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Studying the Gene Expressions of *Bla OXA-51Like* and *Bla OXA-23Like* in *Acinetobacter baumannii*

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Abstract

Genotypic detection of some Antibiotics Resistant genes by polymerase chain reaction (PCR) (20): isolates of *Acinetobacter baumannii* that showed resistance to Ceftaxim, Cefotaxim, Cefepim, and Imipenim were selected. The results showed that 20 isolates of *A. baumannii* possess the *bla-OXA23-like* gene and that all isolates possess this gene with a percentage of 100% and a molecular weight of 605 bp. The current study showed that *A. baumannii* isolates carry 100% of the *bla-OXA51like* gene when studied with 20 isolates that are resistant to antibiotics (Imipenim, Ceftazidime, Cifepime, and Cifexime) that belong to this group of β-lactame with a molecular weight of 382 bp. Gene expression was done for the *bla-*$_{OXA23}$*like and bla-*$_{OXA51}$*like.* The results of gene expression for the *bla-*$_{OXA23}$*like gene show a decrease* in gene expression when treated with Ceftazidime.

Keywords: *Acinetobacter baumannii*, resistance gene, gene expression.

1. Introduction

Class D β-lactamase called (OXA) oxacillinases [1]. There are more than 400 types of these enzymes that contribute to carbapenem resistance. The presence of D-class β-lactamase and Metalo beta lactamase enzymes in *A. baumannii* is one of the most important mechanisms of resistance against Carbapenems in *A.baumannii* [2]. Other types of OXAs include OXA-23, OXA24, OXA-51 and OXA 85 discovered in *A. baumannii. The* OXA-23 enzyme was first discovered in *A. baumannii* isolates in the United States in 1958 [3].

The *bla-OXA23like* gene encodes for OXAs enzymes (a gene prevalent among *A. baumannii*) [4], and in another study conducted in Lebanon, it was shown that 76.5% of this gene was isolated among isolates of *A. baumannii* resistant to anti-carbapenim [5]. In another study in India, it was shown that the OXA-51 enzyme, which is encoded by the *bla-OXA51-like* gene, was isolated from 103 carbapenem-resistant bacterial isolates. This type of enzyme is resistant to penicillins and cephalosporins, as well as cephalosporinase inhibitors, including clavulani acid [6].

2. Materials and methods

2.1. Collection of sample:-

A total of 40 isolates of A. baumannii were obtained from many hospitals in Baghdad, including the Teaching Baghdad Hospital, Pediatric Hospital, AL Shahid Kazy, AL Harery, and teaching clinical laboratories, AL-Yarmok Hospital, and AL Kadhimia Hospital, from the period of November 15, 2018 to February 19, 2019, including sputum 15 (38%), blood 14 (35%), Urine sample (4%), wound swab 4 (10%), pleural effusion fluid, Throat swab and Burn one isolates (2.5%) each of them, Clinical samples were cultured in different media (blood agar, MacConkey agar, and CHROM agar) and incubated at 37°C for 24 h. For diagnosis, we used biochemical tests, including oxidase and catalase, and microscopic examination; for the final detection of isolates, we used the Vitik 2 compact system (France) and the API 20E test (France) [7].

2.2. Genetic detection of the *bla* $_{\text{OXA-23}}$ *like and bla* $_{\text{OXA-51}}$*-like* gene

The sequence (Primers) that was used to detect bla $_{\text{OXA-23}}$ *like* (F: TCTGGTTGTACGGTTCAGC) (R: AGTCTTTCCAAAAATTTTG) 650 bp and *bla* $_{\text{OXA-51}}$*-like* (F:ACAGAARTATTTAAGTGGG) (R:GGTCTACAKCCMWTCC CCA) 500 bp antibiotic resistance genes in a total volume of (25) µl including the forward (1)µl and reverse (1)µl, (2)µl of DNA template, (8.5)µl of deionized-free water (Go Taq master mix) with 12.5 µl. After the PCR products were prepared, they were mixed with a Vortex and placed in the PCR. A thermal-cycling system depending on the conditions and temperatures of the *bla* $_{\text{OXA-23}}$ *like* and *bla* $_{\text{OXA-51}}$*-like* genes optimum [8].

The steps of the PCR reaction were: Step 1 (only one cycle for 5 minutes at a temperature of 95°C for the primary DNA denaturation); Step 2 (30 cycles included: A: 30 sec at 95°C for DNA template denaturation; B: 30 sec at 53°C for the primers to bind to DNA template annealing; C: 40 sec at 72°C for the associated primers to be elongated. Step3 (only one cycle for 7 minutes at 72 °C is needed for the final elongation of the double DNA strip

2.3. Agarose Gel Electrophoresis

After PCR amplification, 5µL of the product of the PCR was transferred to electrophoresis on an agarose gel at a concentration of 2% with Ethidium bromide (0.5) µg/ ml. (5) µl of the PCR product was transferred to the pits designated for it, as well as loaded 5µl of DNA ladder (100 bp) at a voltage of 100 volts for 80 minutes. The gel was examined by using a UV-Transilluminator (300 nm). [9]

2.4. RNA extraction

RNA extraction according [10].

2.5. Real Time PCR and gene expression

As a result of real-time PCR) experiments, the kit was used (one-step RT-qPCR) manufactured by (Promega, USA, as shown in **Table 1**.

Table 1. A result of gene expression *(bla* $_{OXA-23}$ *like* and *bla* $_{OXA-51}$*-like*) by using RT- PCR

Material	Stock constriction	Unit	Final constriction	Unit	Volume sample
qPCR Master Mix	2	X	1	x	5
RT mix	50	X	1	x	0.25
$MgCl_2$	-	-	-	-	0.25
Forward primer	10	µm	1	µm	0.5
Reverse primer	10	µm	1	µm	0.5
Nuclease Free Water	ng/µl	ng/µ	-	ng/µl	1
Total volume	-	-	-	-	10
Aliquoroer for single rxn			9µl of Master mix per tube and add 1µl of Template		

The steps of real-time PCR were: Step 1 (only one cycle for 15 minutes) at a temperature of 37°C for cDNA and for the initial denaturation of DNA (5 minutes) at a temperature of 95°C, Step 2 (40 cycles included): A: 20 sec at 95°C for DNA template denaturation; B: 20 sec at 53 °C for the primers to bind to DNA template annealing; C: 30 sec. at 72°C for the associated primers to be elongated. Step 3 (three cycles for one sec. at 72°C to 95°C for the melted green Calculate the amount of change in the level of gene expression as shown by the following equations:

Folding $=2^{-\Delta\Delta CT}$

$\Delta\Delta CT = \Delta CT$ Treated - ΔCT Control

ΔCT =CT gene - CT House Keeping gene (*16SrRNA*)

3. Results and discussion

3.1. Detection of *bla*-$_{OXA23}$ *like gene*

Twenty isolates of *Acinetobacter baumannii* that showed resistance to (Ceftaxim, Cefotaxim, Cefepim, and Imipenim) belonging to the β-lactam group were selected. The results showed that 20 isolates of *A. baumannii* possess the *bla-OXA23-like* gene, which encodes for the enzyme (Carbapenimase that is responsible for resistance to β-lactam antibiotics, and that all isolates of *A. baumannii* possess this gene at a percentage (100%). with a molecular weight of 605 bp, as shown in **Figure 1** and **Table 2.** Several studies indicated that many genes that encode carbapenemase enzymes that are related to resistance to anti-carbapenems (Imipenim, Meropenim). The resistance of bacteria to these antibiotics occurs due to their production of β-lactamase enzymes that degrade beta-lactams, and this leads to the resistance of *A. baumannii* to

antibiotics. OXA23-like with a percentage (95.4%) [11] Whereas [12] indicated that *A. baumannii* isolated from different clinical cases carries the *bla-OXA23like* gene at a rate of 97.7%. The reason is that this gene has a high prevalence among clinical bacterial isolates.

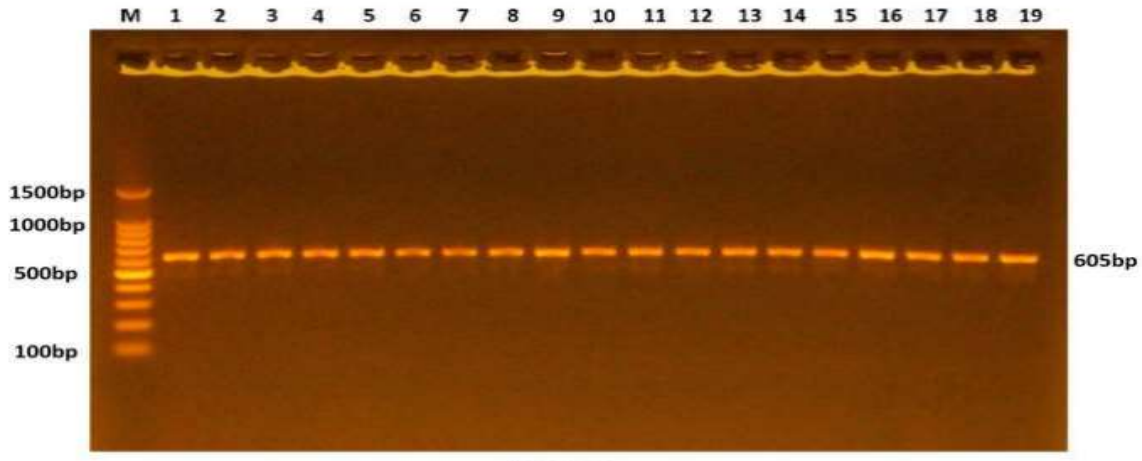

Figure 1. Electrophoresis on agarose gel (2%) of *bla oxa 23 like* gene amplifiers of *A. baumannii* at a voltage of 100 V/cm for 80 minutes

Table 2. Resistance of bacteria to beta-lactam antibiotics due to their possession of the *bla-OXA-23 like* gene

No of isolate	Antibiotics that are bacteria resistant (beta-lactam group)	*bla*-OXA23*like*
1	Ceftazidim ,Imipenim ,Cefepim,Cefotaxim ,Ampicillin , Augmantin	Ve+
2	Ceftazidim ,Imipenim ,Cefepim , Cefotaxim Ampicillin , Augmantin	Ve+
3	Ceftazidim , Imipenim , Cefepim , Cefotaxim Ampicillin , Augmantin	Ve+
4	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim Ampicillin , Augmantin	Ve+
5	Ceftazidim , ,Cefepim ,Cefotaxim , Ampicillin , Augmantin	Ve+
6	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim ,Ampicillin , Augmantin	Ve+
7	Ceftazidim , ,Cefepim ,Cefotaxim Ampicillin , Augmantin	Ve+
8	Ceftazidim , ,Cefepim ,Cefotaxim ,Ampicillin , Augmantin	Ve+
9	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim ,Ampicillin , Augmantin	Ve+
10	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
11	Ceftazidim , ,Cefepim ,Cefotaxim Ampicillin , Augmantin	Ve+
12	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
13	Ceftazidim , ,Cefepim ,Cefotaxim Ampicillin , Augmantin,	Ve+
14	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
15	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim ,Ampicillin , Augmantin	Ve+
16	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
17	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
18	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
19	Ceftazidim ,Imipenim ,Cefepim ,, Cefotaxim Ampicillin , Augmantin	Ve+
20	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+

3.2. Detection of *bla-OXA51 like* gene:-

The current study showed that *A. baumannii* isolates carry 100% of the *bla-OXA51like* gene when studied with 20 isolates that are resistant to antibiotics (Imipenem, Ceftazidime, Cifepime, Cifexime) that belong to this group of β-lactam. *bla-OXA51 like* gene encoding enzymes Carbapenemase. When comparing the doubled bundles with the ladder, it had a molecular weight of 382 bp, as shown in **Figure 2** and **Table 3**. These results were in agreement with the study conducted by [11], where the percentage of the presence of the *bla-OXA51like gene* was 91%, and this was also indicated by researchers [13] when they found that the percentage of the

presence of the gene *bla -OXA 51 like*) in isolates of *A. baumannii* was 91.3%). In another study conducted by [14], they showed that the presence of the gene *bla-OXA51ike*), which encodes for Carbapenimase enzymes, amounted to 100%, which is a result consistent with what we have found in the current study.

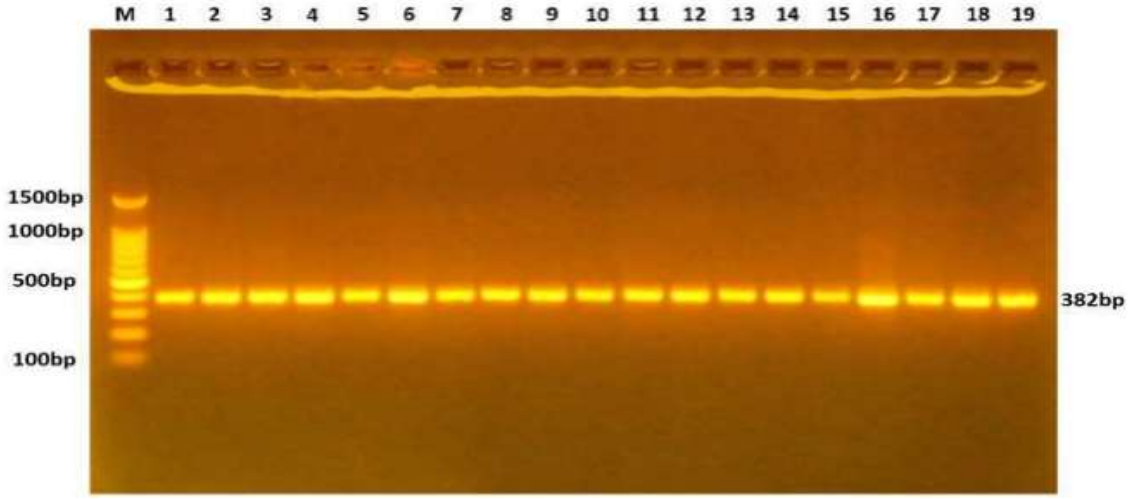

Figure 2. Electrophoresis on agarose gel (2%) of *bla oxa 51 like* gene amplifiers of *A. baumannii* at a voltage of 100 V/cm for 80 minutes

Table 3. Resistance of bacteria to beta-lactams because they possess the *bla-OXA-51 like* gene.

No. of isolate	Antibiotics that are bacteria resistant to beta-lactam group	***bla*-$_{OXA51}$*like***
1	Ceftazidim ,Imipenim ,Cefepim,Cefotaxim ,Ampicillin , Augmantin	Ve+
2	Ceftazidim ,Imipenim ,Cefepim , Cefotaxim Ampicillin , Augmantin	Ve+
3	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim Ampicillin , Augmantin	Ve+
4	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim Ampicillin , Augmantin	Ve+
5	Ceftazidim , ,Cefepim ,Cefotaxim , Ampicillin , Augmantin	Ve+
6	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim ,Ampicillin , Augmantin	Ve+
7	Ceftazidim , ,Cefepim ,Cefotaxim Ampicillin , Augmantin	Ve+
8	Ceftazidim , ,Cefepim ,Cefotaxim ,Ampicillin , Augmantin	Ve+
9	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim ,Ampicillin , Augmantin	Ve+
10	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
11	Ceftazidim , ,Cefepim ,Cefotaxim Ampicillin , Augmantin	Ve+
12	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
13	Ceftazidim , ,Cefepim ,Cefotaxim Ampicillin , Augmantin,	Ve+
14	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
15	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim ,Ampicillin , Augmantin	Ve+
16	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
17	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
18	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+
19	Ceftazidim ,Imipenim ,Cefepim ,, Cefotaxim Ampicillin , Augmantin	Ve+
20	Ceftazidim ,Imipenim ,Cefepim ,Cefotaxim, Ampicillin , Augmantin	Ve+

3.3 Gene expression measurement of the *bla-OXA23* LIKE gene

The gene expression of the anti-β-lactam-resistant *bla-OXA23like* gene was also measured by quantitative polymerase chain reaction (real-time PCR), as the reaction curve was running well with no contaminants, as shown in **Figure 3**. Primers for the gene (*bla-OXA23* and *16S rRNA*) were used using the SYBR green method.

Gene expression measurement of the *bla-OXA23like* gene of *A. baumannii* resistant to beta-lactam antibiotics after it was treated once with the anti-Ceftazedim (0.19 ml/L depending on the MIC concentration). It was noted that there was no increase in gene expression when the bacterial isolate was treated with the antibiotic (Ceftazedim), folding was measured depending on ($2^{-\Delta\Delta CT}$) and again in the absence of the antibiotic (Ceftazedim) as it was (folding based on $2^{-\Delta\Delta CT}$) (1.00), which was considered (control positive) as shown in **Table 4**.

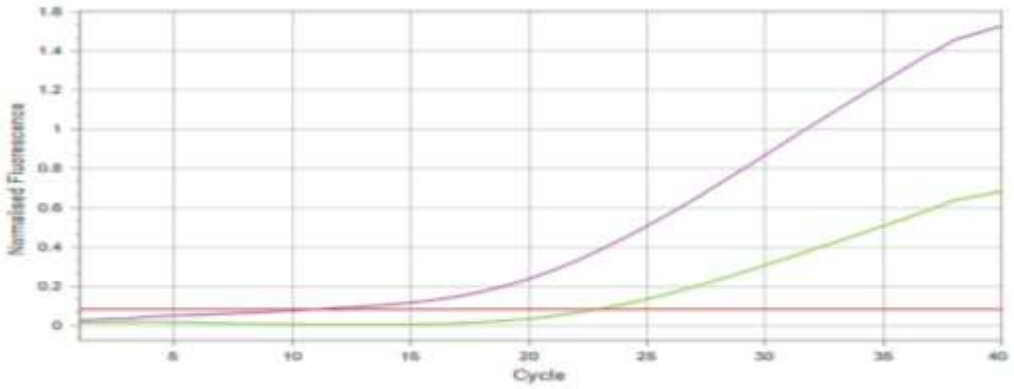

Figure 3. Quantitative polymerase chain reaction curve for *bla-OXA23* gene, the violet color represents the sample treated with the antibiotic Ceftazedim, the green color represents the sample without treatment.

Table 4. Gene expression values of *16SrRNA* and *bla-OXA23 like*

Sample	*16SrRNA* Housekeeping gene CT	*bla*-OXA23 gene CT	ΔCT	ΔΔCT	Folding
positive control Not treated with ceftazidime	6.72	10.96	4.24	0.00	1.00
Treated with the ceftazidime	6.76	22.89	16.13	11.89	0.00

Ct (cycling threshold), ΔCT (delta cycling threshold), ΔΔCT (delta delta threshold)

3.4 Gene expression measurement of the *bla-51OXA like* gene

The gene expression of the β-lactam-resistant *bla-OXA51like* gene was measured by quantitative polymerase chain reaction (Real time PCR) using primers for the gene (*bla-OXA51* and *16S rRNA* on the SYBR green method). The reaction curve was running well with no pollutants, as shown in **Figure 4**.

Measuring the gene expression (*bla-OXA51 like*) of a bacterial isolate of *A. baumannii* resistant to beta-lactam after treatment with ceftazedim at a concentration of 0.19 ml/L depending on the (MIC) concentration, as (folding) was measured based on $2^{-\Delta\Delta CT}$ and again in the absence of the antibiotic (Ceftazedim), where (folding) was based on $2^{-\Delta\Delta CT}$ (1.00) counted as (Control positive) as shown in **Table 5**. An increase in gene expression was observed when the bacterial isolate was treated with the antibiotic Ceftazedim.

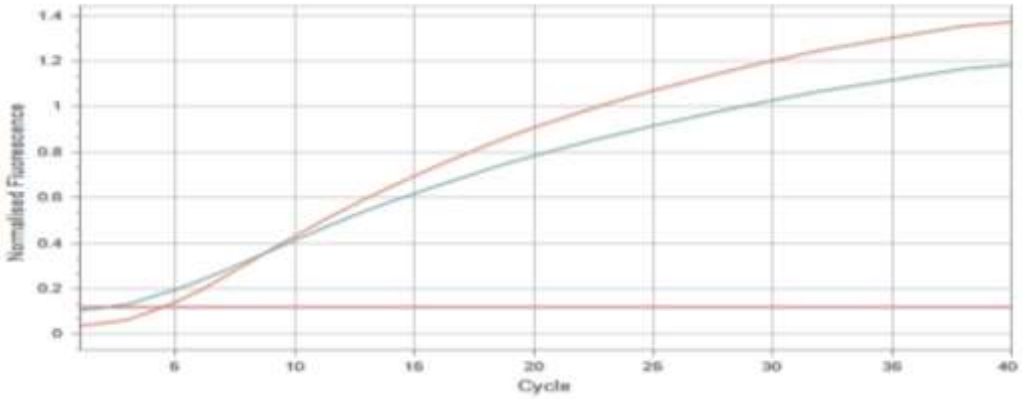

Figure 4. Quantitative polymerase chain reaction curve for *bla-OXA51* gene, blue color represents the sample treated with the antibiotic Ceftazedim, the red color represents the sample without treatment.

Table 5. Gene expression values for *16srRNA* and *bla-OXA51 like* gene

Sample	*16srRNA* Housekeeping gene CT	*bla-OXA51* gene CT	ΔCT	ΔΔCT	Folding
positive control Not treated with ceftazidime	6.72	4.47	2.25-	0.00	1.00
Treated with the ceftazidime	6.76	2.12	4.63-	2.38-	5.21

Ct (cycling threshold), ΔCT (delta cycling threshold), ΔΔCT (delta delta threshold)

4. Conclusion

The gene expression for the *bla-*$_{OXA23}$*like gene showed a* decrease when treated with Ceftazidim, whereas the *bla-*$_{OXA51}$ *like gene*, showed an increase in gene expression when treated with Ceftazidime.

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