

# A New *bla<sub>VIM</sub>* 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<sub>VIM</sub>* gene using different set of primers for different regions of *VIM* gene. The results were found to be PCR positive for the *bla<sub>VIM</sub>* gene. To determine the sequence of *bla<sub>VIM</sub>* gene, DNA sequencing performed. Sequence alignment of *bla<sub>VIM</sub>* gene with previously recorded *bla<sub>VIM</sub>* gene in NCBI- database showed that *P. putida* isolate have different *bla<sub>VIM</sub>* 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  $\beta$ -lactamase enzymes production, which are a group of enzymes capable of hydrolysing the 4-membered  $\beta$ -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 ans Isoelectric focusing of  $\beta$ -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- $\beta$ -lactamase-producing strains. The results were compared to CLSI standard 2008. The isoelectric points of  $\beta$ -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  $\mu$ l dd H<sub>2</sub>O (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  $\mu$ l of supernatant were used as template in the PCR reactions. Master mix was prepared by adding 5  $\mu$ l of tag buffer, 2.5  $\mu$ l of (f and r) primer, 1  $\mu$ l of dNTP(10mM), 5  $\mu$ l of supernatant, 3  $\mu$ l MgCl<sub>2</sub>, and 0.3  $\mu$ l tag polymerase to 30.7  $\mu$ l DDH<sub>2</sub>O (50  $\mu$ 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  $\mu$ 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

Dlnya A. Mohammed is with Biology department, College of Science, University of Sulaimani, Sulaimani City, Kurdistan Region of Iraq. (phone 009647701414371, 00964533290165; e-mail: dlnya.mohamad@univsul.net).

photographed digitally. as was the preparation of recombinant plasmids containing PCR product, and transformation of them into *E. coli* DH5<sup>+</sup>. Plasmids from successful clones were used to determine the sequence of the *bla<sub>VIM</sub>* 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 ([www.ncbi.nlm.nih.gov](http://www.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<sub>VIM</sub>* gene in accordance with *bla<sub>VIM</sub>* 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<sub>IMP</sub>* and *bla<sub>VIM</sub>*) 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<sub>VIM</sub>* 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<sub>VIM</sub>* sequence from Sulaimani hospitals against *bla<sub>VIM2</sub>* gene of *P. putida* class 1 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<sub>VIM</sub>* of *P. putida* isolated from Sulaimani (Fig. 2).

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ATGTTCAAACCTTTGAGTAAGTTATTGGTCTATTGACCGCGCTATCATGGCTATTGCG
GTCTATCATGGCTATTGCGAGTCAGCGAAATTCCGGTC
ATTCTAGCGGTGAGTATCCGACAGTCAGCGAAATTCCGGTC
GGGGAGGTCCGGCTTACAGATTGCCGATGGTGTGGTC
GCATATCGCAACCGCGTCGTTGATGGCGCAGTCACCCGT
CCAATGGTCTCATTGTCGTGATGGTGATGAGTTGCTTGA
TTGATACAGCGTGGGTGCGAAAAAACACGCGGCACTCT
CGCAGGACTTGCAGAACAAATTGGACTTCCTGTAACCGGT
GCAGTCCTCACGCACTTCATGACGACCGCGTCGGCGCGT
TGATGTCCTCGGGCTGGGTGGCACGTACGCATACC
GTCGACACGCCGGTAGCCGAGGTAGAGGGAGCGAGATT
CCCACGCACTCTAGAAGGACTCTCATGAGCGGGAGC
CAGTGCCTCGGTCCAGTAGAACTCTTCTATCCTGGTGC
GCGCATTGACCGACAACTTAGTTGTACGTCCCGTCTGC
GAGTGTGCTCTATGGTGGTTGTGCGATTTATGAGTTGT
CACGCACGTC
TGCAGGGAAACGTGGCCGATGCCGATCTGGCTGAATGGCCC
ACCTCCCATTGAGCGGATTCAACAAACACTACCCGGAAGCA
CAGTTCGTATTCCGGGGCACGGCTTGCCGGCGGTCTAGA
CTTGCTCAAGCACACAAAGCAATGTTGAAAAGCGCACACA
ACGCTCAGTCGTTGAGTAGCAGGCAGATGCCGATAACAT
GAAGTT
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Fig. 1: Complete sequence of the *bla<sub>VIM</sub>* gene in *P. putida* isolate

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Query 1 ATGTTCAAACCTTTGAGTAAGTTATTGGTCTATTGACCGCGCTATCATGGCTATTGCG
Sbjct55 ATGTTCAAACCTTTGAGTAAGTTATTGGTCTATTGACCGCGCTATCATGGCTATTGCG
Query 61 AGTCGGCTCGTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAGCGAAATTCCG
Sbjct613 AGTCGGCTCGTTTCCGTAGATTCTAGCGGTGAGTACGCACAGTCAGCGAAATTCCG
Query121 GTCGGGGAGGTCCGGCTTACAGATTGCCGATGGTGTGGTCGATATCGCAACCGGG
Sbjct673 GTCGGGGAGGTCCGGCTTACAGATTGCCGATGGTGTGGTCGATATCGCAACCGAG
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Query 181 TCGTTTGTATGGCGCAGTCCTACCCGTCATAATGGTCATTGTCGGTGATGGTGATGAGTTG
Sbjct 733 TCGTTTGTATGGCGCAGTCCTACCCGTCATAATGGTCATTGTCGGTGATGGTGATGAGTTG

Query 241 CTTTTGATTGATAACAGCTGGGCTGGAAAAAACACAGCGGCACCTCTCGCGGAGATTGAG
Sbjct 793 CTTTTGATTGATAACAGCTGGGCTGGAAAAAACACAGCGGCACCTCTCGCGGAGATTGAG

Query 301 AAGCAAATTGGACTTCTGTAACCGCTGCAAGTCTCCACGCACTTTCATGACGACCGCTC
Sbjct 853 AAGCAAATTGGACTTCTGTAACCGCTGCAAGTCTCCACGCACTTTCATGACGACCGCTC

Query 361 GGCGCGCTTGTATGTCCTTCAGCGGCTGGGTTGGCAACGTAACGCATACCGTCGACACCGC
Sbjct 913 GGCGCGCTTGTATGTCCTTCAGCGGCTGGGTTGGCAACGTAACGCATACCGTCGACACCGC

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG
Sbjct 973 CGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG

Query 481 AGCGGGGAGCT-GCCACGCTTCGGTCCAGTAGAACTCT-CTTATCCTGGTGTGCGCATT
Sbjct 1033AGCGGGGAGCAGTCG-CGCTTCGGTCCAGTAGAACTCTTCT-ATCCTGGTGTGCGCATT

Query 539 CGACCGACAACCTTAGTTGTGTACGTCCTGTCGAGTGTGCTCTATGGTTGTGCGA
Sbjct 1091CGACCGACAACCTTAGTTGTGTACGTCCTGTCGAGTGTGCTCTATGGTTGTGCGA

Query 599 TTATGAGTTGTGTCAGCACGTCGCGGAGGCTGGCGATGCGATCTGGCTGAATGGC
Sbjct 1151TTATGAGTTGTGTCAGCACGTCGCGGAGGCTGGCGATGCGATCTGGCTGAATGGC

Query 659 CCACCTCCATTGAGGGATTCAACAAACACTACCCGGAAGCAGACAGTTCGTCATTCCGGGC
Sbjct 1211CCACCTCCATTGAGGGATTCAACAAACACTACCCGGAAGCAGTCATTCCGGGC

Query 719 ACGCCCTGGGGGGCTAGACTTGTCTCAAGCACACAAAGAATGGTGTAAAAGCGCACA
Sbjct 1271ACGCCCTGGGGGGCTAGACTTGTCTCAAGCACACAAAGAATGGTGTAAAAGCGCACA

Query 779 CAAATCGCTCAGTCGTTGAGTAG 801
Sbjct 1331CAAATCGCTCAGTCGTTGAGTAG 1353

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**Fig. 2 Sequence alignment of *bla<sub>Vim</sub>* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* class 1 integron (*bla<sub>VIM-2</sub>*), (ACCESSION: AF327064.1)**

Sequence alignment of *bla<sub>Vim</sub>* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* strain DU25165/00 (*bla<sub>VIM-6</sub>*) (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<sub>Vim</sub>* 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 ATGTTCAAACATTGAGTAAGTTATGGCTATTGACCGCTGCTATCATGGCTATTGGG
Sbjct 1 ATGTTCAAACATTGAGTAAGTTATGGCTATTGACCGCTGCTATCATGGCTATTGGG

Query 61 AGTCGGCTCGTTTTGGTCAAGCTTACCGAGTTCGGCTGAGTACCGACAGTCAGGAAATTCCG
Sbjct 61 AGTCGGCTCGTTTTGGTCAAGCTTACCGAGTTCGGCTGAGTACCGACAGTCAGGAAATTCCG

Query 121 GTCGGGGAGGTCGGCTTTACAGATTTCGGATGGTTGGCTCATATCGAACCGG
Sbjct 121 GTCGGGGAGGTCGGCTTTACAGATTTCGGATGGTGTGGCTCATATCGAACCGG

Query 181 TCCTTGTATGTCGCACTTACCGTCAAATGGTCATTGTCGGTGTGGTGATGAGTTG
Sbjct 181 TCCTTGTATGTCGCACTTACCGTCAAATGGTCATTGTCGGTGTGGTGATGAGTTG

Query 241 CTTTTGATTGATAACAGCTGGGTGCAAAAAACACAGCGGCACCTCTCGCGGAGATTGAG
Sbjct 241 CTTTTGATTGATAACAGCTGGGTGCAAAAAACACAGCGGCACCTCTCGCGGAGATTGAG

Query 301 AAGCAAATTGGACTCTGTAACCGCTGCAAGTCTCCACGCACTTTCATGACGACCGGCTC
Sbjct 301 AAGCAAATTGGACTCTGTAACCGCTGCAAGTCTCCACGCACTTTCATGACGACCGGCTC

Query 361 GGCGGGGTTGATGFCCTTCAGGGCTGGGGTGCAACGTAACGCATACCGTCGACACCGC
Sbjct 361 GGCGGGGTTGATGFCCTTCAGGGCTGGGGTGCAACGTAACGCATACCGTCGACACCGC

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG
Sbjct 421 CGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG

Query 481 AGCGGGGAGCT-GCCACGCTTCGGTCCAGTAGAACTCT-CTTATCCTGGTGTGCGCATT
Sbjct 481 AGCGGGGAGCAGTCG-CGCTTCGGTCCAGTAGAACTCTTCT-ATCCTGGTGTGCGCATT

Query 539 CGACCGACAACCTTAGTTGTGTACGTCCTGCGAGTGTGCTCTATGGTTGTGCGA
Sbjct 539 CGACCGACAACCTTAGTTGTGTACGTCCTGCGAGTGTGCTCTATGGTTGTGCGA

Query 599 TTATGAGTTGTCAACGCACTGTCGCGGAGGCTGGCGATGCGCATGCGATCTGGCTGAATGGC
Sbjct 599 TTATGAGTTGTCAACGCACTGTCGCGGAGGCTGGCGATGCGCATGCGATCTGGCTGAATGGC

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Query 659 CCACCTCATTGAGCGGATTCAACAAACACTACCCGGAAGCAGTCGTCATTCCGGGC
Sbjct 659 CCACCTCATTGAGCGGATTCAACAAACACTACCCGGAAGCAGTCGTCATTCCGGGC

Query 719 ACGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG
Sbjct 719 ACGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG

Query 779 CAAATCGCTCAGTCGTTGAGTAGCAGGAGATGCGCATACATGAAGTT
Sbjct 779 CAAATCGCTCAGTCGTTGAGTAGCAGGAGATGCGCATACATGAAGTT

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**Fig. 3 Sequence alignment of *bla<sub>Vim</sub>* sequence from Sulaimani hospital against *Vim6* gene of *p. putida* strain DU25165/00 (*bla<sub>VIM-6</sub>*) (ACCESSION : AY165025.1)**

Sequence alignment of *bla<sub>Vim</sub>* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* strain YMC 98/2/665 class I integron (*bla<sub>VIM-2</sub>*), (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<sub>Vim</sub>* 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 ATGTTCAAACATTGAGTAAGTTATGGCTATTGACCGCTGCTATCATGGCTATTGGC
Sbjct 1455 ATGTTCAAACATTGAGTAAGTTATGGCTATTGACCGCTGCTATCATGGCTATTGGC

Query 61 AGTCGGCTGCTTTTCGCTAGATCTAGCGGTGAGTATCCGACAGTCAGGAAATTCCG
Sbjct 1515 AGTCGGCTGCTTTTCGCTAGATCTAGCGGTGAGTATCCGACAGTCAGGAAATTCCG

Query 121 GTCGGGGAGGTCGGCTTACCGAGTTCGGATGGTTGGCTCATATCGAACCGG
Sbjct 1575 GTCGGGGAGGTCGGCTTACCGAGTTCGGATGGTTGGCTCATATCGAACCGG

Query 181 TCCTTGTATGTCGCACTTACCGCTTACGCAATGGCTCATATGCGATGAGTTG
Sbjct 1635 TCCTTGTATGTCGCACTTACCGCTTACGCAATGGCTCATATGCGATGAGTTG

Query 241 CTTTTGATTGATAACAGCTGGGTGCAAAAAACACAGCGGCACCTCTCGGGAGATTGAG
Sbjct 1695 CTTTTGATTGATAACAGCTGGGTGCAAAAAACACAGCGGCACCTCTCGGGAGATTGAG

Query 301 AAGCAAATTGGACTCTCTGTAACCGCTGCAAGTCTCCACGCACTTTCATGACGACCGCTC
Sbjct 1755 AAGCAAATTGGACTCTCTGTAACCGCTGCAAGTCTCCACGCACTTTCATGACGACCGCTC

Query 361 GGCGGCCTGATGTCCTTCAGGGCTGGGGTGCGAACGTAACGACATACCGTCGACACCGC
Sbjct 1815 GGCGGCCTGATGTCCTTCAGGGCTGGGGTGCGAACGTAACGACATACCGTCGACACCGC

Query 421 CGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG
Sbjct 1875 CGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG

Query 481 AGCGGGGAGCT-GCCACGCTTCGGTCCAGTAGAACTCT-CTTATCCTGGTGTGCGCATT
Sbjct 1935 AGCGGGGAGCAGTCG-CGCTTCGGTCCAGTAGAACTCTTCT-ATCCTGGTGTGCGCATT

Query 539 CGACCGACAACCTTAGTTGTGTACGTCCTGCGAGTGTGCTCTATGGTTGTGCGA
Sbjct 1993 CGACCGACAACCTTAGTTGTGTACGTCCTGCGAGTGTGCTCTATGGTTGTGCGA

Query 599 TTATGAGTTGTCAACGCACTGTCGCGGAGGCTGGCGATGCGCATGCGATCTGGCTGAATGGC
Sbjct 205 TTATGAGTTGTCAACGCACTGTCGCGGAGGCTGGCGATGCGCATGCGATCTGGCTGAATGGC

Query 659 CCACCTCATTGAGCGGATTCAACAAACACTACCCGGAAGCAGTCGTCATTCCGGGC
Sbjct 2113 CCACCTCATTGAGCGGATTCAACAAACACTACCCGGAAGCAGTCGTCATTCCGGGC

Query 719 ACGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG
Sbjct 2173 ACGGCTAGCCGAGGTAGAGGGGAGCAGATTCCCACGCACTCTAGAAGGACTCTCATCG

Query 779 CAAATCGCTCAGTCGTTGAGTAG
Sbjct 2233 CAAATCGCTCAGTCGTTGAGTAG

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**Fig. 4 Sequence alignment of *bla<sub>Vim</sub>* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* strain YMC 98/2/665 class I integron (*bla<sub>VIM-2</sub>*), (ACCESSION: AY907717.1)**

Sequence alignment of *bla<sub>Vim</sub>* 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<sub>Vim</sub>* of p.

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

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Query 1 ATGTTCAAACCTTGGAGTAAGTTATTGGCTATTGACCGCTATCATGGCTATTGGC
Sbjct 5718 ATGTTCAAACCTTGGAGTAAGTTATTGGCTATTGACCGCTATCATGGCTATTGGC

Query 61 AGTCGCCCTCGTTTTCCGTAGATTCTAGCGGTGAGTATCAGCACAGTCAGCGAAATTCCG
Sbjct 5778 AGTCGCCCTCGTTTTCCGTAGATTCTAGCGGTGAGTATCAGCACAGTCAGCGAAATTCCG

Query 121 GTCGGGGAGGTCCGGTTACAGATTCCGATGTTGGTGGCCATATCGAACCGGG
Sbjct 5838 GTCGGGGAGGTCCGGTTACAGATTCCGATGTTGGTGGCCATATCGAACCGGG

Query 181 TCCTTGGATGCCGAGCTTACCGCTCAATGGTCTCATGTCCTGGATGGTGTGAGTTG
Sbjct 5898 TCCTTGGATGCCGAGCTTACCGCTCAATGGTCTCATGTCCTGGATGGTGTGAGTTG

Query 241 CTTTGATTGATAACAGCTGGGTGCGAAAAACACAGCGGCACCTCTCGCGGAGATTGAG
Sbjct 5958 CTTTGATTGATAACAGCTGGGTGCGAAAAACACAGCGGCACCTCTCGCGGAGATTGAG

Query 301 AAAGCAAATTGGACTTCTGTAAACGGCTGAGCTTCCACGACTTCTGACGACCGGGTC
Sbjct 6018 AAAGCAAATTGGACTTCTGTAAACGGCTGAGCTTCCACGACTTCTGACGACCGGGTC

Query 361 GGCGCGTGTGATGCTCCAGCGGCTGGGGTGGCAACAGTACGCATCACCGTGCACACCG
Sbjct 6078 GGCGCGTGTGATGCTCCAGCGGCTGGGGTGGCAACAGTACGCATCACCGTGCACACCG

Query 421 CGGCTAGCCGAGGTAGAGGGGACCGAGATTCCACAGCCTCTAGAGGGACTCTCATCG
Sbjct 6138 CGGCTAGCCGAGGTAGAGGGGACCGAGATTCCACAGCCTCTAGAGGGACTCTCATCG

Query 481 AGCGGGGAGCT-GCCAACCTTCGCTCAGTAGAAGCTCT-TTATCTGGTGTGCGATT
Sbjct 6198 AGCGGGGAGCT-GCCAACCTTCGCTCAGTAGAAGCTCT-TTATCTGGTGTGCGATT

Query 539 CGACCGACAACCTTGTGTAGCTCGGTCTCGAGTTGGTGTCTATGGTGTGTGCGA
Sbjct 6256 CGACCGACAACCTTGTGTAGCTCGGTCTCGAGTTGGTGTCTATGGTGTGTGCGA

Query 599 TTATGAGTTGTCACCGACGCTCGGGGGAGCTGGCGATCCGAATCTGGTGAATGGC
Sbjct 6316 TTATGAGTTGTCACCGACGCTCGGGGGAGCTGGCGATCCGAATCTGGTGAATGGC

Query 659 CCACCTCCATTGAGCGGATTCAACACACTACCCGGAAAGCACAGTTGCTCATCCGGGC
Sbjct 6376 CCACCTCCATTGAGCGGATTCAACACACTACCCGGAAAGCACAGTTGCTCATCCGGGC

Query 719 ACGGCTTGCGGGGGCTTAGACTCTGCTCAACGACACAAACGAAATGGTAAAGCGCACA
Sbjct 6436 ACGGCTTGCGGGGGCTTAGACTCTGCTCAACGACACAAACGAAATGGTAAAGCGCACA

Query 779 CAaatCGCTCAGCTGTGAGTAG
Sbjct 6496 CAaatCGCTCAGCTGTGAGTAG

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Fig. 5 Sequence alignment of *bla<sub>Vim</sub>* sequence from Sulaimani hospitals against *Vim2* gene of *p. putida* transposon Tn1332 (ACCESSION : DQ174113.1)

Sequence alignment of *bla<sub>Vim</sub>* 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<sub>Vim</sub>* 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 ATGTTCAAACCTTGGAGTAAGTTATTGGCTATTGACCGCTATCATGGCTATTG
Sbjct 40 ATGTTCAAACCTTGGAGTAAGTTATTGGCTATTGACCGCTATCATGGCTATTG

Query 59 CGAGTCCGCTCGTTTCCGTAGATTCTAGCGGTGAGTATCCGACAGTCAGCGAAATTCC
Sbjct 98 CAAGTCGGTTAGCCATTCCGGGGAGCGGAGTGTGAGTATCCGACAGTCAGCGAAATTCC

Query 119 CGGTGGGGAGGTCGGCTTACACAGATTCGGCAAGGGTTGGTGTGGCATATCGCAACCG
Sbjct 158 CGGTGGGGAGGTCGGACTTACACAGATTCGGATGGTGTGGTGTGGCATATCGCAACCG

Query 179 GGTGTTTGATGGCGAGCTACCCGTCACATGGCTCATGTCCTGGTGTGAGTAGT
Sbjct 218 AGTCGTTTGATGGCGGGTCTACCCGTCACATGGCTCATGTCCTGGTGTGAGTAGT

Query 239 TGCTTTGATTGATAACAGCTGGGGTGGCAACACAGCGGCACCTCTCGCGGAGATTG
Sbjct 278 TGCTTTGATTGATAACAGCTGGGGTGGCAACACAGCGGCACCTCTCGCGGAGATTG

Query 299 AGAACGAAATTGGACTTCTGTAACCGCTCAGTCTCCACGCACTTCTGACGACCGGG
Sbjct 338 AAAAGCAAATTGGACTTCCGTAACCGCTCAGTCTCCACGCACTTCTGACGACCGGG

Query 359 TCGGCGCGCTGATGTCCTCAGGGGGCTGGGGTGGCAACGTAACGCACTACCGTCGACAC
Sbjct 398 TCGGCGCGCTGATGTCCTCAGGGGGCTGGGGTGGCAACGTAACGCACTACCGTCGACAC

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Query 419 GCGCGCTAGCCGAGGTAGAGGGGGAGGAGATCCACCGCACTCTAGAAGGACTCTCATCG
Sbjct 458 GCGCGCTAGCCGAGGTAGAGGGGGAGGAGATCCACCGCACTCTAGAAGGACTCTCATCG

Query 479 CGAGCGGGGAGCT-GCCAAGCTTCGGTCCAGTAGAAGCTCT-TTATCTGGTGTGCGCA
Sbjct 518 CGAGCGGGGAGCTGGAGCTG-CGCTTCGGTCCAGTAGAAGCTCT-TTATCTGGTGTGCGCA

Query 537 TTGACCCGACAA-CTTAGTTGTCAGCTCCGCTCGCGAGTGTGCTCTATGGTGGTTG
Sbjct 576 TTGACCCGACAACTCTG-TTGTGATACAGTCGGTCAAGCGAACGCTGCTATACGGTGGTTG

Query 596 C-GATTTATGAGTTGTCAGCGACGCTGCGGGGGAGGGTGGCCAGTGGCATCTGGCTGAA
Sbjct 635 CGG-TTCACTGAGTTGTCAGCGACGCTGCGGGGGAGGGTGGCCAGTGGCATCTGGCTGAA

Query 655 TGGGGCACACTTCAATTGAGCGGATTCAACACACTACCGGAAGCACAGTTGCTCATTCG
Sbjct 694 TGGGGCACACTTCAATTGAGCGGATTCAACACACTACCGGAAGCACAGTTGCTCATTCG

Query 715 GGGCACGGCTGCGGGGGCTAGACTTGTGCAAGCACACAAAGATGTTGAAAGCG
Sbjct 754 GGGCACGGCTGCTACCGGGGGCTAGACTTGTGCAAGCACACAGCAACGTTGCAAGACA

Query 775 GACACAAATCGCTCAGTCCTTGACTAGAGCA
Sbjct 814 GACACAAATCGCTCAGTCCTTGACTAGAGCA

```

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

Sequence alignment of *bla<sub>Vim</sub>* 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<sub>Vim</sub>* DNA sequence from Sulaimani hospitals and *bla<sub>Vim</sub>* of *p. putida* strain PFi isolated in Portugal were 792/803 (98%) (Fig. 7). The results showed there were 10 mutations for the *bla<sub>Vim</sub>* 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 1 ATGTTCAAACCTTGGAGTAAGTTATTGGCTATTGACCGCTATCATGGCTATTG
Sbjct 1 ATGTTCAAACCTTGGAGTAAGTTATTGGCTATTGACCGCTATCATGGCTATTG

Query 2 AGTCGCGCTCGTTTCCGTAGATTCTAGCGGTGAGTATCCGACACTCAGCGAAATTCC
Sbjct 2 AGTCGCGCTCGTTTCCGTAGATTCTAGCGGTGAGTATCCGACACTCAGCGAAATTCC

Query 3 GTCGGGGAGGTCCGGCTTACACAGATTGCGATGGTGTGTTGTCGATATCGCAACGGG
Sbjct 3 GTCGGGGAGGTCCGGCTTACACAGATTGCGATGGTGTGTTGTCGATATCGCAACGGG

Query 4 TCGTTGATGGGGAGCTTACCCGTCACATGGTCTATTGTCGATGGTGTGAGTTG
Sbjct 4 TCGTTGATGGGGAGCTTACCCGTCACATGGTCTATTGTCGATGGTGTGAGTTG

Query 5 CTTTGATTGATACAGCTGGGGTGCAGAAAACACAGCGGCACCTCTCGGGAGATTGAG
Sbjct 5 CTTTGATTGATACAGCTGGGGTGCAGAAAACACAGCGGCACCTCTCGGGAGATTGAG

Query 6 AAGCAAATTGGACTTCTGTAAACGCTGAGCTCTCCACGCACTTCTGACGACCCGTC
Sbjct 6 AAGCAAATTGGACTTCTGTAAACGCTGAGCTCTCCACGCACTTCTGACGACCCGTC

Query 7 GGCGGCGTTGATGTCCTCAGGGGGCTGGGGTGGCAACGCTACGCACTACCGTCGACACGC
Sbjct 7 GGCGGCGTTGATGTCCTCAGGGGGCTGGGGTGGCAACGCTACGCACTACCGTCGACACGC

Query 8 CGCGCTAGCCGAGGTAGAGGGGGAGGAGATCCACCGCACTCTAGAAGGACTCTCATCG
Sbjct 8 CGCGCTAGCCGAGGTAGAGGGGGAGGAGATCCACCGCACTCTAGAAGGACTCTCATCG

Query 9 AGCGGGGAGCT-GCCAAGCTTCGGTCCAGTAGAAGCTCT-TTATCTGGTGTGCGATT
Sbjct 9 AGCGGGGAGCT-GCCAAGCTTCGGTCCAGTAGAAGCTCT-TTATCTGGTGTGCGATT

Query 10 CGACCGACAACCTTGTGATACGCTGGGGCTGGGGTGGCAACGCTACGCACTTGTGAGT
Sbjct 10 CGACCGACAACCTTGTGATACGCTGGGGCTGGGGTGGCAACGCTACGCACTTGTGAGT

Query 11 TTTATGAGTTGTCAGCAGCTCTGGGGGGAGGTGGGGAGATGCCATCTGGTGAATGGC
Sbjct 11 TTTATGAGTTGTCAGCAGCTCTGGGGGGAGGTGGGGAGATGCCATCTGGTGAATGGC

Query 12 CCACCTTCAATTGAGCGGATTCAACACACTACCGGAAGCACAGTTGCTCATCCGGG
Sbjct 12 CCACCTTCAATTGAGCGGATTCAACACACTACCGGAAGCACAGTTGCTCATCCGGG

Query 13 ACGGCCTGCGGGGGCTAGACTTGTGTCAGCTCCGCTCTGGAGTGTGCTATGGTGGTTG
Sbjct 13 ACGGCCTGCGGGGGCTAGACTTGTGTCAGCTCCGCTCTGGAGTGTGCTATGGTGGTTG

Query 14 CAAATCGCTCAGTCCTTGAGTAG
Sbjct 14 CAAATCGCTCAGTCCTTGAGTAG

```

Fig. 7 Sequence alignment of Sequence alignment of *bla<sub>Vim</sub>* DNA sequence from Sulaimani hospitals against *Vim* gene of *p. putida* strain PFi class 1 integron (ACCESSION FJ237530)

Sequence alignment of *bla<sub>Vim</sub>* sequence from Sulaimani hospitals against Vim4 gene of *p. putida* strain 283-02 class 1 integron (ACCESSION : FM179466.1).The sequence has a length of about 3329 bp. Identities between the *bla<sub>Vim</sub>* sequence from Sulaimani hospitals and *bla<sub>Vim</sub>4* 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<sub>Vim</sub>* 2 of *p. putida* strain 283-02 which was first published in 2009 by Patzar *et al.*, in Poland [9].

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Query 1      ATGTTCAAACCTT-TTGAGTAAAG-TTATTGGTCTATTTGACCGCGCTCATGGCTATTG
Sbjct 274     ATGTTAAAATTTATT-AGT-AGTTTATTTGGTCTACATGACCCGCTCTGTCAATGGCTTCG

Query 59      CGAGTCGGCTGGCTTITCCCTAGATTTCTAGCGGTGAGTATCCGACAGTCAGGAAATTG
Sbjct 332     CAAGTCGGTAGGCCATTCCGGGAGCCGGTGGTAGTATCCGACAGTCACAGGAATTG

Query 119     CGGTCCGGGAGGTCGGCTTACCCAGATTGCCGAATGGTGTGGTTCGGATATCCGAAACCG
Sbjct 392     CGGTCGAGAGGTCGACCTTACCAAGATTCCGGATGGTGTGGTCCATATCCGAAACCG

Query 179     GGTCTGGTTGATGGCGAGTCCTACCCGTCACATGGTCTCATGGTCTGATGGTGTAGT
Sbjct 452     AGTCGTTGATGGCGGCTTACCCGTCACATGGTCTCATGGTCTGATGGTGTAGT

Query 239     TGCTTTTGATTTGATACACGGCTGGGGTGGAAAAAACACACAGGGCCTTCCTGGGGAGATTG
Sbjct 512     TGCTTTTGATTTGATACACGGCTGGGGTGGAAAAAACACACAGGGCCTTCCTGGGGAGATTG

Query 299     AGAAGCAAATGGACTTCTCTGTAACCGCTGCAGTCTCACCGCACCTTCATGACGACCCG
Sbjct 572     AAAAGCAAATGGACTTCCCTAACCGCTGCAGTCTCACCGCACCTTCATGACGACCCG

Query 359     TCGCGCGCGTTGATGTCCTTCAGCGCGTGGGTGCGAACGTAACGTCACCGCATCACCGTCGACAC
Sbjct 632     TCGCGCGCGTTGATGTCCTTCAGCGCGTGGGTGCGAACGTAACGTCACCGCATCACCGTCGACAC

Query 419     GCCCGCTAGCCGAGGTAGAGGGGAGCAGAGATTCACACGCACTCTCTAGAAAGACTCTCAT
Sbjct 692     GCCGGCTAGCCGAGGCAAGGGGAAACGAGATTCACCGCATCTCTAGAAAGACTCTCAT

Query 479     CGACCGGGGACGT-GCCACGCTTCGGTCAGTAGAACCTCT-CTTATCCTCTGGCTGGCGA
Sbjct 752     CGACCGGGGACGAGT-CGCTTCCTTCAGTAGAGCTCTCT-ATCCCTGGCTGGCGA

Query 537     TTGACCCGACAA-CTTAGTTGATGTCCTCCGCTGCGAGTGTGCTCTATGGTGGTTG
Sbjct 810     TTGACCCGACAACTG-CTTGATATAGCTCCGCTGCGAACCTGCTATAGCTGGTTG

Query 596     C-GATTATGAGTTGTCACGCACGCTCGGGGAGCGTGGCGATGCCGATCTGGCTGAA
Sbjct 869     CCG-TTCATGAGTTGTCACGCACGCTCGGGGAACTGCGCGATGCCGATCTGGCTGAA

Query 655     TGGCCACCCCTCAATTGAGCGGATTCAACACACTACCCGGAAAGCACAGTTCTCATTCG
Sbjct 928     TGGCCACCCCTCCGGTACCGGGATTCAAAACACTACCCGGAAAGCACAGGGTCTCATTCG

Query 715     GGGCACGGCTGCCGGCGCTAGACCTGCTCAAGCACACACGAAATGGTGTAAAAAGCG
Sbjct 988     GGGCACGGCTACCGGGCGCTAGACCTGCTCCAGCACACAGCGAACGTTGTCAAAGCA

Query 775     CACACAAATCGCTCAGTCGGTAGAGTAG
Sbjct 1048    CACAAAATCGCTCAGTCGGTAGAGTAG

```

Fig. 8 Sequence alignment of *bla<sub>Vim</sub>* sequence from Sulaimani hospitals against *Vim*4 gene of *p. putida* strain 283-02 class 1 integron (ACCESSION : FM179466.1)

#### REFERENCES

- [1] Allen, L. H., Herman, G.T. and Friedman, F.H. (2002). *Staphylococcus aureus* Infection and Disease. Kluwer Academic Publishers. New York, Boston, Dordrecht, London, Moscow.
- [2] Dervisoglu, E., Dundar, D.O., Yegenaga, I., Willke, A. ( 2007) Peritonitis due to *Pseudomonas putida* in a Patient Receiving Automated Peritoneal Dialysis". *Infection*.
- [3] Jeong, S. H., Lee, K., Chong, Y. *et al.* (2003). Characterization of a new integron containing VIM-2, a metallo-β-lactamase gene cassette, in a clinical isolate of Enterobacter cloacae. *Journal of Antimicrobial Chemotherapy* 51, 397–400.
- [4] Koh,T.H., Wang,G.C. and Sng,L.H.2004 IMP-1 and a novel metallo-beta-lactamase, VIM-6, in fluorescent pseudomonads isolated in Singapore
- [5] Lawrence, M. and Tierny, J. (1999). Medical diagnosis and treatment. 38<sup>th</sup> Ed. California. San Francisco. California. USA.
- [6] Lee K, Jong Back Lim, Jong Hwa Yum, 2002 *bla<sub>VIM-2</sub>* Cassette-Containing Novel Integrons in Metallo-β-Lactamase-Producing *Pseudomonas aeruginosa* and *Pseudomonas putida* Isolates Disseminated in a Korean Hospital, *Antimicrob Agents Chemother*. April; 46(4): 1053–1058.
- [7] Luzzaro, F., Docquier, J. D., Colinon, C. *et al.* (2004). Emergence in Klebsiella pneumoniae and Enterobacter cloacae clinical isolates of the VIM-4 metallo-β-lactamase encoded by a conjugative plasmid. *Antimicrobial Agents and Chemotherapy* 48, 648–50.
- [8] Otenio, M.H., Da Silva, M.T.L., Marques, M.L.O., Roseiro, J.C., Bidoia, E.D. ( 2005) "Benzene, Toluene, and Xylene Biodegradation by *Pseudomonas putida* CCMI 852". *Brazilian Journal of Microbiology*. P. 258-261.
- [9] Patzer,J.A., Walsh,T.R., Weeks,J., Dzierzanowska,D. and Toleman,M.A. 2009. Emergence and persistence of integron structures harbouring VIM genes in the Children's Memorial Health Institute, Warsaw, Poland, *Antimicrob. Chemother.* 63 (2), 269-273.
- [10] Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo J D, Nordmann P. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. *Antimicrob Agents Chemother*. 2000;44:891–897.
- [11] Poirel, K. Ludovic Cabanne, Louis Collet, and Patrice Nordmann(2006)Class II Transposon-Borne Structure Harboring Metallo-β-Lactamase Gene *blaVIM-2* in *Pseudomonas putida*.*Antimicrobial Agents And Chemotherapy*, p. 2889–2891.
- [12] Papadopoulou,C., Thisiadou,K., Sofianou,D. and Siarkou,V.I.2007.*blaVIM-1* metallo-beta-lactamase gene in *Pseudomonas putida* clinical isolates. *J of microbiology and infectious disease*,vol 4, 118.
- [13] Rasmussen, B. A., Bush, K., Keeney, D. *et al.* (1996). Characterization of IMI-1 β-lactamase, a class A carbapenem-hydrolyzing enzyme from Enterobacter cloacae. *Antimicrobial Agents and Chemotherapy* 40, 2080–6.
- [14] Riccio M L, Franceschini N, Boschi L, Caravelli B, Cornaglia G, Fontana R, Amicosante G, Rossolini G M.(2000) Characterization of the metallo-β-lactamase determinant of *Acinetobacter baumannii* AC-54/97 reveals the existence of *bla<sub>IMP</sub>* allelic variants carried by gene cassettes of different phylogeny. *Antimicrob Agents Chemother*;44:1229–1235
- [15] Sachie Yomoda, Toyoji Okubo, Ayako Takahashi, Masami Murakami, and Shizuko Iyobe(2003) Presence of *Pseudomonas putida* Strains Harboring Plasmids Bearing the Metallo-β-Lactamase Gene *blaIMP* in a Hospital in Japan ,*Journal Of Clinical Microbiology*, p. 4246–4251
- [16] Santos,C., Caetano,T., Ferreira,S. and Mendo,S. 2008. *J. Biology*, University of Aveiro, Campus Santiago, Aveiro 3810, Portugal
- [17] Siarkou,V.I., Papadopoulou,C., Thisiadou,K. and Sofianou,2007. JOURNAL Submitted Laboratory of Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Aristotle University of Thessaloniki, University Campus, Thessaloniki 54124, Greece
- [18] Yan, J. J., Ko, W. W., Chuang, C. L. *et al.* (2005). Metallo-β-lactamase-producing Enterobacteriaceae isolates in a university hospital in Taiwan: prevalence of IMP-8 in Enterobacter cloacae and first identification of VIM-2 in *Citrobacter freundii*. *Journal of Antimicrobial Chemotherapy* 50, 503–11.
- [19] Yum,J.H., Choi,Y., Park,D.-Y., Yong,D., Lee,K. and hong,Y.2005.Department of Laboratory Medicine and Research Institute of Bacterial Resistance, Yonsei University College of Medicine, 134 Shinchondong, Seodaemungu, Seoul 120-752,Korea.

**Dinya A. Mohamed** (M' 09)/ From Iraq- Kurdistan region- Sulaimani in 1970.

*Educational Qualifications:* (beginning with most recent)

1. Ph.D in Microbiology/ Molecular biology . Joined in June 2008 from College of Science - Univ. of Sulaimani
2. M.Sc. in Microbiology, Joined in November, 2002- College of Science - Univ. of Sulaimani

3.B.Sc. in Microbiology, Joined in June 1992- College of Science

Univ. of Mostenseryah

4. High school Sulaimani preparatory for girls, Joined in 1988

She was working in bacteriology department, central lab. In Sulaimani city, Ministry of Health from 1993 to 1999), then asst. Lecturer in Biology dept/ College of Science/ Univ. of Sulaimani. (2003-2008), finally she is now lecturer in Biology dept/ College of Science/ Univ. of Sulaimani. (2008-).

1. Member of Scientific committee 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 collaborated 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 Conference on applied science that held in Kirkuk 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.