



# First case of IMP-15-producing *Pseudomonas aeruginosa* in Switzerland: Challenging laboratory diagnosis using whole genome sequencing (WGS)

Daniel Goldenberger<sup>a,\*</sup>, Danielle Vuichard-Gysin<sup>b</sup>, Kathrin Herzog<sup>c</sup>, Vladimira Hinić<sup>a</sup>, Helena Seth-Smith<sup>a,d</sup>, Adrian Egli<sup>a,d</sup>

<sup>a</sup> Division of Clinical Bacteriology and Mycology, University Hospital Basel and University of Basel, Basel, Switzerland

<sup>b</sup> Division of Infectious Diseases and Hospital Epidemiology, Thurgau Hospital Group, Muensterlingen and Frauenfeld, Switzerland

<sup>c</sup> Laboratory for Clinical Microbiology, Thurgau Hospital Group, Muensterlingen and Frauenfeld, Switzerland

<sup>d</sup> Applied Microbiology Research, Department of Biomedicine, University of Basel, Switzerland

## ARTICLE INFO

### Article history:

Received 14 July 2020

Received in revised form 20 August 2020

Accepted 20 August 2020

### Keywords:

*bla*<sub>IMP-15</sub>

Carbapenemase-producing *Pseudomonas*

*aeruginosa*

Metallo- $\beta$ -lactamase

Switzerland

Cambodia

Whole genome sequencing (WGS)

## ABSTRACT

We report on the first case of multiresistant *Pseudomonas aeruginosa* harbouring the metallo- $\beta$ -lactamase IMP-15 isolated in Switzerland from a patient repatriated from Cambodia. The laboratory diagnosis of IMP-15 was hampered by two negative tests for carbapenemase detection. The carbapenemase gene was subsequently detected by whole genome sequencing and the isolate further characterised by various phenotypic and genotypic analyses.

© 2020 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## Introduction

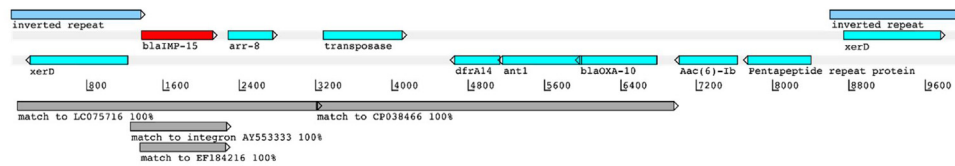
The worldwide emergence of multidrug resistant bacteria is a major threat to public health. *Pseudomonas aeruginosa* is one of the major pathogens causing nosocomial infections like bacteremia and pneumonia. Carbapenemases, especially the metallo- $\beta$ -lactamases (MBLs) such as IMP, VIM, SPM, GIM, NDM, and FIM are one of the most important mechanisms responsible for resistance to  $\beta$ -lactams including carbapenems in *P. aeruginosa*. Moreover, such resistance genes can be transferred to further bacterial species on mobile genetic elements [1]. In 1988, the *bla*<sub>IMP-1</sub> was first documented in Japan on a conjugative plasmid [2]. During the last years, multiple case reports and hospital epidemics have reported *bla*<sub>IMP</sub> variants worldwide. Here, we present the first case of a *P. aeruginosa* harbouring a *bla*<sub>IMP-15</sub> isolated from a patient in Switzerland repatriated from Cambodia.

## Case

We report the case of a 33-year-old male patient from the north-eastern part of Switzerland, who was successfully resuscitated after a cardiac arrest of unknown cause in his holiday apartment in Cambodia on December 24, 2019. After initial supportive treatment at a provincial hospital in Cambodia, he was transferred to an intensive care unit in Bangkok, Thailand, still unconscious and in a critical state, intubated and on vasopressors. Chest radiograph showed bilateral patchy infiltration. Findings on electro-encephalography were compatible with profound diffuse encephalopathy. Repatriation took place on January 3, 2020. On admission onto intensive care unit in Switzerland, the patient was placed under preventive contact isolation measures due to high risk of colonization with multi-drug resistant organisms. A sputum culture from Bangkok revealed growth of *Pseudomonas aeruginosa* resistant against tigecycline and *Acinetobacter baumannii* with intermediate susceptibility to ceftazidime and resistance against tazobactam. Susceptibility testing was otherwise unremarkable. Based on these microbiology results, broad-spectrum parenteral antibiotic treatment with meropenem 500 mg every 12 h (adapted to impaired renal function) was initiated for bilateral pneumonia as per internal guideline. Unfortunately, the patient's condition

\* Corresponding author at: Division of Clinical Bacteriology and Mycology, University Hospital Basel and University of Basel, Petergraben 4, CH-4031, Basel, Switzerland.

E-mail address: [Daniel.Goldenberger@usb.ch](mailto:Daniel.Goldenberger@usb.ch) (D. Goldenberger).



**Fig. 1.** *bla*<sub>IMP-15</sub> containing 10Kb region. The region is flanked by 1355bp inverted repeats covering genes encoding tyrosine recombinase XerD. The *bla*<sub>IMP-15</sub> is shown in red, along with other annotated genes in the region in forward or reverse frames. Matches to database sequences are shown below in grey. Figure was adapted from data visualized in Artemis [5].

deteriorated and he deceased on January 6, 2020. After his death, culture from bronchial aspirate revealed the presence of *Burkholderia cepacia* complex, being phenotypically susceptible to ceftazidime and trimethoprim/sulfamethoxazole only, and *Pseudomonas aeruginosa* resistant to all regularly tested antimicrobials except gentamicin. In addition, a rectal swab performed during routine admission screening showed growth of *Burkholderia cepacia* complex and *Pseudomonas aeruginosa* as well but was otherwise negative for vancomycin resistant enterococci.

The *P. aeruginosa* isolated from bronchial aspirate was sent to the microbiology laboratory of the University Hospital Basel for identification of underlying resistance mechanism. The antimicrobial susceptibility testing performed with Vitek<sup>®</sup> 2 (bioMérieux) revealed resistance to all antimicrobial agents tested except meropenem, interpreted as intermediate, and tobramycin, amikacin and colistin, interpreted as susceptible. The isolate was tested with Xpert<sup>®</sup> Carba-R (Cepheid) and exhibited negative results for the following carbapenemase gene targets: KPC, NDM, VIM, OXA-48-variants and IMP. In order to screen for MBL phenotype, combined Etest<sup>®</sup> strip using imipenem with and without EDTA (Etest<sup>®</sup> MBL IP/IPi; bioMérieux) was performed. Since MBL was excluded with this phenotypic test, we presumed that mechanisms other than carbapenemase production were responsible for carbapenem resistance in this *P. aeruginosa*. Later on, the isolate was submitted to whole genome sequencing (WGS) as part of a study to validate WGS for routine purposes. DNA from isolate 400128-20 was sequenced on both a NextSeq500 platform (Illumina) after Nexteraflex library production (Illumina), resulting in 64x mean coverage and GridION platform (Oxford Nanopore), resulting in 10x mean coverage to aid scaffolding. All reads have been submitted to the ENA under project PRJEB37060. The genome was hybrid assembled using Unicycler v0.3.0b [3], resulting in 18 contigs, annotated with Prokka v1.13 [4] and visualized in Artemis [5]. The isolate belongs to MLST sequence type 155 (<https://pubmlst.org/paeruginosa/>) [6]. The 741-bp-long metallo-beta-lactamase *bla*<sub>IMP-15</sub> gene was identified with 100 % identity, using ABRicate (<https://github.com/tseemann/abricate>) and the NCBI AMRFinderPlus database (doi: 10.1128/AAC.00483-19).

The *bla*<sub>IMP-15</sub> gene is located on a contig of 323Kb, part of the chromosome. The 10Kb region around the *bla*<sub>IMP-15</sub> gene seems to have derived from several plasmids, including LC075716 from *P. aeruginosa* in Vietnam (3.1Kb, 100 %) and CP038466 from *Aeromonas hydrophila* in China (3.8Kb, 100 %). This 10Kb section, flanked by two identical tyrosine recombinase genes, carries a transposase and several other antimicrobial resistance markers (Fig. 1; accession number ERZ1303326; Supplementary file). The identity with the *bla*<sub>IMP-15</sub> class I integron from *P. aeruginosa* in Thailand (accession number AY553333) covers only 997 bp (100 % identity) over the *bla*<sub>IMP-15</sub> gene itself; similarly the match to EF184216 from Mexico [8] covers 889bp (100 % identity) is also over the *bla*<sub>IMP-15</sub> gene (Table 1).

We performed two conventional IMP-targeted PCR tests to confirm the presence of *bla*<sub>IMP-15</sub> gene. The first PCR (A) was described in a study to characterize a hospital epidemic in Mexico in 2008 with *bla*<sub>IMP-15</sub> containing *P. aeruginosa* [8]. The second IMP PCR

**Table 1**

Overview of carbapenemase test results performed on IMP-15 producing *P. aeruginosa* isolate.

Carbapenemase test	Result
Phenotypic tests	
Etest <sup>®</sup> MBL IP/IPi	-
RAPIDEC <sup>®</sup> CARBA NP	+
β-CARBA Test	+
Carbapenem inactivation method mCIM	+
Genotypic tests	
Xpert <sup>®</sup> Carba-R <sup>a</sup>	-
IMP-15 PCR [8]	+
IMP PCR [9,10]	+

<sup>a</sup> Detects KPC, NDM, VIM, OXA-48-variants and IMP (not all IMP subtypes are covered by the assay).

(B) was performed according to references [9,10]. Both PCRs were performed on freshly extracted DNA and showed the expected DNA fragment length of 275bp for PCR (A) and 232bp for PCR (B) on the 4200 TapeStation system (Agilent Technologies). Nucleotide sequencing of both PCR fragments using Sanger sequencing in both directions resulted in 100 % identity to *bla*<sub>IMP-15</sub>. In order to further characterize our isolate, the following phenotypic tests for detection of carbapenemase production were performed: RAPIDEC<sup>®</sup> CARBA NP (bioMérieux), β-CARBA Test (BIO-RAD) and modified Carbapenem Inactivation Method mCIM according CLSI [11]. Interestingly, our isolate showed positive results with all three above mentioned assays. The results of all genotypic and phenotypic tests performed are shown in summary in Table 1.

## Discussion

The MBL variant *bla*<sub>IMP-15</sub> was described for the first time in 2005 in Thailand (GenBank accession no. AY553333) and was associated with a class I integron, genes encoding dihydrofolate reductase (*dhfr*) and aminoglycoside 6'-N-acetyltransferase (*aac* (6')). Further studies reported on IMP-15-producing multiresistant *P. aeruginosa*: three hospital outbreaks in Mexico in 2003–2005 and one case from USA related to one of the Mexican clones in 2005 [7,8,12,13]. The first *bla*<sub>IMP-15</sub> cases in Europe were communicated in 2013 with two *P. aeruginosa* and two *P. putida* strains [14]. In a retrospective study published in 2014, one case from Germany isolated in 2009 was identified [15]. Recently, four *P. aeruginosa* strains containing *bla*<sub>IMP-15</sub> were detected in Lebanon [16,17].

Due to their high transmissibility and potential for causing outbreaks in healthcare settings, timely recognition of patients colonized or infected with carbapenem-resistant gram-negative bacteria (GNB) is key to implementing appropriate preventive measures in order to stop these bacteria from further spread. Active surveillance for detection of asymptomatic colonization is therefore one of several strongly recommended infection control measures and the target population should include patients with recent hospitalization in countries with high prevalence of carbapenem-resistant GNB [18,19]. The carbapenemase activity of this *P. aeruginosa* strain could not be detected with our routine

diagnostic scheme for carbapenemase production using Xpert® Carba-R PCR and Etest® MBL IP/IPI. However, other phenotypic carbapenemase screening tests performed (Table 1) succeeded in detection of this rare carbapenemase type. Concerning the negative result in Xpert® CARBA-R, the manufacturer indicates that the following IMP variants are not covered with this assay: IMP-7, IMP-13, and IMP-14. According to our present data, IMP-15 has to be added to this list until the manufacturer is able to provide a PCR able to detect all IMP variants. The challenging identification of MBL *bla*<sub>IMP-15</sub> in our strain is worrying. Conventional testing with Etest® MBL IP/IPI was not able to detect IMP-15 production in this *P. aeruginosa* isolate in our laboratory, suggesting that such strains could be overlooked and therefore require a high degree of alertness from clinical microbiologists. If the presence of carbapenemase is suspected despite the negative screening test, a second screening test should be considered, or the isolate should be referred to a reference laboratory for further analyses.

Since the development of antimicrobial resistance in GNB is a constantly moving target, the absence of a genetic resistance determinant should be approached with caution, as organisms can harbour resistance mechanisms for which tests were not developed or are false negative.

In this case, the initial microbiology report from Bangkok evoked a false security in the treating physicians. It is therefore of major importance to expand empiric antibiotic therapy in a seriously ill patient if presence of a multi-drug resistant pathogen such as a carbapenemase producer has to be assumed based on epidemiological data or relevant exposure history.

WGS proved to be a powerful tool for revealing mechanism of resistance which could not be detected with conventional, routinely used methods. Unfortunately, this is a relatively novel technology which is still not widely available in the common microbiology laboratory practice.

#### Authors' contributions

DG collected and interpreted laboratory results and wrote the manuscript; DVG collected and interpreted clinical and epidemiological data and wrote the manuscript; KH collected and interpreted laboratory results; VH interpreted laboratory results, supervised phenotypic laboratory testing and reviewed the manuscript; HSS analysed WGS data and wrote the manuscript; AE supervised the study and reviewed the manuscript.

#### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

#### Consent

Written informed consent was obtained from the patient for publication of this case report. A copy of the written consent is available for review by the Editor-in-Chief of this journal on request.

#### Declaration of Competing Interest

The authors report no declarations of interest.

#### Acknowledgements

We thank Berfin Kocyigit, Benjamin Zahm, Daniel Gander and Elisabeth Schultheiss for excellent technical assistance.

Assemblies and searches were performed at sciCORE (<http://scicore.unibas.ch/>) scientific computing center at University of Basel.

#### Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.idcr.2020.e00933>.

#### References

- [1] Hong DJ, Bae IK, Jang IH, Jeong SH, Kang HK, Lee K. Epidemiology and characteristics of metallo-beta-lactamase-producing *Pseudomonas aeruginosa*. *Infect Chemother* 2015;47(2):81–97.
- [2] Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1991;35(1):147–51.
- [3] Wick RR, Judd LM, Gorrie CL, Holt KE. Completing bacterial genome assemblies with multiplex MinION sequencing. *Microb Genom* 2017;3(10):e000132.
- [4] Prokka Seemann T. Rapid prokaryotic genome annotation. *Bioinformatics* 2014;30(14):2068–9.
- [5] Carver T, Harris SR, Berriman M, Parkhill J, McQuillan JA. Artemis: an integrated platform for visualization and analysis of high-throughput sequence-based experimental data. *Bioinformatics* 2012;28(4):464–9.
- [6] Jolley KA, Bray JE, Maiden MCJ. Open-access bacterial population genomics: BIGSdb software, the PubMLST.org website and their applications. *Wellcome Open Res* 2018;3:124.
- [7] Garza-Ramos JU, Sanchez-Martinez G, Barajas JM, Suarez S, Sanchez-Perez A, Rojas-Moreno T, et al. Variability of the bla(IMP-15)-containing integrons, highly related to In95, on an endemic clone of *Pseudomonas aeruginosa* in Mexico. *Microb Drug Resist* 2010;16(3):191–5.
- [8] Garza-Ramos U, Morfin-Otero R, Sader HS, Jones RN, Hernandez E, Rodriguez-Noriega E, et al. Metallo-beta-lactamase gene bla(IMP-15) in a class 1 integron, In95, from *Pseudomonas aeruginosa* clinical isolates from a hospital in Mexico. *Antimicrob Agents Chemother* 2008;52(8):2943–6.
- [9] Ellington MJ, Kistler J, Livermore DM, Woodford N. Multiplex PCR for rapid detection of genes encoding acquired metallo-beta-lactamases. *J Antimicrob Chemother* 2007;59(2):321–2.
- [10] Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis* 2011;70(1):119–23.
- [11] CLSI. M100 Performance Standards for Antimicrobial Susceptibility Testing; 30th ed. Table 3C. Modified Carbapenem Inactivation Methods for Suspected Carbapenemase Production in *Enterobacteriales* and *Pseudomonas aeruginosa*. Wayne, PA: Clinical and Laboratory Standards Institute; 2020.
- [12] Martin CA, Morita K, Ribes JA, Deshpande LM, Sader HS, Castanheira M. IMP-15-producing *Pseudomonas aeruginosa* strain isolated in a U.S. medical center: a recent arrival from Mexico. *Antimicrob Agents Chemother* 2008;52(6):2289–90.
- [13] Quinones-Falconi F, Galicia-Velasco M, Marchiaro P, Mussi MA, Ballerini V, Vila AJ, et al. Emergence of *Pseudomonas aeruginosa* strains producing metallo-beta-lactamases of the IMP-15 and VIM-2 types in Mexico. *Clin Microbiol Infect* 2010;16(2):126–31.
- [14] Gilarranz R, Juan C, Castillo-Vera J, Chamizo FJ, Artilles F, Alamo I, et al. First detection in Europe of the metallo-beta-lactamase IMP-15 in clinical strains of *Pseudomonas putida* and *Pseudomonas aeruginosa*. *Clin Microbiol Infect* 2013;19(9):E424–427.
- [15] Castanheira M, Deshpande LM, Costello A, Davies TA, Jones RN. Epidemiology and carbapenem resistance mechanisms of carbapenem-non-susceptible *Pseudomonas aeruginosa* collected during 2009–11 in 14 European and Mediterranean countries. *J Antimicrob Chemother* 2014;69(7):1804–14.
- [16] Al Bayssari C, Diene SM, Loucif L, Gupta SK, Dabboussi F, Mallat H, et al. Emergence of VIM-2 and IMP-15 carbapenemases and inactivation of oprD gene in carbapenem-resistant *Pseudomonas aeruginosa* clinical isolates from Lebanon. *Antimicrob Agents Chemother* 2014;58(8):4966–70.
- [17] Yaghi J, Fattouh N, Akkawi C, El Chamy L, Maroun RG, Khalil G. Unusually high prevalence of cosecretion of amblar class A and B carbapenemases and nonenzymatic mechanisms in multidrug-resistant clinical isolates of *Pseudomonas aeruginosa* in Lebanon. *Microb Drug Resist* 2020;26(2):150–9.
- [18] Magiorakos AP, Burns K, Rodriguez Bano J, Borg M, Daikos G, Dumpis U, et al. Infection prevention and control measures and tools for the prevention of entry of carbapenem-resistant Enterobacteriaceae into healthcare settings: guidance from the European Centre for Disease Prevention and Control. *Antimicrob Resist Infect Control* 2017;6:113.
- [19] WHO. Guidelines for the prevention and control of carbapenem-resistant Enterobacteriaceae, Acinetobacter baumannii and *Pseudomonas aeruginosa* in health care facilities. 2017 Licence: CC BY-NC-SA 3.0 IGO.