# Emergence of plasmid-encoded VIM-2–producing *Pseudomonas aeruginosa* isolated from clinical samples in Lebanon

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## Abstract

The present study aimed to describe the emergence of carbapenem-resistant *Pseudomonas aeruginosa* isolated from clinical Lebanese patients. The resistance of these isolates is due to the presence of the plasmid-encoded *bla*$_{VIM-2}$ gene. We provide its first description in Lebanon, as well as a description of disruption of the *oprD* gene by mutations.


**Keywords:** Carbapenem resistance, *oprD*gene, *Pseudomonas aeruginosa*, VIM-2
**Original Submission:** 24 October 2018; **Revised Submission:** 6 February 2019; **Accepted:** 13 February 2019
**Article published online:** 22 February 2019

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*Pseudomonas aeruginosa* is an important pathogen that is the main cause of acute nosocomial infections, especially in immunocompromised patients [1]. The resistance of *P. aeruginosa* to carbapenem is becoming a major global threat and is exacerbated by the excessive use of carbapenem [2,3]. This resistance is mainly due to the alteration or loss of the outer membrane porin protein (*oprD*), to the increased expression of the efflux pumps and the production of carbapenemase, mainly Verona integron-encoded metallo-β-lactamase (VIM) and imipenem (IMP) [2,3].

In this study, we report the emergence of carbapenem-resistant *P. aeruginosa* isolated from rectal swabs of 23 intensive care unit patients treated with carbapenem for more than 1 week between October 2016 and February 2017 from Saint-George Hospital in Lebanon. Carbapenem-resistant organisms were screened using agar plates with Ertapenem (2 μg/mL). Four carbapenem-resistant *P. aeruginosa* were isolated and identified by MALDI-TOF MS. Antimicrobial susceptibility testing was performed on Müller-Hinton agar using the disc diffusion method, and Etest was performed to determine the MIC of IMP, as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (https://www.sfm-microbiologie.org/wp-content/uploads/2019/01/CASFM2019_V1.0.pdf). The phenotypic detection of carbapenemase was confirmed using the Carba NP test [4]. The carbapenemase encoding genes were screened by real-time PCR and standard PCR, and were then sequenced. Molecular characterization of the *oprD* gene was performed using PCR amplification and sequencing [5]. Analysis of the sequenced *oprD* gene was compared against the reference strain *P. aeruginosa* PA01 using Multalin alignment software (http://multalin.toulouse.inra.fr/multalin/). Multilocus sequence typing (MLST) was performed to determine the genetic relationship among the clinical isolate as described on the Institute Pasteur's MLST website (https://pubmlst.org/paeruginosa/).

The results indicated that *P. aeruginosa* isolates were resistant to all antibiotics tested except to colistin and fosfomycin, with MICs for IMP >32 μg/mL. All isolates harboured the *bla*$_{VIM-2}$ gene, except *P. aeruginosa* (PA-4) (Table 1). In addition, all isolates had mutations in the *oprD* gene (Fig. 1). MLST analysis revealed that three *P. aeruginosa* (PA-3, PA-6, PA-16), and one *P. aeruginosa* (PA-4) isolates harboured sequence types (ST) 357 and ST233 respectively (Table 1). Conjugal transfer between carbapenemase-producing *P. aeruginosa* and *Escherichia coli* (J35) succeeded, to yield *E. coli* transconjugants harbouring a ~45 kb plasmid, except for the clone ST233, suggesting that these metallo-β-lactamase *bla*$_{VIM-2}$ were plasmid encoded for ST357 and chromosomally encoded for ST233.

Here we describe the emergence of carbapenem-resistant *P. aeruginosa* in Saint-Georges Hospital due to the presence of *bla*$_{VIM-2}$ gene and mutations of the *oprD* gene. These results are in concordance with those previously reported in Lebanon, where Al Bayssari et al. [3,6] have reported the emergence of VIM-2–producing *P. aeruginosa* in humans and animals. Other studies have also shown the spread of VIM-2–producing *P. aeruginosa* in different Lebanese hospitals [7,8]. In addition,



https://doi.org/10.1016/j.nmni.2019.100521

**TABLE 1. Phenotypic and genotypic features of carbapenem-resistant clinical isolates**

| *Pseudomonas aeruginosa* strain | Source | Antibiotic resistance profile | IMP MIC (μg/mL) | Carba NP test | VIM-2 | ST |
|---|---|---|---|---|---|---|
| PA-3 | Rectal swab | TIC, TCC, TZP, CAZ, FEP, IMP, ERT, AK, TOB, CIP, F, DO, SXT, R | >32 | + | + | 357 |
| PA-16 | Rectal swab | TIC, TCC, TZP, CAZ, FEP, IMP, ERT, AK, TOB, CIP, F, DO, SXT, R | >32 | + | + | 357 |
| PA-6 | Rectal swab | TIC, TCC, TZP, CAZ, FEP, IMP, ERT, AK, TOB, CIP, F, DO, SXT, R | >32 | + | + | 357 |
| PA-4 | Rectal swab | TIC, TCC, TZP, CAZ, FEP, IMP, ERT, AK, TOB, CIP, F, DO, SXT,R | >32 | + | − | 233 |

AK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; DO, doxycycline; ERT, ertapenem; F, nitrofurantoin; FEP, cefepime; FF, fosfomycin; IPM, imipenem; R, rifampicin; ST, sequence, type; SXT, trimethoprim/sulfamethoxazole; TCC, ticarcillin/clavulanic, acid; TIC, ticarcillin; TOB, tobramycin; TZP, piperacillin/tazobactam.

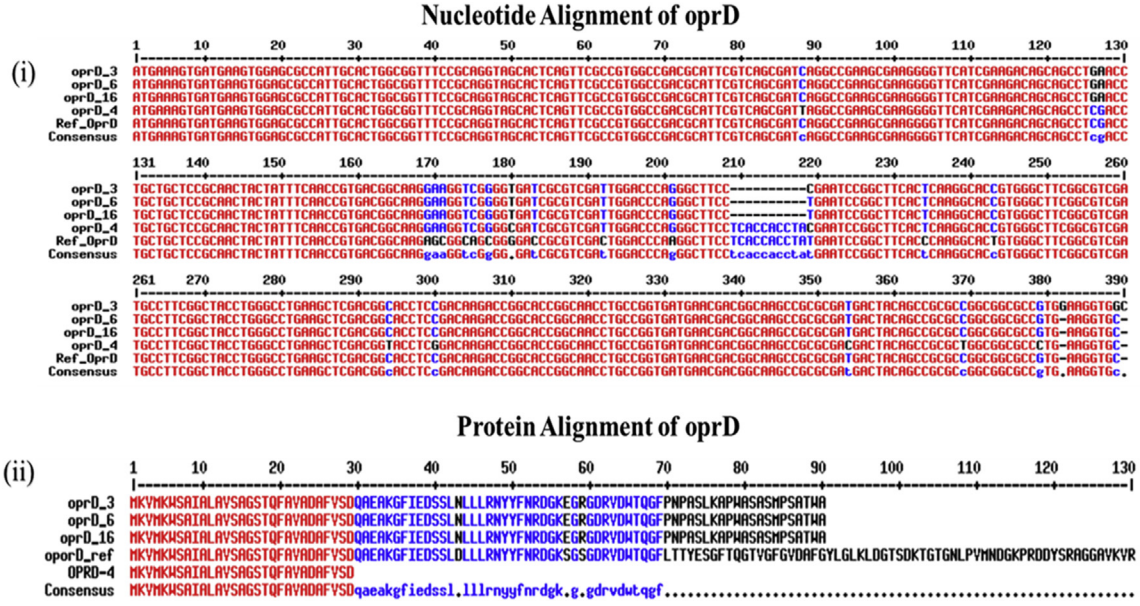


**FIG. 1.** Genetic representation of *oprD* gene. (a) Nucleotide alignment of *oprD* gene and (b) protein alignment of *oprD* gene. For *Pseudomonas aeruginosa* PA-3, PA-16 and PA-6, large deletion of ten nucleotides from position 209 to 218 led to premature stop codon TGA in *oprD,* resulting in truncated polypeptide made of 90 aa residues. For *P. aeruginosa* PA-4, C to T substitution in nucleotide position 88 led to premature stop codon TAG in *oprD,* resulting in truncated polypeptide made of 29 aa residues.

Christophy et al. [9] revealed the presence of the $bla_{VIM}$ gene in *P. stutzeri* collected from cancer patients in North Lebanon. However, none of those studies has revealed a plasmidic location of the $bla_{VIM-2}$ gene. Our study also demonstrated that mutations leading to premature stop codon resulting in a defective protein *oprD* were the main cause of *P. aeruginosa*'s resistance to carbapenem, as described above [3,10]. The main finding in our study was the emergence of *P. aeruginosa* harbouring the VIM-2 plasmid, which has never been detected before in Lebanon, where all detected isolates had the chromosomic $bla_{VIM-2}$ gene, or the studies did not specify the genetic location of the $bla_{VIM}$ gene [3]. MLST analysis showed that the three *P. aeruginosa* isolates harbouring the plasmid-encoded $bla_{VIM-2}$ gene belonged to the ST357 clone, which has been found in different countries of central Europe [11]. However, *P. aeruginosa* ST233, which has the chromosomic $bla_{VIM-2}$ gene, has already been described in Lebanon as well as in various countries in the Mediterranean basin [3,12].

To conclude, our study is the first to report the detection of the plasmid-encoded $bla_{VIM-2}$ gene in Lebanon. This finding poses a serious public health problem because the plasmid containing this β-lactamase is a major source of dissemination of this enzyme. An urgent strategy must be implemented to control the spread of these resistant microorganisms in hospitalized patients.

## Acknowledgements

The authors thank CookieTrad for English-language editorial work. Supported in part by the Lebanese Council for Research and the French government under the 'Investissements

d'avenir' programme managed by the Agence Nationale de la Recherche (Méditerranée Infection 10-IAHU-03).

## Conflict of interest

None declared.

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