

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

Dalanya Asaad Mohammed, and Dara Abdul Razaq

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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ATGTTCAAACCTTTTGAGTAAGTTATTGGTCTATTGGACCGC
GTCTATCATGGCTATTGCGAGTCCGCTCGCTTTTCCGTAG
ATTCTAGCGGTGAGTATCCGACAGTCAGCGAAATCCGGTC
GGGAGGTCCGGCTTTACCAGATTGCCGATGGTGTGGTTC
GCATATCGCAACGCGGTCTGGTGGCGCAGTCTACCCGT
CCAATGGTCTCATTGTCCGTGATGGTGTGATGAGTTGCTTGA
TTGATACAGCGTGGGGTGCAGAAAACACAGCGGCACTTCT
CGCGGAGATTGAGAAGCAAATTGGACTTCTGTAACGCGT
GCAGTCTCCACGCACTTTCATGACGACCGCGTCGGCGGCGT
TGATGTCCTTCGGGCGGCTGGGGTGGCACGTACGCATACC
GTCGACACCGCGGTAGCCGAGGTAGAGGGGAGCGGAT
CCCAGCACTCTCTAGAAGGACTCTCATCGAGCGGGGACG
CAGTGCCTTCGGTCCAGTAGAAGTCTTCTATCCTGGTGTCT
GCGCATTCGACCGACAACCTTAGTTGTGTACGTCCTGCTGC
GAGTGTGCTCTATGGTGGTTGTGCGATTTATGAGTTGT
CACGCAAGTC
TGCGGGGAACGTGGCCGATGCCGATCTGGCTGAATGGCCC
ACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCA
CAGTTCGTCATTCCGGGGCACGGCTTGCCGGGCGGTCTAGA
CTTGCTCAAGCACACAACGAATGTTGTAAGGCGCACACA
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 ATGTTCAAACCTTTTGAGTAAGTTATTGGTCTATTGGACCGCTCTATCATGGCTATTGCG
Sbjct55 ATGTTCAAACCTTTTGAGTAAGTTATTGGTCTATTGGACCGCTCTATCATGGCTATTGCG
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 AAGCAAATTGGACTTCTCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC
Sbjct 853 AAGCAAATTGGACTTCTCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC

Query 361 GCGCGGCTTGATGTCTTTCAGCGCGCTGGGGTGGCAACGTACGCATCACCGTGACACGCG
Sbjct 913 GCGCGGCTTGATGTCTTTCAGCGCGCTGGGGTGGCAACGTACGCATCACCGTGACACGCG

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG
Sbjct 973 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG

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

Query 539 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGTTGGTGTGCGGA
Sbjct 1091CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGTTGGTGTGCGGA

Query 599 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC
Sbjct 1151TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC

Query 659 CCACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCTCGTATTCCGGGGC
Sbjct 1211CCACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCTCGTATTCCGGGGC

Query 719 ACGGCTGCCGGGCGGTCTAGACTTGTCTAAGCACACAACGAATGTTGAAAAAGCGCAC
Sbjct 1271ACGGCTGCCGGGCGGTCTAGACTTGTCTAAGCACACAACGAATGTTGAAAAAGCGCAC

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 ATGTTCAAACCTTTTGAAGTAAATTTGGTCTATTGACCGCGTCTATCATGGCTATTGCG
Sbjct 1 ATGTTCAAACCTTTTGAAGTAAATTTGGTCTATTGACCGCGTCTATCATGGCTATTGCG

Query 61 AGTCCGCTCGCTTTTTCCGTAGATTTCTAGCGGTGAGTATCCGACAGTCAGCGAAATTCGG
Sbjct 61 AGTCCGCTCGCTTTTTCCGTAGATTTCTAGCGGTGAGTATCCGACAGTCAGCGAAATTCGG

Query 121 GTCGGGAGTCCCGCTTTACAGATTTCCGATGGTGTGGTGTGCGATATCGCAACCGGG
Sbjct 121 GTCGGGAGTCCCGCTTTACAGATTTCCGATGGTGTGGTGTGCGATATCGCAACCGGG

Query 181 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG
Sbjct 181 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG

Query 241 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG
Sbjct 241 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG

Query 301 AAGCAAATTGGACTTCTCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC
Sbjct 301 AAGCAAATTGGACTTCTCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC

Query 361 GCGCGGCTTGATGTCTTTCAGCGCGCTGGGGTGGCAACGTACGCATCACCGTGACACGCG
Sbjct 361 GCGCGGCTTGATGTCTTTCAGCGCGCTGGGGTGGCAACGTACGCATCACCGTGACACGCG

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG
Sbjct 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG

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

Query 539 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGTTGGTGTGCGGA
Sbjct 539 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGTTGGTGTGCGGA

Query 599 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC
Sbjct 599 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC

Query 659 CCACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCTCGTATTCCGGGGC
Sbjct 2113 CCACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCTCGTATTCCGGGGC

Query 719 ACGGCTGCCGGGCGGTCTAGACTTGTCTAAGCACACAACGAATGTTGAAAAAGCGCAC
Sbjct 2173 ACGGCTGCCGGGCGGTCTAGACTTGTCTAAGCACACAACGAATGTTGAAAAAGCGCAC

Query 779 CAAATCGCTCAGTCGTTGAGTAG
Sbjct 2233 CAAATCGCTCAGTCGTTGAGTAG
    
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Query 659 CCACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCTCGTATTCCGGGGC
Sbjct 659 CCACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCTCGTATTCCGGGGC

Query 719 ACGGCTGCCGGGCGGTCTAGACTTGTCTAAGCACACAACGAATGTTGAAAAAGCGCAC
Sbjct 719 ACGGCTGCCGGGCGGTCTAGACTTGTCTAAGCACACAACGAATGTTGAAAAAGCGCAC

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 ATGTTCAAACCTTTTGAAGTAAATTTGGTCTATTGACCGCGTCTATCATGGCTATTGCG
Sbjct 1455 ATGTTCAAACCTTTTGAAGTAAATTTGGTCTATTGACCGCGTCTATCATGGCTATTGCG

Query 61 AGTCCGCTCGCTTTTTCCGTAGATTTCTAGCGGTGAGTATCCGACAGTCAGCGAAATTCGG
Sbjct 1515 AGTCCGCTCGCTTTTTCCGTAGATTTCTAGCGGTGAGTATCCGACAGTCAGCGAAATTCGG

Query 121 GTCGGGAGTCCCGCTTTACAGATTTCCGATGGTGTGGTGTGCGATATCGCAACCGGG
Sbjct 1575 GTCGGGAGTCCCGCTTTACAGATTTCCGATGGTGTGGTGTGCGATATCGCAACCGGG

Query 181 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG
Sbjct 1635 TCGTTTGATGGCGCAGTCTACCCGTCCTCAATGGTCTCATTGTCCGTGATGGTGATGAGTTG

Query 241 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG
Sbjct 1695 CTTTTGATTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACCTTCTCGCGGAGATTGAG

Query 301 AAGCAAATTGGACTTCTCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC
Sbjct 1755 AAGCAAATTGGACTTCTCTGTAACGCGTGCAGTCTCCACGCACCTTTCATGACGACCGCGTC

Query 361 GCGCGGCTTGATGTCTTTCAGCGCGCTGGGGTGGCAACGTACGCATCACCGTGACACGCG
Sbjct 1815 GCGCGGCTTGATGTCTTTCAGCGCGCTGGGGTGGCAACGTACGCATCACCGTGACACGCG

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG
Sbjct 1875 CGGCTAGCCGAGGTAGAGGGGAGCGAGATCCCACGCACCTCTAGAAAGGACTCTCATCG

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

Query 539 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGTTGGTGTGCGGA
Sbjct 1993 CGACCGACAACCTAGTTGTGTACGTCCCGTCTCGGAGTGTGCTCTATGTTGGTGTGCGGA

Query 599 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC
Sbjct 205 TTTATGAGTTGTCAACGACGCTCTCGGGGACGCTGGCCGATGCCGATCTGGCTGAATGGC

Query 659 CCACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCTCGTATTCCGGGGC
Sbjct 2113 CCACCTCCATTGAGCGGATTCAACAACACTACCCGGAAGCACAGTTCTCGTATTCCGGGGC

Query 719 ACGGCTGCCGGGCGGTCTAGACTTGTCTAAGCACACAACGAATGTTGAAAAAGCGCAC
Sbjct 2173 ACGGCTGCCGGGCGGTCTAGACTTGTCTAAGCACACAACGAATGTTGAAAAAGCGCAC

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  AGTCCGCTCGCTTTTCCGTAAGTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCGG
Sbjct 5778 AGTCCGCTCGCTTTTCCGTAAGTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCGG

Query 121 GTCGGGGAGGTCGGGCTTTACAGAGTTGCCGATGGTGTGGTTCGCATATCGCAACGCGG
Sbjct 5838 GTCGGGGAGGTCGGGCTTTACAGAGTTGCCGATGGTGTGGTTCGCATATCGCAACGCGG

Query 181 TCGTTTATGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCGGTATGGTATGAGTTG
Sbjct 5898 TCGTTTATGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCGGTATGGTATGAGTTG

Query 241 CTTTTGATTTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGTTGAG
Sbjct 5958 CTTTTGATTTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGTTGAG

Query 301 AAGCAAATTTGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGGTC
Sbjct 6018 AAGCAAATTTGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGGTC

Query 361 GCGCGCTTGTATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACACGC
Sbjct 6078 GCGCGCTTGTATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACACGC

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTTAGAAGGACTCTCATCG
Sbjct 6138 CGGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTTAGAAGGACTCTCATCG

Query 481 AGCGGGGACGT-GCCACGCTTCGGTCCAGTAGAAGTCT-CTTATCTGGTGTGGCGATT
Sbjct 6198 AGCGGGGACGTAGT-GCCTTCGGTCCAGTAGAAGTCT-ATCTGGTGTGGCGATT

Query 539 CGACCGACAACCTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTGCGA
Sbjct 6256 CGACCGACAACCTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTGCGA

Query 599 TTTATGAGTTGTCAAGCGAGTCTCCGGGAGCGTGGCCGATGCGCATCTGCTGAATGCG
Sbjct 6316 TTTATGAGTTGTCAAGCGAGTCTCCGGGAGCGTGGCCGATGCGCATCTGCTGAATGCG

Query 659 CCACCTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCAATCCGCGGGC
Sbjct 6376 CCACCTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCAATCCGCGGGC

Query 719 ACGCCCTGCGCGCGCTTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGCAC
Sbjct 6436 ACGCCCTGCGCGCGCTTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGCAC

Query 779 CAAATCGCTCAGTCTGTGAGTAG
Sbjct 6496 CAAATCGCTCAGTCTGTGAGTAG
    
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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   ATGTTAAAGTTATT-AGT-AGTTTATTGGTCTACATGACCGCGTCTGTATGGCTGTGCG

Query 59  CGAGTCCGCTCGCTTTTCCGTAAGTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCG
Sbjct 98  CAAGTCCGTTAGCCCAATCCGCGGAGCGAGTGGTGGTATCCGACAGTCAAGGAAATTCG

Query 119 CGTCCGGGAGGTCGGGCTTTACAGAGTTGCCGATGGTGTGGTTCGCATATCGCAACGC
Sbjct 158 CGTCCGGGAGGTCGGGCTTTACAGAGTTGCCGATGGTGTGGTTCGCATATCGCAACGC

Query 179 GGTCCGTTGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCGGTATGGTATGAGT
Sbjct 218 AGTCCGTTGATGGCGCGGTCTACCCGTCCTAATGGTCTCATTTGTCGGTATGGTATGAGT

Query 239 TGCTTTTGTATGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGATTG
Sbjct 278 TGCTTTTGTATGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGATTG

Query 299 AGAAGCAAATTTGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGCG
Sbjct 338 AAAAGCAAATTTGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGCG

Query 359 TCGGCGCGGTTGATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACAC
Sbjct 398 TCGGCGCGGTTGATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACAC
    
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Query 419 GCCCGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTCTAGAAGGACTCTCAT
Sbjct 458 GCCCGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTCTAGAAGGACTCTCAT

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

Query 537 TTCGACCGACAA-CTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTG
Sbjct 576 TTCGACCGACAACTCG-GTGTATACGTCCTCGTCCAGGACGCTATACGCTGGTGTG

Query 596 C-GATTATGAGTTGTCAAGCGAGTCTGCGGGGAGCGTGGCCGATGCCGATCGGCTGAA
Sbjct 635 CCG-TTCATGAGTTGTCAAGCGAGTCTGCGGGGAGCGTGGCCGATGCCGATCGGCTGAA

Query 655 TGGCCCACTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCAATCCG
Sbjct 694 TGGCCCACTCCGTTGAGCGGATTCACAACTACCCGGAAGCACAGGTCGTCAATCCG

Query 715 GGGCAGCGCTGCCGGCGGTCTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGG
Sbjct 754 GGGCAGCGCTTACCGCGGCTTAGACTTCTCAAGCACACAGGATTTGTAAGGCGG

Query 775 CACAAATCGCTCAGTCTGTGAGTAGA
Sbjct 814 CACAAATCGCTCAGTCTGTGAGTAGA
    
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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   AGTCCGCTCGCTTTTCCGTAAGTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCGG
Sbjct   AGTCCGCTCGCTTTTCCGTAAGTCTAGCGGTGAGTATCCGACAGTCAAGGAAATTCGG

Query   GTCGGGGAGGTCGGGCTTTACAGAGTTGCCGATGGTGTGGTTCGCATATCGCAACGCGG
Sbjct   GTCGGGGAGGTCGGGCTTTACAGAGTTGCCGATGGTGTGGTTCGCATATCGCAACGCGG

Query   TCGTTTATGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCGGTATGGTATGAGTTG
Sbjct   TCGTTTATGATGGCGCAGTCTACCCGTCCTAATGGTCTCATTTGTCGGTATGGTATGAGTTG

Query   CTTTTGATTTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGATTGAG
Sbjct   CTTTTGATTTGATACAGCGTGGGGTGCAGAAAAACACAGCGGCACTTCTCGCGGAGATTGAG

Query   AAGCAAATTTGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGGTC
Sbjct   AAGCAAATTTGACTTCTCTGTAACCGCTGCGAGTCTCCACGCACTTTTCATGACACCGGTC

Query   GCGCGCTTGTATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACACGC
Sbjct   GCGCGCTTGTATGTCCTTCAGCGGCTGGGGTGGCAACGTACGCATCACCCTCGACACGC

Query   CGGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTTAGAAGGACTCTCATCG
Sbjct   CGGCTAGCCGAGGTAGAGGGGAGCGAGATTCCACGCACTCTTAGAAGGACTCTCATCG

Query   AGCGGGGACGT-GCCACGCTTCGGTCCAGTAGAAGTCT-CTTATCTGGTGTGGCGATT
Sbjct   AGCGGGGACGTAGT-GCCTTCGGTCCAGTAGAAGTCT-ATCTGGTGTGGCGATT

Query   CGACCGACAACCTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTGCGA
Sbjct   CGACCGACAACCTTAGTTGTGTACGTCCTCGTCCGAGTGTGCTCTATGGTGGTGTGCGA

Query   TTTATGAGTTGTCAAGCGAGTCTCCGGGAGCGTGGCCGATGCCGATCTGGCTGAATGCG
Sbjct   TTTATGAGTTGTCAAGCGAGTCTCCGGGAGCGTGGCCGATGCCGATCTGGCTGAATGCG

Query   CCACCTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCAATCCGCGGGC
Sbjct   CCACCTCCATTGAGCGGATTCACAACTACCCGGAAGCACAGTTCGTCAATCCGCGGGC

Query   ACGCCCTGCGCGCGCTTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGCAC
Sbjct   ACGCCCTGCGCGCGCTTAGACTTCTCAAGCACACAAAGATTTGTAAGGCGCAC

Query   CAAATCGCTCAGTCTGTGAGTAG
Sbjct   CAAATCGCTCAGTCTGTGAGTAG
    
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1. Member of Scientific commiti in biology dept.
2. Member of Biology Syndicate of Kurdistan.
3. Member of Kurdistan universities association
4. Member of Iraqi DNA based diagnosis Research center coloborated with Jorden Institute of DNA based diagnosis.

Published Research :

- 1- The use of Bacillus cereus phospholipase C in prophylaxis and treatment of thromboplastin induced thrombosis in mice. Kurdistan Academic J. 2004.
- 2- Emergency of vancomycin resistant Staphylococcus aureus burned patients in Emergency hospital in Sulaimani city, Kurdistan region, Iraq. Journal of Karkok University.
- 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.
- 4- Comparative analysis of the Tn1546 element from newly isolated and identified vancomycin resistant Staphylococcus aureus strain isolated from burn suffering human patients hospitalized at intensified care unit Sulaimani Central Hospital, Iraq. FEBS – June 2010.

Conference Attended:

- 1- The 2nd Kurdistan Conference on Biological Science that was held in Dohuk university in 6-8/4/2008.
- 2- The First science Confereance on applied science that held in Kirkoke University in 24-26/3/2009.
- 3- Participated in (2nd power-lab workshop and new technique in bio-science). Eqlem Danesh Co. on des.29-30, 2008 in shahid Behashti medical science, university, Tehran, Iran
- 4-Participated in “The 2010 International Conference on Bioinformatics and Biomedical Science“ in 16-18 April 2010, Chengdu, China.
- 5- Participated in “International Conference on Biological Science and Engineering” in 24-26 Nov. 2010 , Venice, Italy.