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The Expression of *pmr* A gene in *Acinetobacter baumannii* Bacteria that Responsible for Colistin Resistance

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Abstract

A total of Forty isolates of *Acinetobacter baumannii* was Collected from many Hospitals (Teaching Baghdad Hospital, Teaching Pediatric Hospital, Al-Shahid Kazy Al-Harery and teaching clinical laboratories, Al-Yarmok hospital and Al- Kadhimia hospital) in Baghdad at the period 15/11/2018 to 19/2/2019, including sputum 15 isolates (37.5%), Blood 14 isolates (53%), wound swab 4 isolates (15%), Urine 4 isolates (10%), Burns one isolate (2.5%), pleural effusion fluids one isolate (2.5%) and throat swab one isolate (2.5%) .

The current study showed that six isolates of *A. baumannii* that were resistant to colistin (polymyxin) carry the (*pmrA*) gene at product size was 175bp. The gene expression of the *pmr A* gene for bacteria was measured once it was treated with a Colistin (polymyxin) antibiotic at a concentration (<=0.5µl) of 0.48 ml and without treated by using MIC (minimum inhibitory concentration) method as it was measured. It was observed that there was no increase in the gene expression of *pmrA* gene after the bacterial isolate was treated with Colistin (polymyxin).

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التعبيرالجيني لـ *pmr* A في بكتريا *Acinetobacter baumannii* المسؤول عن مقاومة مضاد الكولستين

رنا مجاهد عبد الله واركان عدنان مهدي

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الخلاصة

بعد التشخيص تم الحصول على اربعون عزلة لبكتريا *Acinetobacter baumannii* جمعت من عده مستشفيات (مستشفى بغداد التعليمي، مستشفى حماية الاطفال التعليمي، مشتشفى الشهيد غازي الحريري ، المختبرات التعليمية، الكاظمية التعليمي ومستشفى اليرموك التعليمي) في بغداد للمدة من 2018/11/15 حتى 2/19/ 2019 وشملت العزلات من البلغم 15 عزلة (37.5٪) ومن عينات الدم 14 (35٪) ومن مسحات الجروح 4 عزلة (15٪) ، من الادرار 4 عزلة (10٪)، من حالات الحروق عزلة واحده (2.5٪)، ومن سوائل الجسم عزلة واحدة (2.5٪) ومسحة البلعوم عزلة واحده (2.5٪). أظهرت الدراسة الحالية أن ست عزلات من بكتريا *A. baumannii* المقاومة للكولسين (بولي ماكسين) تحمل الجين (*pmrA*) وكان حجم الحزم (175) زوج قاعده. تم قياس التعبير الجيني لجين *pmr A* للعزلة البكتيرية *A. baumannii* المقاومة للكوليسين (بولي ماكسين) عند معالجتها بمضاد الكولسين بتركيز0.5=> ماكيروليتر وبحجم 0.48 مل وبدون معالجة اعتمادًا على طريقة التركيز المثبط الادنى (MIC). لوحظ انه لا توجد اية زيادة في التعبير الجيني لجين *pmrA* بعد ان تم معاملة العزلة البكتيرية بمضاد الكولسين(بولي ماكسين).

Introduction

Acinetobacter baumannii are Gram negative bacteria that are non-lactose fermenting, cocobacilli, Aerobic bacteria, belonging to the family of Moraxellacea. [1] *A. baumannii* non-motile bacteria, not produce oxidase, urease and indole, while it Produce catalase positive. Many environmental bacterial isolates grow at a temperature from 20 °C to 30 °C, while *A. baumannii* bacteria grow at a temperature of 44 °C [2].

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A. baumannii is opportunistic organism that infects humans in society and hospitals, especially patients who suffer from a defect in the immune system (immunocompromised), especially patients with burn infections and patients hospitalized in intensive care (ICU). It plays an important role in many infections, including blood stream infection, pneumonia, meningitis, soft tissues, skin infection, endocarditis and urinary tract infection (UTI) [3].

Colistin (polymyxin) is an important antibiotic in the treatment of infection caused by the highly multidrug resistant MDR-mediated bacterium including *A. baumannii*, which produces the carbapenimase enzyme [4].

With the frequent use of Colistin in the treatment of infection, *A. baumannii* bacteria were resistant to Colistin [5]. In Gram-negative bacteria, resistance against Colistin (polymyxin) is acquired through changes in target sites for Colistin that targets (lipopolysaccharide LPS), as there are two types of resistance mechanisms against colistin [6]. First, inhibition of lipid A (which is the main component of lipopolysaccharide) in Gram-negative bacteria, which leads to loss of LPS, which is the target of the antibiotic (Colistin) mutations in the (*pmrA*) and PM gene stimulate. The *pmrC* gene that adds the (Phospho-ethanolamine) group (PEtn) to (Hepta-acylated) for lipid A as mutations in these two genes (*pmrAB*) activate the second mechanism of resistance [7].

The aims of study is to isolate of *Acinetobacter baumannii* from different pathologies sources and to the express the gene of *pmr*A which that resistance genes.

Material and Methods

Collection of Sample

A total of 40 isolates of *A. baumannii* was obtained from many Hospitals (Teaching Baghdad Hospital, Teaching Pediatric Hospital, Al-Shahid Kazy Al-Harery and teaching clinical

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laboratories, Al-Yarmok hospital and Al- Kadhimia hospital) in Baghdad city within the period 15 November 2018 to 19 February2019, including sputum 15 (37.5%), blood 14 (35%), Urine sample 4 (10%), wound swab 4 (10%), pleural effusion fluid, Throat swab and Burn one isolates (2.5%) each of them, Clinical samples were diagnostic by media culture, biochemical tests, microscopic examination Vitik 2 and API 20E Test.

pmr A gene detection by PCR

Sequence primers used to detect of *pmr*A F: (ATGACAAAAATCTTGAT GATT GAAGA T) R: (CCCATCATAGGCAATCCTA AATCCA) [8]. A volume PCR solution was 25 µl including (Forward and Revere one µl for each of them, 2 µl DNA template, 8.5 µl Deionized water and 12.5 µl master mix) [9]. Steps of PCR reaction in Table (1). After that 5µL of the PCR product was transferred to the agarose gel electrophoresis at a concentration 2% with Ethidium bromide (0.5) µg/ ml, as well as loaded 5µl of DNA Ladder (100) bp. at (100) volts for (80) minutes. The gel was examined using a UV-Transilluminator (300 nm) [10].

Table 1: Optimum conditions for polymerase chain reaction of the *pmrA* gene**.**

STEP	PROGRAM	
1	Only one cycle for 5 minutes at a temperature of 95°C	
2	30 cycle included:	
	A	30 sec at 95°C
	B	30 sec at 57°C
	C	40 sec. at 72°C
3	Only one cycle for 7 minute at 72°C	

RNA extraction

RNA extraction according to [8].

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Gene expression by using Real Time PCR

The kit was used (One Step RT-qPCR) manufactured by (Promega, USA). The mix reaction of Real time PCR in Table (2)

Table 2: Mix Reaction of Real Time PCR

Material	Sample volume (µl)
qPCR Master Mix	5
RT mix	0.25
MgCl2	0.25
Template	1
Forward primer	0.5
Reverse primer	0.5
Nuclease Free Water	2.5
Total volume	10

Steps of Real time PCR shown in table (3).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

$\Delta CT =$ CT gene - CT House Keeping gene (*16SrRNA*).

Table 3: Optimum Conditions for Real Time PCR Reaction of *pmrA* gene

step	**Program**	
1	Only one cycle for 15 minutes at a temperature of 37°C	
	5 minutes at a temperature of 95°C for the Initial denaturation of DNA	
2	40 cycle included	
	A	20 sec at 95°C
	B	20 sec at 37°C
	C	30 sec. at 72°C
3	Melted green, Three cycles for one sec. at 72°C to 95°C.	

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Results and Discussion

All Forty isolates were isolated as the following: sputum 15 isolates (37.5%), Blood 14 isolates (35%), wound swab 4 isolates (10 %), Urine 4 isolates (10%), Burns 1 isolate (2.5%), pleural effusion fluids one isolate (2.5%) and throat swab one isolate (2.5%). This result was agreement with [11]. All isolates were catalase positive and oxidase negative. Cultured on MaCconky agar and after 24 hours, 37 C showed smooth and mucoid colonies and pink color. In blood agar, the isolates showed mucoid colonies and elevated, white color tended to gray color. *A. baumannii* is characterized gram negative, cocobacilli. For final identification Vitik 2 system was used. Our study found the highest isolate of *A, baumannii* the most widespread was among the sputum samples (15) isolates (37.5%), as this result was in agreement with [12] where the highest isolation of *A. baumannii* from sputum samples (56.3%). In another study co [13] it was found that the highest isolation of *A. baumannii* was among sputum samples with a percentage of (59.1%). The study of [14] have shown that these bacteria can resist dehydration when they are in several places in the hospital such as curtains, patient beds and medical equipment, and it has been proven that hospital workers are more likely to spread the disease through contamination of their hands.

The percentage of isolates bacteria from blood samples was (14) out of a total of (40) bacterial isolates, with a percentage of (35%) in the study. In a study by [12] it was high, the percentage of isolation of *A. baumannii* from blood samples was (23.35%).

As for the percentage of bacterial isolation from wound samples that were taken from patients with wound infection after performing surgical operations in a number of Baghdad hospitals, it amounted to (10%) an average of (4) isolates out of a total of (40) isolates. The reason may be due to the lack of hygiene before the operation or the lack of interest of the medical staff by not wearing sterile medical gloves in hospitals, which leads to contamination of wounds with these bacteria [15]. In another study of [16] in Iraq the bacteria isolated from wound

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infection only 4 isolates (10%) and this result agreed with the current study. After examining the bacteria in urine samples of patients with urinary tract infection, it was found that the number of isolates was (4) at a rate of (10%). The researchers also explained that the isolates of bacteria from UTI samples amounted to (13). Bacterial isolate out of a total of (111) bacterial isolates belonging to *Acinetobacter baumannii* (7.11) %.

The lowest percentage of isolates of bacteria from burns samples came from only one isolate at a rate of 2.5% of the total number of isolates and an average of one isolate from chest fluids and one isolate from throat swabs at a rate of (2.5%) for each of them. These results are consistent with [17] as the lowest percentage of isolates was from body fluids, which amounted to (10) isolates from a total of (111) isolates belonging to *A. baumannii* at a rate of (9%). This result is in agreement with the [12] as the lowest percentage of bacteria isolation from throat swabs samples was (8%). The current study showed that only six isolates of *A. baumannii* that were resistant to colistin (polymyxin). The results of our study showed that only six isolates that are resistant to colistin carry the (*pmrA*) gene at percentage (15%) the bands are equal in product size 175bp as shown in figure (1).

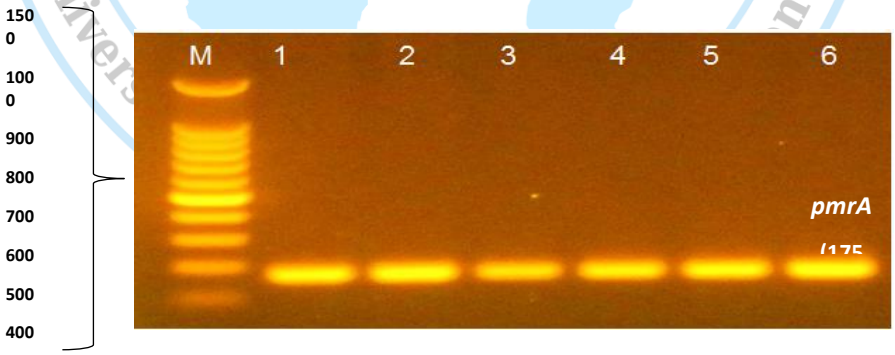

Figure 1: Agarose gel Electrophoresis of the *pmrA* gene of *A. baumannii*, at a voltage of 100 volts for 80 minutes. M: ladder, 1, 2, 3, 4, 5, 6 the positive isolate.

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Mavroidi *et al.* [18] showed that *A. baumannii* possesses the (*pmrA*) gene by (28.5%). While among researchers [19] during their study, they did not have *A. baumannii* to the (*pmrA*) gene, and the result was (0%), and this result was inconsistent with the results of the current study. The gene expression of the (*pmrA*) gene responsible for colistin (polymyxin) resistance was measured by real time PCR using (*pmrA*) and *16S rRNA* primers (control Positive) (method SYBR green). The interaction curve was running well with no contaminants as shown in Fig. (2).

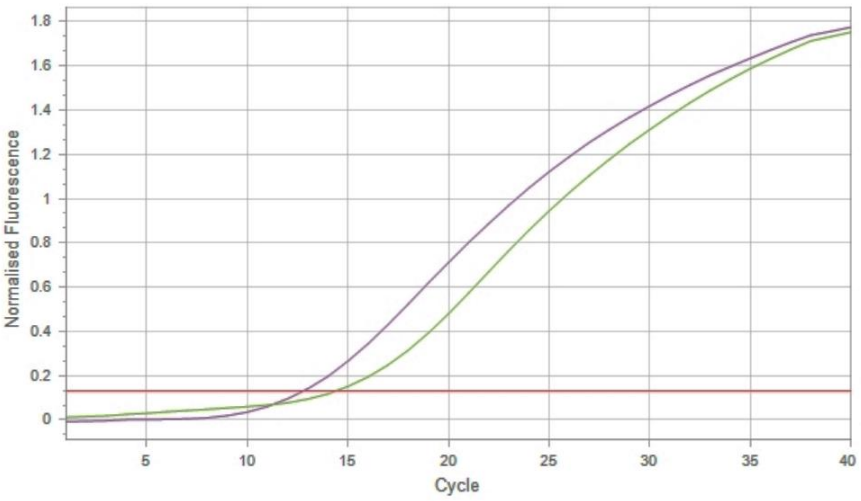

Figure 2: The quantitative polymerase chain reaction curve of the *pmrA* gene, violet color: sample treated with colicin, green color: sample without treatment

The gene expression of the *pmrA* gene of the bacterial isolate of *A. baumannii* colistin-resistant bacteria was measured once it was treated with a Colistin antibiotic at a concentration (<=0.5µl) of 0.48 ml depending on the minimum inhibitory concentration (MIC) as it was measured. Measuring Folding depending on ($2^{-\Delta\Delta CT}$) with treatment of Colistin, and without treatment of the Colistin antibiotic. The folding without treatment

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depending on ($2^{-\Delta\Delta CT}$) was 1.00 (Positive control) and the folding with treatment 0.32 that means when the isolate was treated with (Colistin) the result showed no increase in gene expression all result as shown in table (4).

The researchers [8] in their study they measured the gene expression of the *pmrA* gene in *A. baumannii* isolate that is resistant to Colistin and another isolate that was sensitive to colistin, as the value of ($2^{-\Delta\Delta CT}$) for the *pmrA* gene in the bacterial isolate resistant to the Colistin was 249.68, while the value of ($2^{-\Delta\Delta CT}$) for the bacterial isolate sensitive to the Colistin was 0.309145. It was found that the gene expression value of *pmrA* gene in the Colistin -resistant isolate was significantly increased. While there was no increase in the gene expression of the bacterial isolate sensitive to Colistin. The result was inconsistent with the current study.

Table 4: gene expression values for *16S rRNA* gene and *pmrA* gene

Samples	*16 SrRNA* (CT)	*pmr*A gene (CT)	ΔCT	ΔΔCT	Folding
Untreated with colicin antibiotic	7.96	12.48	4.88	0.00	1.00
Treatment with the colicin antibiotic	7.93	14.47	6.54	1.66	0.32

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

Conclusions

The current study showed that all isolates of *A. baumannii* that were resistant to colistin (polymyxin) carry the (*pmrA*) gene the bands are equal in product size 175bp. While there was no increase in *pmrA* gene expression after treatment bacteria with Colistin antibiotic.

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