Egypt. J. Vet. Sci. **Vol. 56**, No. 7, pp. 1457 -1465 (2025)

Egyptian Journal of Veterinary Sciences

https://ejvs.journals.ekb.eg/

Identification of blaOxa23 Gene in *Acinetobacter baumannii* Isolated From Clinical and Hospitals Environment in Mosul City-Iraq

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Abstract

ACINETOBACTER BAUMANNII an opportunistic pathogen and identified multidrug-resistant bacteria poses a significant global medical threat due to their ability to survive in a hospital environment cause nosocomial infections and develop various resistance mechanisms as an acquisition of multiple genes to resist antibiotics, especially carbapenem, it can hydrolyze this antibiotic because it has class D β-lactamases (oxacillinases). Our study focused on determining the existence of the blaOxa23 gene in strains of *Acinetobacter baumannii* resistant to many antibiotics isolated from various clinical samples in Mosul City, IRAQ**,** Three hundred samples (environmental and clinical) were collected from hospitals and the Burns Center in Mosul City, Iraq, During September 21, 2023, and January 1, 2024.The samples were cultured on MacConkey medium as a differentiation medium for Gram-negative bacteria, and biochemical tests, IMVIC test, and molecular diagnosis were performed. Out of 200 clinical sample and 100 environmental sample 24 isolates of *Acinetobacter baumannii* were detected, DNA was extracted, and PCR was applied to discover the genes of blaOxa23 . The outcomes revealed possesses the blaOxa23 gene in all selected bacterial strains resulting in a product size of 501 base pairs, our results indicate a high incidence of the *blaOxa23*-like gene, serving as a resistance marker to all ten antibiotics(Amikacin, Azithromycin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, meropenem, Levofloxacin and piperacillin _tazobactam) among *Acinetobacter baumannii* in the study region**.**

Keywords: Beta-lactamase, DNA extraction, Outbreak, Acquired resistance gene.

Introduction

A. baumannii is a significant bacterial individual of the *Acinetobacter* genus and is known to colonize the skin and cause infections in various body systems, including the bloodstream, urinary tracts, and other soft tissues. This is particularly prevalent in immunocompromised individuals, contributing to 20% of healthcare-associated infections globally [1,2].

These Gram-negative bacteria are oxidase-negative, strictly aerobic, non-fermentative, non-pigmented, and catalase-positive, and they appear in the form of pale yellow or gray colonies [3].

In the United States of America, *A. baumannii* poses a substantial threat, causing approximately 7,000 infections and 500 deaths annually. Its high importance is characterized by being one of the causes of nosocomial infections, it has the potential to develop the mechanisms of resistance against several antibiotics, especially against carbapenem, and resistance to high-severity conditions a capacity for developing robust resistance mechanisms, it has been isolated from several places such as catheters and ventilation systems, leading to conditions like urinary tract skin soft-tissue infections pneumonia, and bacteremia [4, 5,6].

Understanding the characteristics and prevalence of *Acinetobacter baumannii* is crucial, in devising strategies to address and prevent its impact on healthcare facilities. The persistent misuse and improper application of antibiotics play a role, in fostering bacterial resistance mechanisms. These mechanisms encompass acquiring resistance genes

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(Received 13/04/2024, accepted 08/05/2024)
DOI: 10.21608/EJVS.2024.282669.2003

generating hydrolyzed enzymes reducing the permeability of membranes to antibiotics modifying targets increasing efflux pump expression and forming biofilms. As a result, this bacterium can elude the effects of antibiotics adapt to conditions, and ultimately propagate infections [7].

The bacteria can also develop resistance to AmpC cephalosporinases, extended-spectrum β-lactamases, type D chromosomal OXA-51, and plasmid OXA-23, OXA-24, OXA-58, OXA-143, and OXA-134 Carbapenemases, as well as the type A Carbapenemases (KPC and GES) and type B Carbapenemases (metallo-β-lactamases) [8,9].

As one of the significant beta-lactamase resistance genes found in bacteria, this gene is normally situated on the bacterial chromosome and is sometimes present on plasmids [10].

A precise and speedy diagnosis of the strains of *A. baumannii* carrying blaOxa23 can give us vital knowledge about how these microorganisms could be managed and what type of therapy should be followed to control the rise of nosocomial infections [11,12].

In this study, we have highlighted the potential of different bacterial isolate of *A. baumannii* that were isolated from various hospitals and burn centers in Mosul City to produced bla oxa23 gene which indicates resistance to many antibiotics.

Material and Methods

Ethical approval

Ethical approval for our work was secured from the Ministry of Health and Environment, Nineveh Health Directorate, under reference number (37160) on September 20, 2023

Sample collection

Three hundred swabs were taken from different clinical specimens such as burns, respiratory infections, urinary tract infections, and surgical wounds. Were obtained from Ibn Sina Hospital, AL-Salam General Teaching Hospital, and Mosul Specialized Burn Center in Iraq from September 2023 to January 2024.

We took 100 environmental swabs, and 200 clinical (134 females and 66 males) were transferred by swab with media and then cultured on MacConkey medium. One hundred Swab was taken from burn infection, one hundred from the hospital environment, 27 from wound and surgical, 45 from urinary tract infection, 5 from CSF, and 23 from Respiratory tract infection. Subsequently, we transferred them to the Biology Department in the College of Science at the University of Mosul for diagnostic and identification purposes.

Samples culturing

Bacterial samples streaking was done on the MacConkey agar As a selective media for gram negative bacteria and incubated at 37 °C overnight under aerobic conditions. Culture reports classified bacterial isolates into lactose-fermenting and non-fermenting categories. Lactose non-fermenting isolates were selected, subcultured on MacConkey agar, and incubated overnight. Subsequently, these cultures were kept for further analysis.

Isolation and Identification of Acinetobacter baumanii

A single pure colony was initially subjected to standard bacteriological tests, encompassing assessments of the morphology of the colony on MacConkey medium, Gram stain, catalase, and oxidase tests [13].

Based on morphological properties, the isolates primarily identified as *Acinetobacter baumannii*. Subsequently, it was further confirmed using the Vitek 2 system and ultimately molecularly identified through using 16S rRNA sequence

Molecular detection of Acinetobacter baumannii

DNA extraction

To perform the DNA extraction, bacterial species were cultured on nutrient agar and incubated at 37°C for 24 hours. A full loop of inoculum was taken, Genomic DNA was extracted using a genomic DNA isolation kit supplied by the Geneaid company. Steps were followed as recommended by the manufacturer. Concentration and purity of genomic DNA was measured then DNA was stored at -20 °C until further used.

Polymerase chain reaction

PCR was conducted in a 20 µL volume reaction using GoTaq G2 Green Master Mix supplied by Promega (USA). The universal AGAGTTTGATCMTGGCTCAG primers 27F and 1522R AAGGAGGTGATCCARCCGCA were used to amplify the full region of the 16S rRNA gene [14].

The concentration of primers (1 µM each) and the total amount of template DNA (100 ng) were added as recommended by the manufacturer. The PCR program for the 16S rRNA gene was set as follows: initial denaturation at 95 °C for 3 min followed by 30 cycles of amplification including a denaturation step at 95 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 1 min. A final extension step

was set at 72 °C. PCR products were separated on 1% agarose gel and stained with Midori Green Advance DNA stain. A 100 bp DNA marker (New England Biolabs, UK) was used as a molecular weight marker. PCR products for the 16S rRNA gene were purified and sent for sequencing [14].

Sequences and record new strain

This species belonging to this genus were diagnosed by 16S rRNA gene using the Genetic Analyzer 3130 from Hitachi to determine the sequence, and then a blast was made at the National Center National Center for Biotechnology Information NCBI, and isolates were recorded for the first time.

Antibiotic susceptibility test

Ten Antibiotics (Amikacin, Azithromycin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, meropenem, Levofloxacin and piperacillin _tazobactam)used for susceptibility test . Disc diffusion method was used for this test based on the Clinical and Laboratory Standards Institute (CLSI) guidelines from the year 2023. *Acinetobacter baumannii* isolates were cultured on brain heart infusion agar. After an incubation period at 37°C for 18-24 hours, single colonies, approximately 3-5 in number, were selected and transferred into a tube containing 4 ml of physiological saline solution to obtain the bacterial suspension, to activate the bacteria and were incubated at 37 °C in the tube (0.5) of the standard McFarland tubes for 24-18 hours [15].

On the next day, a bacterial suspension was prepared, in which the bacterial count was equivalent to bacterial culture, and placed in a plate containing the1.5 x 10^8 cells/cm² [16].

A sterile swab was immersed in the suspension and then streaked on the Mueller-Hinton agar plate, and left the pales at room temperature for 15-20 minutes then we placed the antibiotic discs using forceps and the plates were incubated at 37 °C for 24 hours, and then the inhibition zones (mm) was measured using an electronic calibrator and the resulting data were compared with the CLSI guidelines 2023.

Molecular detection of blaOxa23 gene

Genomic DNA was extracted from bacterial isolates using the Genomic DNA extraction kit (Geneaid, Taiwan) following the manufacturer's instructions. The DNA extraction process involved the use of GD columns and followed a protocol that included sample preparation and bacterial cell lysis. Subsequently, DNA was bound to the membrane while contaminants remained suspended. Washing steps were performed to remove contaminants, leaving the DNA bound to the membrane. The final step involved the elution of pure genomic DNA, which was then ready for subsequent reactions. The samples were stored at -20°C until PCR was conducted [17,18].

Preparation of primers used in this study

The primer used to detect the bla oxa23 gene in *Acinetobacter baumanii* as shown in Table 1.

Polymerase Chain Reaction (PCR)

10 (µl) Master Mix. (2x) 4 (ul) Nuclease Free Water.

1 (µl) of 10 µM Forward Primer. 1 (ul) of 10 µM Reverse Primer.

4 (µl) Extracted DNA.

PCR program used to detect blaOxa23 according to Abouelfetouh [19] In Table 2 after the reaction was complete, the results of the PCR were electrophoresis using agarose gel and then viewed by ultraviolet light.

Agarose gel electrophoresis technique

The preparation of gel was achieved by dissolving 0.4 g of agarose powder in a 40 ml TAE solution pH value of 7.8, the mixture was heated with stirring using magnetic stirrers to melt all the ingredients and then poured onto a special tray with a comb left to harden at room temperature and become ready later (20).

Results

Three hundred swabs were collected from various sources, including burns, respiratory infections, urinary tract infections, and surgical wounds, as well as from the hospital's environment at Ibn Sina Hospital, AL-Salam General Teaching Hospital, and the Burn Center in Mosul city-IRAQ. These swabs were directly cultured on MacConkey agar, chosen as a selective medium for Gram-negative bacteria. Among the collected samples, 158 had no growth, while 142 samples demonstrated growth on MacConkey agar.

Within the growing samples, 88 isolates exhibited non-lactose fermenting or very weak fermenting characteristics, and 54 were identified as lactose-fermenting samples, as detailed in Table 3.

The presumptive identification of bacterial isolates relied on the shape of colony morphology (Figure 1) and Gram staining reactions. Subsequently, biochemical tests (indole, oxidase tests, methyl red, Voges-Proskauer, citrate and catalase).

Confirmation was also achieved through the Vitek 2 system, followed by molecular diagnosis using the the16S rRNA and PCR methods (Figure 2), Molecular diagnosis revealed two new isolates of *Acinetobacter baumannii* isolate No. 5 and No. 9 which were registered in (NCBI) as a new strain with no. (OR:835244.1) and no. (OR:835243.1)

Distribution of Acinetobacter baumanii

Out of 300 samples 142 sample were obtained from clinical sources and the hospital environment based on phenotypic and biochemical tests and the diagnosis was then confirmed using the vitek2 system, and then by molecular diagnosis where 24 isolates of *Acinetobacter baumannii*, were obtained. (8%) according to the biochemical test, vitek2system, and molecular diagnosis.,

Antibiotic Sensitivity

By CLSI guidelines from 2023, all 24 isolates of *Acinetobacter baumannii* were subjected for screening of antibiotic resistance against 10 antibiotics (Table 4). The results showed theirs high abilities to resist antibiotics as shown in Fig. (3) and Table (4).

High resistance rates were observed for all of the antimicrobial agents Studied, including Amikacin, Azithromycin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Imipenem, meropenem, Levofloxacin and piperacillin _tazobactam.

Detection of blaOxa23

Three isolates of *Acinetobacter baumannii (*isolate number 3,5,9) were chosen for the molecular detection of the blaOxa23 gene. The outcomes revealed all isolate possess this gene 501 bp Fig.(4).

Regarding the blaOXA-23- gene, the current result that all strains of *Acinetobacter* (100%) possessing the blaOXA-23-like gene. Respectively. blaOXA-23 of *A. baumannii* also reported from diverse countries, the OXA-23 is considered the first in the resistance among carbapenem OXA-type lactamases recognized in *A. baumannii* bacteria Different countries were exposed to outbreaks caused by the strains of *A. baumannii* which containing bla OXA-23- genes [21].

It has been found that carbapenem-resistant genes can transfer between different species, and it has been emphasized that effective identification of carbapenem-resistant strains is one of the important strategies to control their spread and infection. The Blaoxa-23-like gene was one of these strategies for antibiotic [22].

DNA sequencings

Two isolates belong to *Acinetobacter baumannii* that have blaoxa23 gen record as a new strain in National Center for Biotechnology Information (NCBI) as (AwAm1 and (AwAm2) and number OR835243.1 and OR835244.1 as shown in Fig. (5).

Discussion

High rates of resistance by bacteria were observed for most of the antimicrobials used in our study, include Amikacin, Azithromycin, Ceftazidime, Ceftriaxone, Ciprofloxacin, Gentamicin, Imepenem, meropenem, Levofloxacin and piperacillin _tazobactam. Antimicrobial Resistance Considerably restricts the available treatment options, especially resistance to carbapenem, which is Considered to be the first option to treat severe infections due to *Acinetobacter.baumannii* Nosocomial infection [23].

The Inappropriate and incorrect administration of antimicrobial agents and lack of appropriate infection control Strategies may be the possible reasons behind increasing resistant rate of Acinetobacter. Baumannii to common used Antimicrobial drugs and increase rat of mortality [24].

The researchers found Basatian_Tashkan [25] that the Acinetobacter baumannii have resisted most of the antibiotics used, and this is the same thing we found in our results, because they possess resistance genes that they may have acquired from their external environment or conjugation. Strateva [26] have explained that these bacteria have resisted the following antibiotics: Imipenem (100%), Meropenem (100%) and levofloxacin (100%), Piperacillin (100%), Ceftazidime (98.4%), and Amikacin (96.6%) and Tetracycline (91.6%).

The resistance genes of carbapenem are very important, many outbreaks have been found due to strains similar to blaoxA-23. It has been shown in our current study that all resistant Acinetobacter baumanii strains contained this gene at a rate of 100% [27].

blaOXA-23 represented a new subset of the OXA family. It has been identified in outbreaks of carbapenem-resistant Acinetobacter in many countries. Regarding blaOXA-23- gene, the current result found all strains of Acinetobacter (100%) were possessing bla OXA-23-like gene. Respectively. blaOXA-23- gene in isolates of *A.baumannii* also reported from diverse countries, the OXA-23 is considered the first in the resistance among carbapenem OXA-type lactamases recognized in *A. baumannii* bacteria Different countries were exposed to outbreaks caused by the strains of *A. baumannii*

which containing bla OXA-23- genes[22]. *Acinetobacter baumannii* Isolates had blaOXA-23 genes (Fig. 4) . In the study in mosul Iraq ,The isolates blaOXA-23 positive *A. baumannii* exhibit resistance to all tested antimicrobials and Appeared to be MDR(multi drug resistance)(Table 4) this may making a significant issue for decision of treatment.

We concluded from our research that *Acinetobacter baumannii* possess *blaOxa23* and with an increase, which negatively affects public health because after a while they will become resistant to a wide range of antibiotics, so awareness should be in the use of antibiotics and commitment to health-related matters, especially when being in hospitals with patients.

Acknowledgment

The researchers express their appreciation to everyone who participated to the completion of this work, and supporting of University of Mosul.

Conflicted interested: none

Funding statement: Self-funding

Author`s contribution: The first researcher participated in and carried out the practical aspect in addition to publication mission. The second researcher completed the task of designing, statistical analysis, making tables, and writing.

TABLE 1. Primer used in bla oxa23 gene detection

Primer name	Nucleotide sequence (5'_3')	Size of the amplicons(bps)	References
Oxa23 F	GAT CGG ATTGGA GAA CCA GA	501bp	Abouelfetouh,2019
Oxa23 R	ATT CTT GAC CGC ATT TCCAT		

TABLE 2. PCR program

Gene name	Initial denaturation	Denaturation	Cycles	Aneling	Extension	Final extension
blaOxa23	95°C/1min	95°C/15s	30	52°C/10s	72°C/10s	72°C/10min

TABLE 3.Prevalence of gram-negative bacterial growth in Mosul city hospitals

Category	No.	%
Lactose fermenting isolate	54	18%
Non-lactose fermenting isolate	88	29.3%
No growth	158	52.6%
Total	300	100%

Fig. 1. *Acinetobacter baumanii* on MacConkey agar

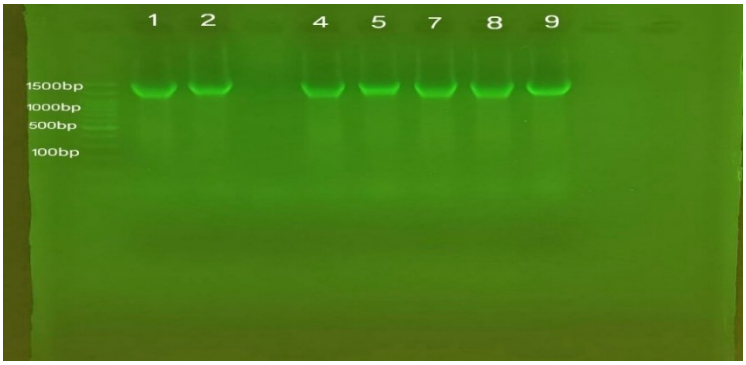

Fig. 2. The PCR output of the 16S rRNA

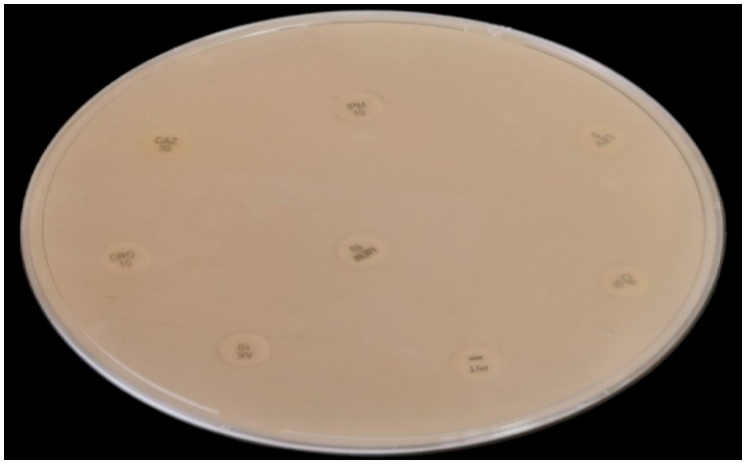

Fig. 3. Antibiotic susceptibility in *Acinetobacter baumannii*

TABLE 4. Antibiotic Sensitivity test of *Acinetobacter baumannii*

Antibiotic name	**Antibiotic group**	**Inhibition zone** (mm)
Amikacin- Ak(10)	Aminoglycosides	R(zero)
Azithromycin –AZM (15)	Macrolides	R(Zero)
Ceftazidime-CAZ(30)	Cephems	R(Zero)
Ceftriaxone -CRO(10)	Cephems	R(Zero)
Ciprofloxacin -CIP(10)	Fluoroquinolones	R(Zero)
Gentamicin -CN(10)	Aminoglycosides	R(Zero)
Imipenem -IPM(10)	Carbapenems	R(Zero)
Levofloxacin -LEV(5)	Fluoroquinolones	R(Zero)
Meropenem -MEM(10)	Carbapenems	R(Zero)
Piperacillin _tazobactam-P/T (30/6)	B-lactam+ inhibitors	R(Zero)

R= resistance

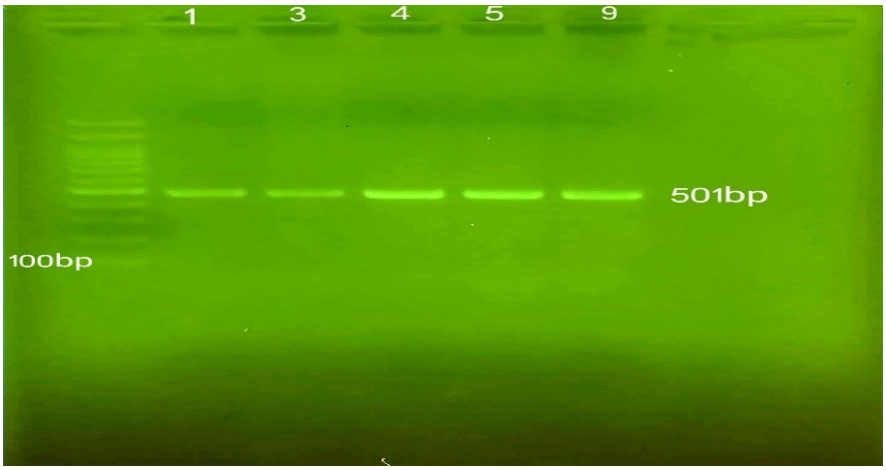

Fig. 4. electrophoresis of Oxa23 at 501bp on agarose gel technique

11/27/23, 11:55 PM Acinetobacter baumannii strain AwAm2 16S ribosomal RNA gene, partial s - Nucleotide - NCBI

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Here's how you know

Nucleotide

GenBank

Acinetobacter baumannii strain AwAm2 16S ribosomal RNA gene, partial sequence

GenBank: OR835244.1
FASTA Graphics

Go to:

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              ORGANISM  Acinetobacter baumannii
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              Moraxellaceae; Acinetobacter; Acinetobacter calcoaceticus/baumannii
                          complex.
            REFERENCE   1  (bases 1 to 796)
              AUTHORS   Sulaiman,A.I. and Saeed,A.
  TITLE     Detection the Bacterial agents of nosocomial infections in Mosul -
                          Iraq
              JOURNAL   Unpublished
            REFERENCE   2  (bases 1 to 796)
              AUTHORS   Sulaiman,A.I. and Saeed,A.
              TITLE     Direct Submission
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      781 ctctgatgct ttagtg
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Fig. 5. New strain record in NCBI

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الكشف عن blaOxa23 gene في *Acinetobacter baumannii* المعزولة من عينات سريرية وبيئة مستشفيات مدينة الموصل - العراق

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الخلاصة

تعد *Acinetobacter.baumannii* احد البكتريا المسببة للأمراض الانتهازية ذات المقاومة للأدوية المتعددة وتشكل تهديدًا طبيًا عالميًا كبيرًا نظرًا لقدرتها على البقاء في بيئة المستشفى وتسبب عدوى المستشفيات وتطور آليات المقاومة المختلفة كاكتساب الجينات متعددة المقاومة للمضادات الحيوية، وخاصة الكاربابينيم، حيث يمكنها ان تقوم بتحليل هذا المضاد الحيوي لأنه يحتوي على فئة دال من انزيمات البيتالاكتاميز ،ركزت دراستنا على تحديد وجود جين (*blaOxa23*) في سلالات بكتريا *Acinetobacter.baumannii* المقاومة للمضادات الحيوية المعزولة من عينات سريرية مختلفة في مدينة الموصل .العراق. وتم جمع300عينة سريرية وبيئية من المستشفيات ومركز الحروق في مدينة الموصل في الفترة ما بين 21ايلول2023و1كانون الثاني2024وتم الكشف عن 24عزلة من بكتريا الراكدة البومانية وتم بعدها استخلاص الحمض النووي واستخدام تقنية PCR لاكتشاف هذا الجين .

تم اختيار عزلات مختلفة من *Acinetobacter.baumannii* للكشف الجزيئي لهذا الجين، بينت النتائج التي تم الكشف عنها ان جميع عزلات الراكدة البومانية التي حددت جزيئيا كانت تمتلك الجين*blaOxa23* وعند base pairs 501،تشير نتائجنا الى وجود نسبة عالية من الجين والذي يعمل كمؤشر مقاومة لجميع المضادات الحيوية تقريبا في *Acinetobacter.baumannii* في موقع الدراسة.

الكلمات المفتاحية: انزيمات البيتالاكتاميز، استخلاص الحمض النووي،التفشي،جينات المقاومة المكتسبة.