

A New *bla*_{VIM} Gene in a *Pseudomonas putida* Isolated from ENT Units in Sulaimani Hospitals

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Abstract—A total of twenty tensile biopsies were collected from children undergoing tonsillectomy from teaching hospital ENT department and Kurdistan private hospital in sulaimani city. All biopsies were homogenized and cultured; the obtained bacterial isolates were purified and identified by biochemical tests and VITEK 2 compact system. Among the twenty studied samples, only one *Pseudomonas putida* with probability of 99% was isolated. Antimicrobial susceptibility was carried out by disk diffusion method, *Pseudomonas putida* showed resistance to all antibiotics used except vancomycin. The isolate further subjected to PCR and DNA sequence analysis of *bla*_{VIM} gene using different set of primers for different regions of *VIM* gene. The results were found to be PCR positive for the *bla*_{VIM} gene. To determine the sequence of *bla*_{VIM} gene, DNA sequencing performed. Sequence alignment of *bla*_{VIM} gene with previously recorded *bla*_{VIM} gene in NCBI- database showed that *P. putida* isolate have different *bla*_{VIM} gene.

Keywords—Clinical isolates, Putida, Sulaimani, Vim gene.

I. INTRODUCTION

MICROORGANISMS might exhibit resistance to drugs by many different mechanisms. The most important mechanism is β -lactamase enzymes production, which are a group of enzymes capable of hydrolysing the 4-membered β -lactam ring of beta-lactam antibiotics [1], which can be either chromosomally encoded or plasmid mediated [11]. Several novel MBLs were identified, including VIM-1 from *P. aeruginosa* and IMP-2 from *Acinetobacter baumannii* in Italy [15], VIM-2 from *P. putida* in France [119], and IMP-3 from *Shigella flexneri* in Japan. The spread of MBLs in gram-negative rods has been described in several other countries and is becoming an emerging threat [7]. It remains unknown whether these MBLs have appeared in other countries. The aim of the study is to identify the molecular mechanism of the multidrug resistant *P. putida* among the isolates.

II. METHODS

A. Isolation and Identification

Samples were collected from Teaching Hospital (ENT Dept.) and Kurdistan Private Hospital. Biopsies were taken after tonsillectomy. Biopsy was transferred to laboratory in a sterile container which contains normal saline. Samples were

prepared for bacteriological examination by homogenization and centrifugation. Prepared samples were cultured on nutrient agar, and then single colonies were selected and inoculated on selective media for the purpose of obtaining pure cultures. Isolate identification performed microscopically, biochemical tests, and then the identification confirmed using VITEK 2 compact system.

B. Antimicrobial susceptibility and Isoelectric focusing of β -lactamase: Antibiotic-containing discs (BBL, Cockeysville, MD, USA) were used for routine antibiograms by disc diffusion assay. MICs of antimicrobial agents were determined by the agar dilution method. *Escherichia coli* ATCC 25922 were used as MIC reference strain. Modified Hodge and EDTA-disc synergy tests were performed for the screening of metallo- β -lactamase-producing strains. The results were compared to CLSI standard 2008. The isoelectric points of β -lactamases were determined by loading cell sonicates to precast pH 3 to 10 gels. The gel was overlaid with a filter paper soaked in 20 mM EDTA for 5 min, before the imipenem (0.5 mg/L)-containing Mueller–Hinton agar was added. In this manner, inhibition of imipenem-hydrolysing activities could be observed.

C. Molecular methods

Amplification of *VIM* gene of *pseudomonas putida* by direct colony PCR: A single bacterial colony which is previously cultured on nutrient agar was dissolved in 50 μ l dd H₂O (MQ). The cells suspension was incubated at 37°C water bath for at least 3 min. The cells were disrupted by heating by the insertion of the PCR tube containing the bacterial suspension into the thermocycler using the following program: 2 cycles for 10 min. at 99°C heating and 1 cycle for 5 min. at 4°C cooling. The samples were centrifuged at 13000 rpm for 10 minutes. The pellet was discarded and 5 μ l of supernatant were used as template in the PCR reactions. Master mix was prepared by adding 5 μ l of tag buffer, 2.5 μ l of (f and r) primer, 1 μ l of dNTP(10mM), 5 μ l of supernatant, 3 μ l MgCl₂, and 0.3 μ l tag polymerase to 30.7 μ l DDH₂O (50 μ l total volume in a sterile 0.5 ml PCR tube on ice). The PCR reactions were inserted into the PCR programs: A- PCR for the detection of VIM-type metallo-lactamase genes was carried out with primers VIM-DIA/f and VIM-DIA/r in a 50 μ l volume Reaction parameters were as follows: Annealing at 55°C for 60s extension at 72°C for 90s denaturation at 94°C for 50s for 25 cycles. The samples were analyzed by gel electrophoresis at 80V for 1 hr. The gel running was stopped and the DNA was visualized, and the DNA bands were

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photographed digitally. as was the preparation of recombinant plasmids containing PCR product, and transformation of them into *E. coli* DH5, Plasmids from successful clones were used to determine the sequence of the *bla_{VIM}* gene by the dideoxynucleotide-chain termination method, with an automatic DNA sequencer (ABI 3700, in Adden institute for molecular biology techniques/ Tehran- Iran The determination of the sequence was repeated with more than two clones from independent amplicons. Both strands were sequenced. Sequence alignment of *VIM* gene: Homology searches were conducted between the sequence of other reported sequences of *VIM* gene for *P. Putida* and other Gram negative bacteria in database of NCBI using BLAST program which is available at the NCBI online at (ww.ncbi.nlm.nih.gov) and the sequence of the same gene of the natural isolates.

III. RESULTS AND DISCUSSION

The results showed that out of 20 samples, only one was positive for *Pseudomonas putida*, the identification levels (Confidence and probability) by VITEK 2 compact system was 99%. *Pseudomonas putida*, being that the bacterium rarely colonizes mucosal surfaces but from other previously reported cases, it was determined that risk factors for developing such infections include the insertion of catheters, intubation, and/or intravascular devices [3]. *P. putida* infection was found in contaminated bottle of StaKleer. StaKleer is an anti-fog solution used on mirrors and endoscopes to prevent condensation from occurring, allowing for the proper visualization of ear and nose tissues. Sometimes unopened bottles of the solution at the clinic were found to be contaminated with *Pseudomonas putida* [9]. Disc diffusion testing revealed that *Pseudomonas putida* local isolate was resistant to most β -lactams, including ampicillin, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, cefalothin, cefoxitin, cefotaxime, ceftazidime and aztreonam. The isolate was also resistant to tobramycin, intermediate to gentamicin, but susceptible to amikacin and ciprofloxacin. MICs of imipenem and meropenem for the isolate were 4 mg/L, and that of aztreonam was 64 mg/L. MICs of ampicillin, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, cefalothin, cefoxitin, cefotaxime and ceftazidime were >128 mg/L. Isoelectric focusing of extract of the isolate showed two β -lactamase bands of pI ~5.3 and 9.0. The isolate showed positive modified Hodge and EDTA-disc synergy tests, and the only pI 5.3 band was no longer present when the gels were overlaid with EDTA, which are findings suggesting a metallo- β -lactamase. The band of pI ~9.0 was likely to be chromosomal AmpC cephalosporinase. A plasmid harbouring a carbapenem resistance determinant was not detected (data not shown). These results suggest that a metallo- β -lactamase gene may be located on the chromosome. VIM-2 metallo- β -lactamase has no hydrolytic activity against aztreonam, but the MIC of aztreonam for *P.putida* was 64 mg/L, which is higher than the resistant breakpoint. This result was possibly due to production of a chromosomal cephalosporinase (pI ~9.0). The gel electrophoresis analysis showed a band about 800 bp for *bla_{VIM}* gene in accordance with *bla_{VIM}* gene sequence. Carbapenem-hydrolyzing metallo- β -lactamases, especially

IMP-type and VIM-type metallo- β -lactamases, are clinically important, because these enzymes effectively hydrolyze almost all β -lactam antibiotics except monobactams, conferring resistance to penicillins and cefepime in addition to carbapenems on pathogenic bacteria. Since genes encoding these metallo- β -lactamases (*bla_{IMP}* and *bla_{VIM}*) and their variant genes have become easy to detect using the PCR method, since 1989 the dissemination of these genes in clinical isolates has been widely observed in gram-negative bacteria, especially in *Pseudomonas aeruginosa* and other non-glucose-fermenting bacteria [16]. Multiple-drug resistance *P. putida* isolates producing VIM-type metallo- β -lactamases were reported in Italy as a causative species of nosocomial infections. [14, 19, 4, 8]. Luzzaro *et al.*, 2004 [7] reported that the sizes of the integron carrying the *bla_{VIM}* varied among the isolates from 3 to 6 kb. Prevalence of metallo- β -lactamase-producing *P.putida* is an important clinical problem, representing a reservoir of genetic determinants of multi-drug resistance. The *P. putida* isolate PCR product which has been amplified and used as template for sequence reaction (Fig.1). The result of sequence alignment of *bla_{VIM}* sequence from Sulaimani hospitals against *bla_{VIM2}* gene of *.putida* class I integron which published by Lee *et al.* in 2002, in korea, (ACCESSION: AF327064.1) showed that the sequence has a length of about 3057 bp., identities were 792/803 (98%), which indicate that there were 11 mutations in *bla_{VIM}* of *p. putida* isolated from Sulaimani (Fig. 2).

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ATGTTCAAAC TTTT GAGTAAGTTATTGGTCTATTGGACCGC
GTCTATCATGGCTATTGCGAGTCCGCTCGCTTTTCCGTAG
ATTCTAGCGGTGAGTATCCGACAGTCAGCGAAATCCGGTC
GGGGAGGTCCGGCTTTACCAGATTGCCGATGGTGTGGTTC
GCATATCGCAACGCGGTGCTTTGATGGCGCAGTCTACCCGT
CCAATGGTCTCATTGTCCGTGATGGTGTGATGAGTTGCTTTGA
TTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCT
CGCGGAGATTGAGAAGCAAATTGGACTTCTGTAACGCGT
GCAGTCTCCACGCACTTTCATGACGACCGCGTCCGGCGCGT
TGATGTCTTCGGGCGGCTGGGGTGGCACGTACGCATACC
GTCGACACCGCGGTAGCCGAGGTAGAGGGGAGCGGAT
CCCAGCACTCTCTAGAAGGACTCTCATCGAGCGGGGACG
CAGTGCCTTCGGTCCAGTAGAACTTCTATCCTGGTGTCT
GCGCATTCGACCGACAACCTTAGTTGTGTACGTCCTGCTGC
GAGTGTGCTCTATGGTGGTTGTGCGATTTATGAGTTGT
CACGCACGTC
TGCGGGGAACGTGGCCGATGCCGATCTGGCTGAATGGCCC
ACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCA
CAGTTCGTCATTCCGGGGCACGGCTTGCCGGGCGGTCTAGA
CTTGCTCAAGCACACAACGAATGTTGTA AAAAGCGCACACA
ACGCTCAGTCGTTGAGTAGCAGGCAGATGCGGCATAACAT
GAAGTT

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Fig. 1: Complete sequence of the *bla_{VIM}* gene in *P. putida* isolate

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Query 1 ATGTTCAAAC TTTT GAGTAAGTTATTGGTCTATTGGACCGC
Sbjct55 ATGTTCAAAC TTTT GAGTAAGTTATTGGTCTATTGGACCGC
Query 61 AGTCCGCTCGCTTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAGCGAAATCCG
Sbjct613 AGTCCGCTCGCTTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAGCGAAATCCG
Query121 GTCGGGAGGTCCGGCTTTACCAGATTGCCGATGGTGTGGTTCGCATATCGCAACGCGG
Sbjct673 GTCGGGAGGTCCGGCTTTACCAGATTGCCGATGGTGTGGTTCGCATATCGCAACGCGG

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Query 181 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG
Sbjct 733 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG

Query 241 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG
Sbjct 793 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG

Query 301 AAGCAAATTGGACTTCCCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC
Sbjct 853 AAGCAAATTGGACTTCCCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC

Query 361 GCGCGGCTTGATGTCCCTTACGCGCGTGGGGTGGCAACGTACGCATCACCGTGACACGCG
Sbjct 913 GCGCGGCTTGATGTCCCTTACGCGCGTGGGGTGGCAACGTACGCATCACCGTGACACGCG

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG
Sbjct 973 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG

Query 481 AGCGGGGACGT-GCCACGCTTCGGTCCAGTAGAACTCT-CTTATCCTGGTGTGCGCATT
Sbjct 1033AGCGGGGACGAGT-GCCTTCGGTCCAGTAGAACTCTTCT-ATCCTGGTGTGCGCATT

Query 539 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGGTGGTGTGCGGA
Sbjct 1091CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGGTGGTGTGCGGA

Query 599 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC
Sbjct 1151TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC

Query 659 CCACCTCCATTGAGCGGATTCACAACTACCAGCAAGCAGTTCGTCATTCCGGGGC
Sbjct 1211CCACCTCCATTGAGCGGATTCACAACTACCAGCAAGCAGTTCGTCATTCCGGGGC

Query 719 ACGGCTGCCGGCGGTCTAGACTTGTCAAGCACACAAAGATGTTGATAAAGCGCAC
Sbjct 1271ACGGCTGCCGGCGGTCTAGACTTGTCAAGCACACAAAGATGTTGATAAAGCGCAC

Query 779 CAAATCGCTCAGTCGTTGAGTAG 801
Sbjct 1331CAAATCGCTCAGTCGTTGAGTAG 1353
    
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Fig. 2 Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* class 1 integron (*bla_{VIM-2}*), (ACCESSION: AF327064.1)

Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim6* gene of *p. putida* strain DU25165/00 (*bla_{VIM-6}*) (ACCESSION: AY165025.1). Sequence has a length of about 828 bp. Identities were 821/830 (98%) (Fig 4). The results showed that there were 9 mutations for the *bla_{Vim 6}* of *p. putida* strain DU25165/00 which was first published by Koh *et al.*, in (2004)(5) in Singapore (Fig 3).

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Query 1 ATGTTCAAACCTTTTGAAGTATTGGTCTATTGACCGCTCTATCATGGCTATTGCG
Sbjct 1 ATGTTCAAACCTTTTGAAGTATTGGTCTATTGACCGCTCTATCATGGCTATTGCG

Query 61 AGTCCGCTCGCTTTTCCGTAGATTTACGCGGTGAGTATCCGACAGTCAGCGAAATTCGG
Sbjct 61 AGTCCGCTCGCTTTTCCGTAGATTTACGCGGTGAGTATCCGACAGTCAGCGAAATTCGG

Query 121 GTCGGGAGTCCGCGTTTACAGATTTCCGATGGTGTGGTGCATATCGCAACGCGG
Sbjct 121 GTCGGGAGTCCGCGTTTACAGATTTCCGATGGTGTGGTGCATATCGCAACGCGG

Query 181 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG
Sbjct 181 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG

Query 241 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG
Sbjct 241 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG

Query 301 AAGCAAATTGGACTTCCCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC
Sbjct 301 AAGCAAATTGGACTTCCCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC

Query 361 GCGCGGCTTGATGTCCCTTACGCGCGTGGGGTGGCAACGTACGCATCACCGTGACACGCG
Sbjct 361 GCGCGGCTTGATGTCCCTTACGCGCGTGGGGTGGCAACGTACGCATCACCGTGACACGCG

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG
Sbjct 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG

Query 481 AGCGGGGACGT-GCCACGCTTCGGTCCAGTAGAACTCT-CTTATCCTGGTGTGCGCATT
Sbjct 481 AGCGGGGACGAGT-GCCTTCGGTCCAGTAGAACTCTTCT-ATCCTGGTGTGCGCATT

Query 539 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGGTGGTGTGCGGA
Sbjct 539 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGGTGGTGTGCGGA

Query 599 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC
Sbjct 599 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC

Query 659 CCACCTCCATTGAGCGGATTCACAACTACCAGCAAGCAGTTCGTCATTCCGGGGC
Sbjct 2113 CCACCTCCATTGAGCGGATTCACAACTACCAGCAAGCAGTTCGTCATTCCGGGGC

Query 719 ACGGCTGCCGGCGGTCTAGACTTGTCAAGCACACAAAGATGTTGATAAAGCGCAC
Sbjct 2173 ACGGCTGCCGGCGGTCTAGACTTGTCAAGCACACAAAGATGTTGATAAAGCGCAC

Query 779 CAAATCGCTCAGTCGTTGAGTAG
Sbjct 2233 CAAATCGCTCAGTCGTTGAGTAG
    
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Query 659 CCACCTCCATTGAGCGGATTCACAACTACCAGCAAGCAGTTCGTCATTCCGGGGC
Sbjct 659 CCACCTCCATTGAGCGGATTCACAACTACCAGCAAGCAGTTCGTCATTCCGGGGC

Query 719 ACGGCTGCCGGCGGTCTAGACTTGTCAAGCACACAAAGATGTTGATAAAGCGCAC
Sbjct 719 ACGGCTGCCGGCGGTCTAGACTTGTCAAGCACACAAAGATGTTGATAAAGCGCAC

Query 779 CAAATCGCTCAGTCGTTGAGTAG
Sbjct 779 CAAATCGCTCAGTCGTTGAGTAG
    
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Fig. 3 Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospital against *Vim6* gene of *p. putida* strain DU25165/00 (*bla_{VIM-6}*) (ACCESSION : AY165025.1)

Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* strain YMC 98/2/665 class I integron (*bla_{VIM-2}*), (ACCESSION: AY907717.1). Sequence has a length of about 5325 bp. Identities were 792/803 (98%).The results showed that there were 11 mutations for the *bla_{Vim 2}* of *p. putida* strain YMC 98/2/665 which was first identified in Korea in 2005 by Yan (19) (Fig. 4).

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Query 1 ATGTTCAAACCTTTTGAAGTATTGGTCTATTGACCGCTCTATCATGGCTATTGCG
Sbjct 1455 ATGTTCAAACCTTTTGAAGTATTGGTCTATTGACCGCTCTATCATGGCTATTGCG

Query 61 AGTCCGCTCGCTTTTCCGTAGATTTACGCGGTGAGTATCCGACAGTCAGCGAAATTCGG
Sbjct 1515 AGTCCGCTCGCTTTTCCGTAGATTTACGCGGTGAGTATCCGACAGTCAGCGAAATTCGG

Query 121 GTCGGGAGTCCGCGCTTACAGATTTCCGATGGTGTGGTGCATATCGCAACGCGG
Sbjct 1575 GTCGGGAGTCCGCGCTTACAGATTTCCGATGGTGTGGTGCATATCGCAACGCGG

Query 181 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG
Sbjct 1635 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG

Query 241 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG
Sbjct 1695 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG

Query 301 AAGCAAATTGGACTTCCCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC
Sbjct 1755 AAGCAAATTGGACTTCCCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC

Query 361 GCGCGGCTTGATGTCCCTTACGCGCGTGGGGTGGCAACGTACGCATCACCGTGACACGCG
Sbjct 1815 GCGCGGCTTGATGTCCCTTACGCGCGTGGGGTGGCAACGTACGCATCACCGTGACACGCG

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG
Sbjct 1875 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG

Query 481 AGCGGGGACGT-GCCACGCTTCGGTCCAGTAGAACTCT-CTTATCCTGGTGTGCGCATT
Sbjct 1935 AGCGGGGACGAGT-GCCTTCGGTCCAGTAGAACTCTTCT-ATCCTGGTGTGCGCATT

Query 539 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGGTGGTGTGCGGA
Sbjct 1993 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGGTGGTGTGCGGA

Query 599 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC
Sbjct 205 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC

Query 659 CCACCTCCATTGAGCGGATTCACAACTACCAGCAAGCAGTTCGTCATTCCGGGGC
Sbjct 2113 CCACCTCCATTGAGCGGATTCACAACTACCAGCAAGCAGTTCGTCATTCCGGGGC

Query 719 ACGGCTGCCGGCGGTCTAGACTTGTCAAGCACACAAAGATGTTGATAAAGCGCAC
Sbjct 2173 ACGGCTGCCGGCGGTCTAGACTTGTCAAGCACACAAAGATGTTGATAAAGCGCAC

Query 779 CAAATCGCTCAGTCGTTGAGTAG
Sbjct 2233 CAAATCGCTCAGTCGTTGAGTAG
    
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Fig. 4 Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* strain YMC 98/2/665 class I integron (*bla_{VIM-2}*), (ACCESSION: AY907717.1)

Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* transposon Tn1332 (ACCESSION: DQ174113.1). The sequence has a length of about 11132 bp. Identities were 792/803 (98%) (Fig. 5). The results showed that there were 11 mutations for the *bla_{Vim}* of *p.*

putida transposon Tn1332 which was first published in 2006 by Poirel *et al.*, in France[11].

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Query 1   ATGTTCAAACCTTTGAGTAAGTTATTGGTCTATTGACCGCGTCTATCATGGCTATTGCG
Sbjct 5718 ATGTTCAAACCTTTGAGTAAGTTATTGGTCTATTGACCGCGTCTATCATGGCTATTGCG

Query 61  AGTCCGCTCGCTTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCGG
Sbjct 5778 AGTCCGCTCGCTTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCGG

Query 121 GTCGGGGAGGTCGGGCTTTACAGAGATTGCCGATGGTGTGGTTCGCATATCGCAACGGGG
Sbjct 5838 GTCGGGGAGGTCGGGCTTTACAGAGATTGCCGATGGTGTGGTTCGCATATCGCAACGGGG

Query 181 TCGTTTATGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCCTGATGGTGTAGTTG
Sbjct 5898 TCGTTTATGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCCTGATGGTGTAGTTG

Query 241 CTTTTGATTTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGTTGAG
Sbjct 5958 CTTTTGATTTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGTTGAG

Query 301 AAGCAAATGGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGGTC
Sbjct 6018 AAGCAAATGGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGGTC

Query 361 GCGCGCTTGTATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACACGC
Sbjct 6078 GCGCGCTTGTATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACACGC

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTCTAGAAGGACTCTCATCG
Sbjct 6138 CGGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTCTAGAAGGACTCTCATCG

Query 481 AGCGGGAGCGT-GCCACGCTTCGGTCCAGTAGAAGTCT-CTTATCTGGTGTGGCGATT
Sbjct 6198 AGCGGGAGCGAGT-GCCTTCGGTCCAGTAGAAGTCTTCT-ATCTGGTGTGGCGATT

Query 539 CGACCGACAACCTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTGCGA
Sbjct 6256 CGACCGACAACCTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTGCGA

Query 599 TTTATGAGTTGTACGCAAGCTTCGCGGGAGCGTGGCCGATGCGCATCTGCTGAATGGC
Sbjct 6316 TTTATGAGTTGTACGCAAGCTTCGCGGGAGCGTGGCCGATGCGCATCTGCTGAATGGC

Query 659 CCACCTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCATTCCGGGGC
Sbjct 6376 CCACCTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCATTCCGGGGC

Query 719 ACGCCCTGCGGGCGGCTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGCAC
Sbjct 6436 ACGCCCTGCGGGCGGCTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGCAC

Query 779 CAAATCGCTCAGTCTGTAGTAG
Sbjct 6496 CAAATCGCTCAGTCTGTAGTAG
    
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Fig. 5 Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* transposon Tn1332 (ACCESSION : DQ174113.1)

Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim1* gene of *p. putida* strain A2580/277 (VIM-1) gene, (ACCESSION : EU118150.1). The sequence has a length of about 843 bp, Identities were 738/809 (91%) (Fig. 6). The results showed that there were 71 mutations for the *bla_{Vim1}* of *p. putida* strain A2580/277 which was first identified in Greece by Papadopoulou *et al.* in 2007[12].

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Query 1   ATGTTCAAACCTTTGAGTAAGTTATTGGTCTATTGACCGCGTCTATCATGGCTATTGCG
Sbjct 40   ATGTTAAAAGTTATT-AGT-AGTTTATTGGTCTACATGACCGCGTCTGTATGGCTGTGCG

Query 59  CGAGTCCGCTCGCTTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTC
Sbjct 98  CAAGTCCGTTAGCCCAATCCGGGGAGCGAGTGGTGGTATCCGACAGTCAAGGAAATTC

Query 119 CGTCCGGGAGGTCGGGCTTTACAGAGATTGCCGATGGTGTGGTTCGCATATCGCAACGC
Sbjct 158 CGTCCGGGAGGTCGGGCTTTACAGAGATTGCCGATGGTGTGGTTCGCATATCGCAACGC

Query 179 GGTCCGTTGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCCTGATGGTGTAGT
Sbjct 218 AGTCCGTTGATGGCGCGTCTACCCGTCCTAATGGTCTCATTTGTCCTGATGGTGTAGT

Query 239 TGCTTTTGTATGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGATTG
Sbjct 278 TGCTTTTGTATGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGATTG

Query 299 AGAAGCAAATGGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGCG
Sbjct 338 AAAAGCAAATGGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGCG

Query 359 TCGCGCGGCTGATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACAC
Sbjct 398 TCGCGCGGCTGATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACAC
    
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Query 419 GCCCGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTCTAGAAGGACTCTCAT
Sbjct 458 GCCCGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTCTAGAAGGACTCTCAT

Query 479 CGAGCGGGGACGT-GCCACGCTTCGGTCCAGTAGAAGTCT-CTTATCTGGTGTGCGGCA
Sbjct 518 CGAGCGGGGACGAGT-GCCTTCGGTCCAGTAGAAGTCTTCT-ATCTGGTGTGCGGCA

Query 537 TTCGACCGACAA-CTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTG
Sbjct 576 TTCGACCGACAACTCG-GTGTATACGTCCTCGTCCAGGACGCTATACGCTGGTGTG

Query 596 C-GATTATGAGTTGTACGCAAGTCTGCGGGGAGCGTGGCCGATGCCGATCGGCTGAA
Sbjct 635 CCG-TTCATGAGTTGTCAAGCAAGTCTGCGGGGAACTGGCCGATGCCGATCGGCTGAA

Query 655 TGGCCCACTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCGTCATTCCG
Sbjct 694 TGGCCCACTCCGTTGAGCGGATTCAAAAACACTACCCGGAAGCACAGGTCGTCATTCCC

Query 715 GGGCAGCGCTGCCGGCGGCTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGC
Sbjct 754 GGGCAGCGCTTACCGGCGGCTAGACTTCTCAAGCACACAGGAACTTTGTAAGGCGC

Query 775 CACAAATCGCTCAGTCTGTAGTAGA
Sbjct 814 CAAAAATCGCTCAGTCTGCCGATGAGA
    
```

Fig. 6 Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim1* gene of *p. putida* strain A2580/277 (VIM-1) gene, (ACCESSION : EU118150.1)

Sequence alignment of *bla_{Vim}* DNA sequence from Sulaimani hospitals against *Vim* gene of *p. putida* strain PFi class 1 integron (ACCESSION FJ237530). The sequence has a length of about 1904 bp. Identities between the *bla_{Vim}* DNA sequence from Sulaimani hospitals and *bla_{Vim2}* of *p. putida* strain PFi isolated in Portugal were 792/803 (98%) (Fig. 7). The results showed there were 10 mutations for the *bla_{Vim2}* of *p. putida* strain PFi which include transversion, deletion and insertion).The information about this sequence was first submitted by Santos *et al.*(2008) in Portugal [16].

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Query   ATGTTCAAACCTTTGAGTAAGTTATTGGTCTATTGACCGCGTCTATCATGGCTATTGCG
Sbjct   ATGTTCAAACCTTTGAGTAAGTTATTGGTCTATTGACCGCGTCTATCATGGCTATTGCG

Query   AGTCCGCTCGCTTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCGG
Sbjct   AGTCCGCTCGCTTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCGG

Query   GTCGGGGAGGTCGGGCTTTACAGAGATTGCCGATGGTGTGGTTCGCATATCGCAACGGGG
Sbjct   GTCGGGGAGGTCGGGCTTTACAGAGATTGCCGATGGTGTGGTTCGCATATCGCAACGGCG

Query   TCGTTTATGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCCTGATGGTGTAGT
Sbjct   TCGTTTATGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCCTGATGGTGTAGT

Query   CTTTTGATTTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGATTGAG
Sbjct   CTTTTGATTTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGATTGAG

Query   AAGCAAATGGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGGTC
Sbjct   AAGCAAATGGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGGTC

Query   GCGCGCTTGTATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACACGC
Sbjct   GCGCGCTTGTATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACACGC

Query   CGGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTCTAGAAGGACTCTCATCG
Sbjct   CGGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTCTAGAAGGACTCTCATCG

Query   AGCGGGGAGT-GCCACGCTTCGGTCCAGTAGAAGTCT-CTTATCTGGTGTGGCGATT
Sbjct   AGCGGGGAGCGAGT-GCCTTCGGTCCAGTAGAAGTCTTCT-ATCTGGTGTGGCGATT

Query   CGACCGACAACCTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTGCGA
Sbjct   CGACCGACAACCTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTGCGA

Query   TTTATGAGTTGTACGCAAGCTTCGCGGGAGCGTGGCCGATGCCGATCTGGCTGAATGGC
Sbjct   TTTATGAGTTGTACGCAAGCTTCGCGGGAGCGTGGCCGATGCCGATCTGGCTGAATGGC

Query   CCACCTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCATTCCGGGGC
Sbjct   CCACCTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCATTCCGGGGC

Query   ACGCCCTGCGGGCGGCTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGCAC
Sbjct   ACGCCCTGCGGGCGGCTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGCAC

Query   CAAATCGCTCAGTCTGTAGTAG
Sbjct   CAAATCGCTCAGTCTGTAGTAG
    
```

Fig. 7 Sequence alignment of Sequence alignment of *bla_{Vim}* DNA sequence from Sulaimani hospitals against *Vim* gene of *p. putida* strain PFI class I integron (ACCESSION FJ237530)

Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim*4 gene of *p. putida* strain 283-02 class I integron (ACCESSION : FM179466.1). The sequence has a length of about 3329 bp. Identities between the *bla_{Vim}* sequence from Sulaimani hospitals and *bla_{Vim}* of *p. putida* strain 283-02 isolated in Poland were 736/807 (91%) (Fig. 8). The results showed that there were 71 mutations for the *bla_{Vim}* 2 of *p. putida* strain 283-02 which was first published in 2009 by Patzar *et al.*, in Poland [9].

Query 1	ATGTTCAAACCTT-TGTAAGAA-TTATTGGTCTATTGACCGCTCTATCATGGCTATTG
Sbjct 274	ATGTTAAAGTTATT-AGT-AGTTATTGCTACATGACCGCTCTGTATGGCTGTCG
Query 59	CGAGTCOCCTGCCTTTTCCGAGTATTCTAGCGTGAGTATCCGACAGTCAAGAAATTC
Sbjct 332	CAAGTCOCCTGATCCCAATCCGAGGAGCCGAGTGTGATATCCGACAGTCAAGAAATTC
Query 119	CGGTCCGGAAGGTCCGGCTTTACAGATTGCGCATGGTGTGTTGTCGCAATCCGCAACGC
Sbjct 392	CGGTCCGGAAGGTCCGCAATTTACAGATTGCGCATGGTGTGTTGTCGCAATCCGCAACGC
Query 179	GGTGGTTTGATGGCGCAGTCTACCCGTCCAATGGTCTCATTGTCCGTGATGGTGAATGAT
Sbjct 452	AGTGGTTTGATGGCGCAGTCTACCCGTCCAATGGTCTCATTGTCCGTGATGGTGAATGAT
Query 239	TGCTTTTGATTGATACAGCGTGGGGTGGCAAAAACACAGCGGCACCTTCTCCGCGAGATTG
Sbjct 512	TGCTTTTGATTGATACAGCGTGGGGTGGCAAAAACACAGCGGCACCTTCTCCGCGAGATTG
Query 299	AGAAGCAAATGGACTTCTGTFAACGCTGAGTCTCCACGCACTTTCATGACGACCGCG
Sbjct 572	AAAAGCAAATGGACTTCCCGTFAACGCTGAGTCTCCACGCACTTTCATGACGACCGCG
Query 359	TCGGCGCGCTTGATGTCCTTCAGCGCGCTGGGGTGGCAACGTACGCATCACCGTCAACAC
Sbjct 632	TCGGCGCGCTTGATGTCCTTCAGCGCGCTGGGGTGGCAACGTACGCATCACCGTCAACAC
Query 419	GCCGGCTAGCCAGGTAGAGGGAGCGAGATGCCACGCACTCTCTAGAAGGACTCTCTCAT
Sbjct 692	GCCGGCTAGCCAGGTAGAGGGAGCGAGATGCCACGCACTCTCTAGAAGGACTCTCTCAT
Query 479	CGAGCGGGAGCT-GCCACGCTCCCGTCCAGTAACTTCT-TTATCCTGTGTCTGGCGCA
Sbjct 752	CGAGCGGGAGCT-GCCTCGTCCAGTAACTTCT-TTATCCTGTGTCTGGCGCA
Query 537	TTGACCACGAAA-CTTAGTTGTGTACGCTCCCGTCTCCGAGTGTGCTCATGGTGGTTTGTG
Sbjct 810	TTGACCACGAAA-CTTAGTTGTGTACGCTCCCGTCTCCGAGTGTGCTCATGGTGGTTTGTG
Query 596	C-GATTATGAGTTGTACACGCAGTCTCCGGGGAGCGGTGGCCGATGCCGATCTGGCTGAA
Sbjct 869	CCG-TTCATGAGTTGTACACGCAGTCTCCGGGGAGCGGTGGCCGATGCCGATCTGGCTGAA
Query 655	TGCCCACCTCCCTTGGAGGGATTCAACAACACTACCCGGAAGCAGTGTGCTCATTCGC
Sbjct 928	TGCCCACCTCCCTTGGAGGGATTCAACAACACTACCCGGAAGCAGTGTGCTCATTCGC
Query 715	GGGACGCGCTGCGCGGCGGTCTAGACTTGCTCAAGCACACAACGAATGTTGTAAGAGCG
Sbjct 988	GGGACGCGTCTACCGGCGGTCTAGACTTGCTCAAGCACACAACGAATGTTGTAAGAGCG
Query 775	CACACAAATCGCTCAGTCTGTTAGATAG
Sbjct 1048	CACAAAAATCGCTCAGTCTGCGGAGTAG

Fig. 8 Sequence alignment of *bla_{Vim}* sequence from Sulaimani hospitals against *Vim*4 gene of *p. putida* strain 283-02 class I integron (ACCESSION : FM179466.1)

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- 3- Comparison of Tn1546 element of vancomycin resistant Staphylococcus aureus isolated from burned patients in Sulaimani hospital. Published in International conference proceeding on bioinformatics and biomedical technology -April 2010.
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- 5- Participated in “International Conference on Biological Science and Engineering” in 24-26 Nov. 2010 , Venice, Italy.