF system and whole genome sequencing defined it as *P. kurunegalensis*. Antibiotic susceptibility testing revealed susceptibility only to tobramycin and colistin. All phenotypic tests for carbapenemase and metallo-beta-lactamase (MBL) production were positive. Multiplex PCR was performed to search for nine common carbapenemase encoding genes whereas the variable region of the integron was determined by DNA sequencing. Molecular assays confirmed the presence of *blaVIM-2* located within a typical Class I integron including also an *aacA29b* aminoglycoside N(6')-acetyltransferase casette.

Despite *P. putida* not being a common pathogen, it still could survive in hospital conditions causing difficult-to-treat infections and becoming a source of resistant genes, including MBL-encoding genes.

Keywords

extensively drug resistance, lung abscess, *P. putida* group, *P. kurunegalensis*, VIM-2 metallo-beta-lactamase

INTRODUCTION

Pseudomonas putida, until recently considered an opportunist with low pathogenic potential, is often misdiagnosed as *Pseudomonas aeruginosa* (85% genome similarity) due to similar pigment production.[1] Cases of difficult-to-treat nosocomial infections, including bacteremia, pneumonia, urinary tract infections and others, caused by multidrug-resistant (MDR) and carbapenem-resistant strains, are increasingly reported in a number of European, Asian, and American hospitals, mostly in critically ill and immu-

nocompromised patients, but also in newborns.[2-7]

For the period of 10 years (till February 15, 2022) 76 cases of *P. putida* were reported from the Chest and Respiratory Disease Hospital, Ankara, Turkey – 17% resistant to meropenem and 14% to imipenem. All samples were pulmonary in origin.[8]

Exacerbation of bronchiectasis by *Pseudomonas putida* complicating COVID-19 disease is reported by *Georgakopoulou et al.* in 2021. Antimicrobial susceptibility testing showed that this strain is pansusceptible. The patient presented with improvement after three days of piperacillin-tazobactam administration.[9]

VIM-2 metallo-β-lactamase-producing *Pseudomonas putida* are detected in Blattella germanica cockroaches in an Algerian hospital, Africa, confirming their prevalence in a hospital environment.[10]

The first VIM-5-producing *P. putida* in the Balkan Peninsula was isolated in Turkey in 2008. A 25-year-old patient with esophageal perforation due to a vehicular accident was hospitalized in the chest surgery clinic of Firat University Medical Center in Turkey. He developed central venous catheter infection due to carbapenem-resistant *P. putida* strain. Antimicrobial susceptibility testing performed by disk diffusion and E-test showed resistance to all β-lactams including imipenem and meropenem. The patient subsequently received combined thera y with ceftazidime, ciprofloxacin and amikacin, and recovered fully.[11]

Since then, to the best of our knowledge, no other cases of VIM-producing *P. putida* have been reported in our neighboring countries.

Similar strains have been detected in other European countries. Between January 2004 and May 2007, *P. putida* isolates were reported in two hospitals in Belgium in ten patients (five VIM-4 producing from hospital 1 and five VIM-2 producing in hospital 2, respectively) with serious underlying diseases (liver cirrhosis, meningitis, polytrauma, cancer, etc.), who have been treated for more than 10 days in intensive care units and had received prior broad-spectrum antimicrobial therapy.

All but one of these strains were isolated from urine and the last from a tracheal aspirate, and all showed high-level resistance to imipenem and meropenem. In eight of the patients, the isolates were considered to be colonization, five of the cases were fatal. The other two patients with urinary infection underwent colistin therapy and were successfully cured.[5]

Three isolates of *Pseudomonas putida* harboring blaVIM-2 were isolated from surfaces in pediatric women's sanitary facilities at the Hospital Infante D. Pedro, Aveiro, Portugal.[12]

The first two carbapenem-resistant blaVIM-2 producing *Pseudomonas putida* from Spain were reported at the University Hospital Complex of Santiago de Compostela. Both were isolated from immunocompromised patients with serious underlying diseases and hospitalized for more than 15 days. Both isolates showed susceptibility only to amikacin and colistin.[13]

Here we report the first proven VIM-producing *P. putida* in our country in 2017 from the largest Bulgarian hospital – St George University Hospital in Plovdiv.

CASE REPORT

The strain was isolated from a 59-year-old woman, hospitalized in the Second thoracoabdominal Surgery Department at St George University Hospital in Plovdiv, with a clinical diagnosis of right-sided lung abscess. She has been repeatedly treated in outpatient and hospital settings for frequent bronchitis and pneumonia with antibiotics – amoxicillin/clavulanic acid, gentamicin, ciprofloxacin, imipenem/cilastatin. She was admitted to the hospital with a fever up to 38.9°C, coughing with expectoration and shortness of breath, stabbing pains in the right chest, and easy fatigue.

The imaging studies (X-ray and fibrobronchoscopy) demonstrated evidence of a right-sided lung abscess. No tumor cells were detected. There was no evidence of an endobronchial proliferative process. Functional examination of breathing was in normal limits.

Laboratory tests

Data of leukocytosis, increased procalcitonin and ERS (markers of inflammation) ***(Table 1)****.*

On the second day of surgery, the level of immunoglobulin IgM started decreasing, whereas the other immunoglobulins IgA and IgG stayed within normal limits ***(Table 2)****.*

Table 1. Preoperative laboratory blood tests

Indicator	Result	Reference range
White blood cells	↑13.9×10^9/l	3.5–10.5×10^9/l
Red blood cells	4.8×10^{12}/l	4.5–69×10^{12}/l
Platelets	310×10^9/l	140–400×10^9/l
Neutrophils	↑77 %	40–70 %
Monocytes	8%	1–14%
Lymphocytes	33%	22–48%
Albumin	38 g/l	35–55 g/l
Procalcitonin	↑2.94 ng/ml	<0.5 ng/ml
ERS (Westergren)	↑26 mm/h	≤20 mm/h for women over 50 years old

Table 2. Serum immunoglobulins on the second day after surgery

Indicator	Result	Reference range
IgM	↓0.248 g/l	0.4–2.3 g/l
IgA	1.363 g/l	0.7–4.5 g/l
IgG	10.350 g/l	7–16 g/l

Operative intervention

Thoracotomy with bilobectomia dextra was performed. A chest drain was placed on active aspiration.

Therapy

The treatment was started with cefotaxime (2×1 g. I.V.) + flagyl (3×500 mg I.V.) daily.

Material was taken for microbiological examination intraoperatively (punctate from the abscess cavity).

Microbiological laboratory result

P. putida were susceptible only to colistin and tobramycin.

After the microbiological result, a correction of the antibiotic therapy was conducted and the patient was treated with tobramycin 80 mg/2 ml twice a day in intravenous infusion for 8 days.

The chest drain was removed on time. The operative wound healed primarily.

The patient was discharged 11 days after surgery with improved general condition – permanently afebrile, without subjective complaints, able to eat and walk.

Microbiological and genetic analysis

The identification and antimicrobial susceptibility of the isolate were performed by Vitek 2 automated system with subsequent MALDI-TOF analysis (both BioMérieux, France) which confirmed it as P. putida. The whole genome sequencing performed later defined it as P. kurunegalensis, belonging to the Pseudomonas putida group.[14] The genome has been deposited in GenBank – BioSample: SAMN37404455.[15]

For the detection of total carbapenemase production, the Modified Hodge test[16] and the Modified CarbaNP v3 test[17] were used. Screening for metallo-beta-lactamase production was performed with an E-test imipenem/imipenem+EDTA (Liofilchem).

Multiplex PCR for nine carbapenemase-encoding genes (VIM, IMP, SIM, GIM, SPM, NDM, GES, KPC, OXA-48) was us ed to determine the type of enzyme, and the variable region of class I integron was determined by DNA sequencing.

The P. kurunegalensis isolate demonstrated resistance to the entire beta-lactam group, including the two carbapenems imipenem and meropenem (MIC>16 μg/ml), preserving susceptibility only to colistin (MIC <2 μg/ml) and tobramycin (MIC <4 μg/ml), EUCAST 2017 – and was characterized as extensively drug resistant (XDR).

*The phenotypic methods applied were positive for carbapenemase **(Fig. 1)** and metallo-beta-lactamase production **(Fig. 2)**, respectively. Capillary electrophoresis by multiplex PCR confirmed the presence of blaVIM **(Fig. 3)** and DNA sequencing (Sanger method) identified it as blaVIM-2 located in the typical class I integron, also including the aacA29b aminoglycoside N(6')-acetyltransferase cassette. The presence of this acetyltransferase explains the resistance to the aminoglycosides amikacin and gentamicin **(Fig. 4)**.*

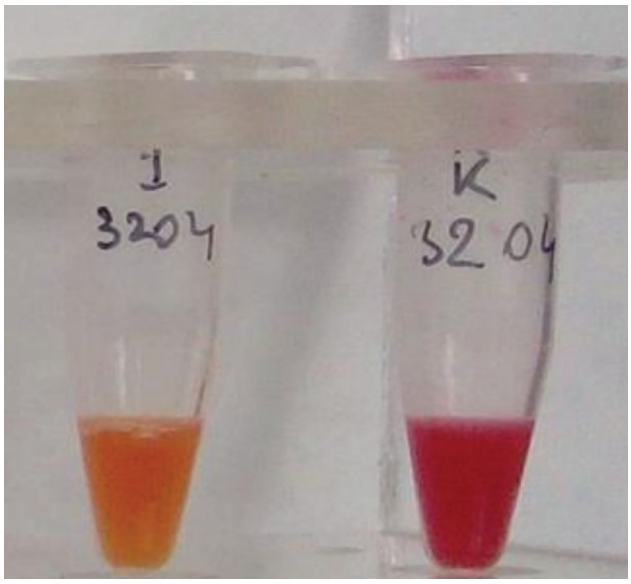

Figure 1. Modified Carba NP v3 test for carbapenemase production: right (in red) positive *P. putida*, left (in yellow) KPC negative control.

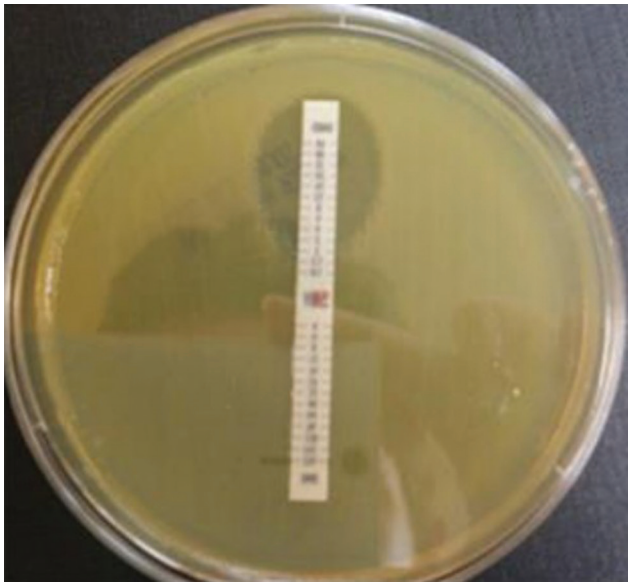

Figure 2. Positive E-test with imipenem/imipenem+EDTA (Liofilchem) for metallo-beta-lactamase production.

DISCUSSION

Although *P. putida* is not a frequent pathogen, it successfully survives in hospital settings and is associated with severe and difficult-to-treat infections, sometimes fatal, due to innate and acquired resistance to a number of antimicrobial agents.[18-22] This often makes difficult or hinders timely therapy. Strains *P. putida* reported in the international literature were isolated mostly from inpatients, but also from outpatients.[22,23] In Tan et al.'s study[22], 24 of the 32 (75%) hospitalized patients were infected with MDR strains *P. putida*. In case of traumatic injuries, these microorganisms

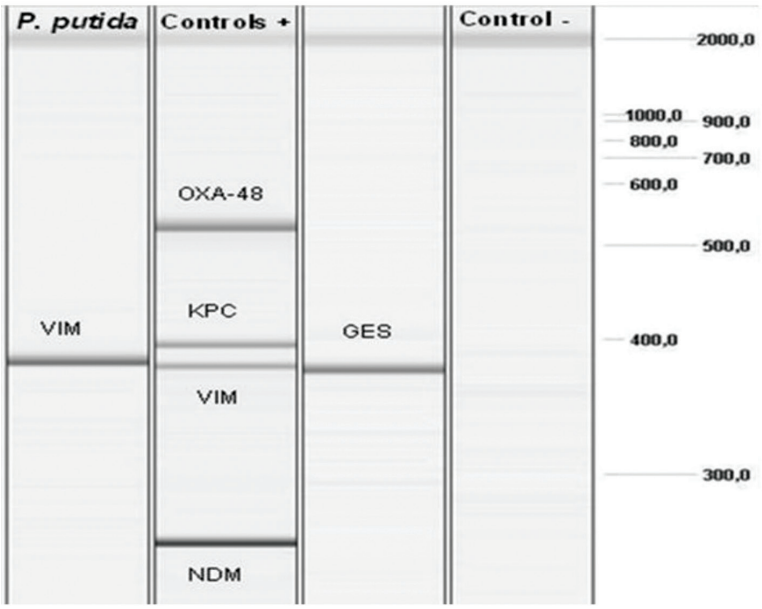

Figure 3. Capillary electrophoresis by multiplex PCR for the screening of 9 genes encoding carbapenemases (OXA-48, KPC, VIM, NDM, SIM, GIM, IMP, GES, SPM) of the *P. putida* isolate. The result is positive for the presence of VIM-coding genes.

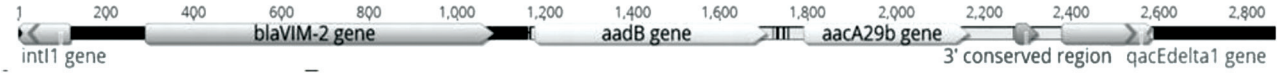

Figure 4. Class 1 integron encoding the VIM-2 gene in association with the gene encoding the acetyltransferase – *aacA29b* in our *P. putida*.

may cause infections in young healthy people.[23]

Until recently, *P. putida* was considered a bacterium with low toxicity and weak pathogenicity. However, some studies indicate that the mortality rate in patients with bacteremia and underlying disease can be as high as 40%.[6,19,22]

At the same time, some carbapenem-resistant isolates are a source of MBL-encoding genes, which requires strict epidemiological and microbiological control in the hospital environment.[24,25]

CONCLUSION

The presented clinical case emphasizes the need for precise microbiological diagnosis of *Pseudomonas putida*, thorough performance and interpretation of antimicrobial resistance tests. Determination of the production and the type of carbapenemases is particularly important for the adequate antibiotic therapy. Patients infected with carbapenem-resistant strains should be reported and be a subject of strict infection control measures.

Ethical approval

All procedures performed in the present study were in accordance with the ethical standards of the institution and with the 1964 Helsinki Declaration and its later amendments. Written informed consent was obtained from the patient.

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REFERENCES

1. Chakrabarty AM, Gunsalus IC. Transduction and genetic homology between Pseudomonas species putida and aeruginosa. J Bacteriol 1970; 103(3):830–2.
2. Agata ED. [Pseudomonas aeruginosa and other Pseudomonas species]. Mandell, Douglas y Bennett. Enfermedades infecciosas. Principios y práctica 2018; 2656–70 [Spanish].
3. Aguilar C, Maramba-lazarte C. A cross-sectional analysis of neonatal bacteriemia in the neonatal intensive care unit of the Philippine general hospital. PIDSP J 2011; 12(1):17–27.
4. Picollo M, Ferraro DK, Pérez G, et al. [Pseudomonas putida bacteremia in children: a case series]. Enferm Infecc Microbiol Clin 2023; 41(4):221–4 [Spanish].
5. Bogaerts P, Huang TD, Rodriguez-Villalobos H, et al. Nosocomial infections caused by multidrug-resistant Pseudomonas putida isolates producing VIM-2 and VIM-4 metallo-β-lactamases. J Antimicrob Chemother 2008; 61(3):749–51.

6. Kim SE, Park S-H, Park HB, et al. Nosocomial *Pseudomonas putida* bacteremia: high rates of carbapenem resistance and mortality. Chonnam Med J [Internet]. 2012; 48(2):91. Available from: https://synapse.koreamed.org/DOIx.php?id=10.4068/cmj.2012.48.2.91
7. Almuzara M, Radice M, de Gárate N, et al. VIM-2–producing Pseudomonas putida, Buenos Aires. Emerg Infect Dis 2007; 13(4):668.
8. Baykal H, Çelik D, Ülger AF, et al. Clinical features, risk factors, and antimicrobial resistance of pseudomonas putida isolates. Med (United States) 2022; 101(48):E32145.
9. Georgakopoulou V, Avramopoulos P, Papalexis P, et al. Exacerbation of bronchiectasis by Pseudomonas putida complicating COVID-19 disease: A case report. Exp Ther Med 2021;22(6):1–4.
10. Loucif L, Cherak Z, Chamlal N, et al. First detection of VIM-2 metallo-β-lactamase-producing Pseudomonas putida in Blattella germanica cockroaches in an Algerian hospital. Antimicrob Agents Chemother 2017; 61(8):10–128.
11. Poirel L, Yakupoğullari Y, Kizirgil A, et al. VIM-5 metallo-beta-lactamase-producing Pseudomonas putida from Turkey. Int J Antimicrob Agents [Internet] 2009: 33(3):287 [cited 2016 Nov 15]. Available from: http://www.ijaaonline.com/article/S0924857908003348/fulltext
12. Santos C, Caetano T, Ferreira S, et al. Tn5090-like class 1 integron carrying blaVIM-2 in a Pseudomonas putida strain from Portugal. Clin Microbiol Infect 2010; 16(10):1558–61.
13. Treviño M, Moldes L, Hernández M, et al. Nosocomial infection by VIM-2 metallo-beta-lactamase-producing Pseudomonas putida. J Med Microbiol [Internet] 2010; 59(Pt 7):853–5. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20360397
14. Girard L, Lood C, Höfte M, et al. The ever-expanding pseudomonas genus: Description of 43 new species and partition of the pseudomonas putida group. Microorganisms 2021; 9(8):1766.
15. Genome assembly ASM3202744v1. Available from: https://www.ncbi.nlm.nih.gov/ datasets/genome/GCA_032027445.1
16. Amjad A, Mirza IA, Abbasi SA, et al. Modified Hodge test: A simple and effective test for detection of carbapenemase production. Iran J Microbiol 2011; 3(4):189–93.
17. Ivanova K. [Beta-lactamases with carbapenemase activity – microbiological and molecular genetic studies]. (PhD dissertation). Sofia, 2017 [Bulgarian].
18. Lombardi G, Luzzaro F, Docquier JD, et al. Nosocomial infections caused by multidrug-resistant isolates of Pseudomonas putida producing VIM-1 metallo-β-lactamase. J Clin Microbiol 2002; 40(11):4051–5.
19. Yoshino Y, Kitazawa T, Kamimura M, et al. Pseudomonas putida bacteremia in adult patients: Five case reports and a review of the literature. J Infect Chemother 2011; 17(2):278–82.
20. Anaissie E, Fainstein V, Miller P, et al. Pseudomonas putida. Newly recognized pathogen in patients with cancer. Am J Med 1987; 82(6):1191–4.
21. Thomas BS, Okamoto K, Bankowski MJ, et al. A lethal case of Pseudomonas putida bacteremia due to soft tissue infection. Infect Dis Clin Pract 2013; 21(3):e13–5.
22. Tan G, Xi Y, Yuan P, et al. Risk factors and antimicrobial resistance profiles of Pseudomonas putida infection in Central China, 2010-2017. Medicine (Baltimore) 2019; 98(44):e17812.
23. Carpenter RJ, Hartzell JD, Forsberg JA, et al. Pseudomonas putida war wound infection in a US Marine: a case report and review of the literature. J Infect 2008; 56(4):234–40.
24. Peter S, Oberhettinger P, Schuele L, et al. Genomic characterisation of clinical and environmental Pseudomonas putida group strains and determination of their role in the transfer of antimicrobial resistance genes to Pseudomonas aeruginosa. BMC Genomics 2017; 18(1):1–11.
25. Hong JS, Yoon EJ, Song W, et al. Molecular characterization of Pseudomonas putida group isolates carrying blaVIM-2 disseminated in a University Hospital in Korea. Microb Drug Resist 2018; 24(5):627–34.

Первый представитель группы *Pseudomonas Putida*, производящий VIM, из крупнейшей болгарской больницы

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Резюме

Здесь мы описываем первого обнаруженного представителя группы *Pseudomonas putida*, продуцирующего VIM-2 – *Pseudomonas kurunegalensis* из крупнейшей болгарской больницы – УМБАЛ „Св. Георги“ в Пловдиве.

59-летняя пациентка с правосторонним абсцессом лёгкого была госпитализирована во Вторую клинику торакоабдоминальной хирургии. Она неоднократно лечилась от лёгочных инфекций. Пунктат из полости абсцесса был взят для микробиологического исследования. Процесс идентификации и чувствительность к антимикробным препаратам были выполнены с помощью Vitek 2. Видовая группа *P. putida* была подтверждена с помощью системы MALDI-TOF, а секвенирование всего генома определило её как *P. kurunegalensis*. Тестирование чувствительности к антибиотикам выявило чувствительность только к тобрамицину и колистину. Все фенотипические тесты на продукцию карбапенемазы и металло-бета-лактамазы (MBL) были положительными. Мультиплексная PCR была проведена для поиска девяти общих генов, кодирующих карбапенемазу, тогда как вариабельная область интегрона была определена с помощью секвенирования ДНК. Молекулярные анализы подтвердили наличие *blaVIM*-2, расположенного внутри типичного интегрона класса I, включая также кассету аминогликозид N(6')-ацетилтрансферазы *aacA29b*.

Несмотря на то, что *P. putida* не является распространённым патогеном, он всё же может выживать в условиях больницы, вызывая трудно поддающиеся лечению инфекции и становясь источником резистентных генов, включая гены, кодирующие MBL.

Ключевые слова

широкая лекарственная устойчивость, абсцесс лёгкого, группа *P. putida*, *P. kurunegalensis*, металло-бета-лактамаза VIM-2