

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.

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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 μ g/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_VI.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* PAO1 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 μ g/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,

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

<i>Pseudomonas aeruginosa</i> 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, eropenem; F, nitrofurantoin; FEP, cefepime; FF, fosfomycin; IMP, imipenem; R, rifampicin; ST, sequence type; SXT, trimethoprim/sulfamethoxazole; TCC, ticarcillin/clavulanic acid; TIC, ticarcillin; TOB, tobramycin; TZP, piperacillin/tazobactam.

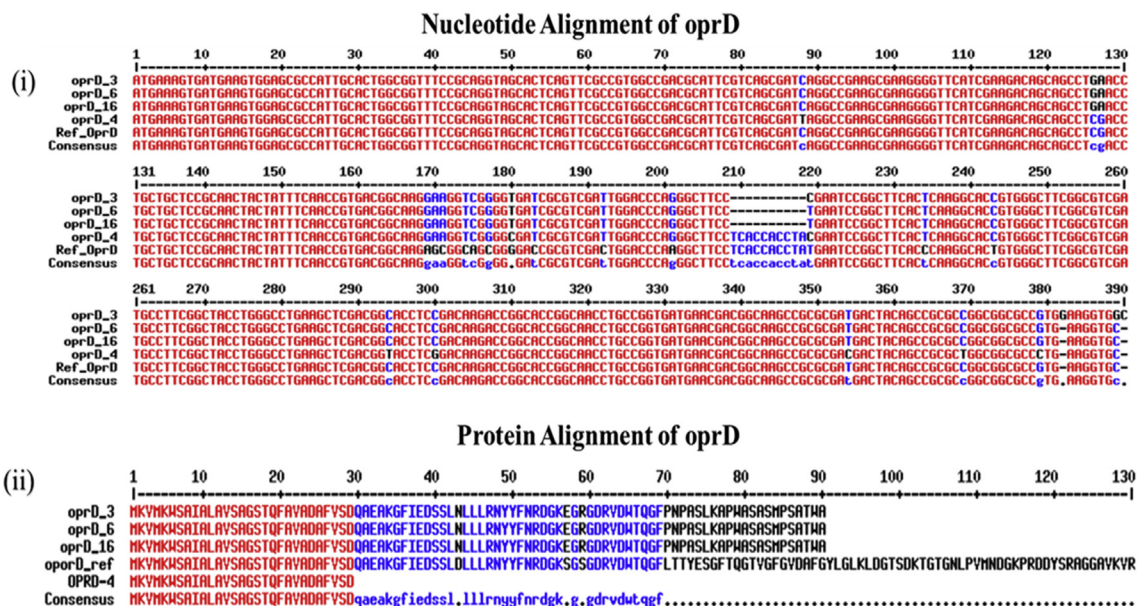


FIG. 1. Genetic representation of *oprD* gene. (a) Nucleotide alignment of *oprD* gene and (b) protein alignment of *oprD* gene. For *Pseudomonas aeruginosa* PA-3, PA-16 and PA-6, large deletion of ten nucleotides from position 209 to 218 led to premature stop codon TGA in *oprD*, resulting in truncated 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 chromosomal *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 chromosomal *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.

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Conflict of interest

None declared.

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