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Molecular Characterization of bla OXA-51 Gene among Acinetobacter baumannii Isolates in Babylon Province

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ORIGINAL STUDY

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Molecular Characterization of *bla*$_{OXA-51}$ Gene among *Acinetobacter baumannii* Isolates in Babylon Province

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Abstract

Background: *Acinetobacter baumannii*, identified as an ESKAPE pathogen, is part of a group of bacteria known for their high antibiotic resistance and significant role in nosocomial infections.

Objectives: The objective of the current study is to investigate the genetic sequences of the *blaOXA-51* gene in *A. baumannii* isolates.

Materials and Methods: 600 specimens were collected during the period from September 2022 to December 2022 from three hospitals in Babylon Province/Iraq.

Results: In the present study, 20 (3.33%) out of 600 isolates from (urine, Burn, blood and wound)in percentage 6(30%), 5(25%), 5(25%), and 4(20%) respectively. The identification of bacteria by VITEK 2 system and PCR techniques for the detection of *bla*$_{oxa-51}$ gene and nucleotide sequence of nucleotide to *blaoxa-51gene*. The specimens were immediately inoculated on MacConkey agar and then on CHROM agar incubated overnight at 37°C under aerobic conditions, and the isolated bacteria were identified by VITEK 2 system. The detection of antimicrobial activity using the Vitek 2 system. The susceptibility of *A. baumannii* isolates to antibiotics, including highly resistant for Amikacin, Cefepime, Ceftazidime, and Ciprofloxacin, Piperacillin, Ticarcillin, and Ticarcillin/Clavulanic Acid. Colistin 33.33%, Imipenem 93.3%, Meropenem 86.67%, and Tobramycin 80%. Genetically, PCR for the detection of *bla*$_{OXA-51}$ genes among *A. baumannii* isolates revealed that 5 isolates were registered in GenBank.

Conclusion: PCR of *A. baumannii* performed by *blaOXA-51* Gene, Housekeeping genes *blaOXA-51* are considered unique gene to *A. baumannii* and *blaOXA-51* is considered a better molecular marker for the study of phylogenetic and taxonomic relationships at the species level, precisely for this bacterium. The detection of blaOXA-51 gene provides a correct and convenient method of identifying *A. baumannii*.

Keywords: A.baumannii, blaOXA-51, VITEK 2 system, CHROMID

1. Introduction

Acinetobacter baumannii, an opportunistic bacterial pathogen, has become increasingly associated with hospital-acquired infections [1]. This rise in incidence is partly linked to infected combat troops returning from conflict zones, notably during the Iraq War, leading to the nickname "Iraqibacter." The pathogen's sudden prominence in military treatment facilities marked the beginning of a significant increase in cases, raising alarms in the medical community. *A. baumannii's* profile has been further elevated by the dramatic rise in multidrug-resistant (MDR) strains, leading to its designation as a "red alert" human pathogen. The phenomenon of MDR pathogens is now a serious concern for both nosocomial (hospital-acquired) and community-acquired infections [2]. *A. baumannii* is a key member of the "ESKAPE" group, which includes Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae,

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Pseudomonas aeruginosa, and Enterobacter spp [3]. This acronym underscores the pathogens' ability to "ESKAPE" the effects of antimicrobial drugs, complicating treatment efforts and threatening public health. *A. baumannii's* resistance to antibiotics is largely due to intrinsic mechanisms such as the presence of *blaOXA-51*-like genes in all its isolates. These genes encode β-lactamase enzymes that degrade β-lactam antibiotics, including carbapenems, which are often used as last-resort treatments. The *blaOXA-51*-like genes are not only unique to *Acinetobacter baumannii* but are also consistently found in all its strains. This makes the detection of these genes a potentially simple and reliable method for identifying *A. baumannii* in clinical settings, surpassing current biochemical identification methods in ease and accuracy [4]. The medical community can better combat this threat and safeguard public health. The identification of *blaOXA-51*-like genes as a diagnostic marker represents a promising step towards more effective management and control of *Acinetobacter baumannii* infections, highlighting the need for continued innovation and vigilance in the face of this persistent challenge [5].

2. Materials and methods

2.1. Clinical specimens and culture characteristics

A cross-sectional study were conducted involving 300 clinical specimens collected from patients who attended the hospital in Hilla city: Al-Hilla General Teaching, from September 2022 to December 2022. They've been collected from burns, wounds, urine, and blood. When the samples was collected, they were immediately transported to the laboratory. Streaking of samples was done in MacConkey agar as well as nutrient agar, which were ann incubated at 37°C in the aerobic environment for 24 hours. Bacterial colonies with different morphologies were individually isolated and treated with Gram staining to be examined under a light microscope. These suspect colonies were later subcultured on CHROMID agar for another 24 hours at 37°C. The bacterial growth and colorations were documented after using the Vitek 2 compact system. This approach was multifaceted that entailed the assessment and classification of *A. baumannii* observed in the specimens taken from the patients.

2.2. VITEK- 2 system for identification of Acinetobacter baumannii

Acinetobacter baumannii identification was conducted utilizing the Vitek 2 system, following the manufacturer's guidelines (BioMérieux, France). The procedure involved inoculating bacteria onto a MacConkey agar plate and then incubating at 37°C for 24 hours. Subsequently, a bacterial suspension was prepared by transferring 1-3 colonies from the growth onto test tubes containing 3 mL of normal saline, with adjustment of the suspension's turbidity to a McFarland standard of 0.5. The susceptibility of *A. baumannii* isolates to various antibiotics (Amikacin, Cefepime, Ceftazidime, Ciprofloxacin, Colistin, Gentamicin, Imipenem, Meropenem, Piperacillin, Ticarcillin, Ticarcillin/Clavulanic Acid, and Tobramycin) was assessed using the VITEK 2 Compact system with Gram Negative Susceptibility software version 5.01.and AST-GN76 (*A. baumannii* cards).

2.3. DNA isolation and amplification

Genomic DNA of isolated bacteria was extracted using the classical protocol by Presto Mini gDNA Bacteria Kit (Geneaid, USA).

2.3.1. Primer

The primers utilized in this study were produced by Macrogen company located in Korea. To prepare the working solution, the primers were diluted from stock using TE buffer to achieve a concentration of 10 picomoles per microliter, after which they were stored at −20°C. The oligonucleotide primer for all genes investigated in this research were sourced from prior studies and are detailed in (Table 3). This table includes the primer sequences for each studied gene, along with their corresponding amplicon size in base pairs (bp) and the respective reference. These PCR primers were employed for detecting subtypes of system in clinical isolates of *A. baumannii*.

2.4. Molecular detection of blaOXA-51 gene by PCR

Screening of *A. baumannii* infection genetically by Conventional PCR, a sequencing study was performed according to the following steps: *A. baumannii* isolates DNA extraction from specimens, the achieved procedure according to the method recommended by the manufacturing company (Promega /USA).Primer preparation for PCR, Nuclease-free water to dissolve all lyophilized primer, as shown in (Table 1). Initially, the primer stock solution by adding 300 microliters of nuclease-free water to the primer tube, resulting in a final concentration of 100 picomoles per microliter. Next, the working solution according to the instructions provided by the primer manufacturer's (Macrogen, Korea). To do this, add 10 μL of the primer stock solution (stored at −20°C) to 90 μL of nuclease-free water, resulting in a working primer solution with a concentration of 10 pmol/μL.

Table 1. Selected primers for bla_{oxa-51} gene in this study.

Primer Name	Sequence (5-3)	PCR-product (bp)	Reference
bla_{OXA-51}	F5′-TAA TGC TTT GAT CGG CCT TG-3′ R5′-TGG ATT GCA CTT CAT CTT GG-3′	353	[6]

The amplification conditions for the two rounds of the PCR were as follows: initial denaturation at 94/3 min, followed by 25 cycles of amplification at 94/30 sec, 57/30 sec, and 72°C for 40 sec, followed by a final extension step at 72°C for 5 min in a PCR Thermocycler.

2.5. Standard sequencing

DNA sequencing was conducted to analyze genetic changes in the *blaOXA-51* gene of a local A. *baumannii* isolate, comparing it with NCBI-GenBank *A. baumannii* strains. The gene sequencing followed amplification via the PCR method. The PCR products were purified from agarose gel using the Spin Column DNA Gel Extraction Kit (Biobasic, Canada) as follows:

Specific PCR products were excised from the gel with a clean, sharp scalpel and transferred into a 1.5 mL microcentrifuge tube.

Forty-four microliters of Binding Buffer II was added to the gel fragment, incubated at 60°C for 10 minutes, and shaken until the agarose gel completely dissolved.

The mixture was added to the EZ-10 column, allowed to stand for 2 minutes, centrifuged at 10,000 rpm for 2 minutes, and the flow-through was discarded.

Fifty-four microliters of Wash Solution was added to each tube and centrifuged at 10,000 rpm for 1 minute. The solution was discarded.

This washing step was repeated and centrifuged at 10,000 rpm for another minute to eliminate residual Wash Buffer.

The column was placed in a 1.5 mL microcentrifuge tube, and 30 μL of Elution Buffer was added to the center of the column. After incubating at room temperature for 2 minutes, the tube was centrifuged at 10,000 rpm for 2 minutes to elute the PCR product, which was then stored at −20°C.

The purified PCR product samples were sent to Macrogen Company in Korea for Sanger sequencing using an automated DNA sequencer. The results were received via email and analyzed using the MEGA [6] software program. Genetic changes, phylogenetic tree analysis, and multiple sequence alignment analysis were performed based on NCBI-BLAST alignment identification. The sequences obtained in this study were deposited in GenBank under accession numbers LC600868, LC600869, LC600870, LC600871, and LC600872, along with reference strains for *A. baumannii* of the *blaOXA-51* gene.

Table 2. Showed Antibiotic susceptibility percentage of A. baumannii isolates.

Antibiotics	Resistance %	Sensitive%
Ticarcillin	100%	0%
Ticarcillin Clavulanic Acid	100%	0%
Pipracillin	100%	0%
Ceftazidime	100%	0%
Cefepime	100%	0%
Imipenem	93.33%	6.67%
Meropenem	86.67%	13.33%
Amikacin	100.00%	0%
Gentamicin	93.33%	6.67%
Tobramycin	80.00%	20.00%
Ciprofloxacin	100%	0%
Colistin	33.33%	66.67%

2.6. Ethical approval

Verbal consent was obtained from each patient before sampling. This study was approved by the Committee of Publication Ethics at the College of Medicine, University of Babylon, Iraq. University of Babylon. and hospital ethics committee under document number [IRB: 6-24, 7/9/2022].

3. Results

3.1. Clinical specimens and culture characteristics

In this study, 20(3.33%) out of 600 isolates from (urine, Burn, blood, and wound)in percentage 6 (30%), 5(25%), 5(25%) and 4(20%) respectively, as in (Fig. 1). The culture characteristics on blood, MacConky, chromagar selective media for *A.baumannii are shown* in (Fig. 2).

3.2. Antibiotic susceptibility test by VITEK 2 compact system

The results of Antibiotics Susceptibility Test by VITEK -2 system as in Table 2.

3.3. Genetically detection of blaOXA-51 gene by PCR

The *blaOXA-51* gene was detected by polymerase chain reaction (PCR) with specific primers with a

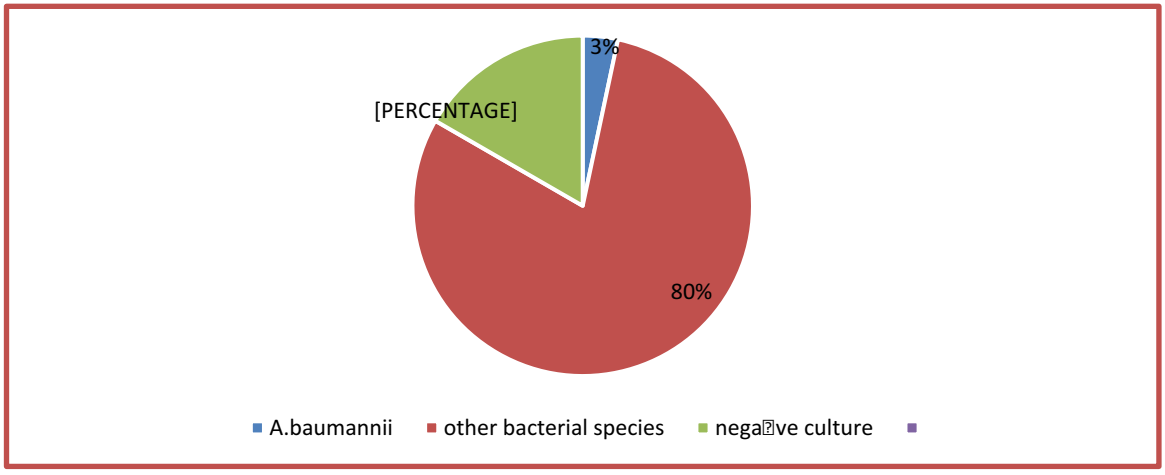


Fig. 1. Percentage of isolation rate in A. baumannii from different clinical specimens.

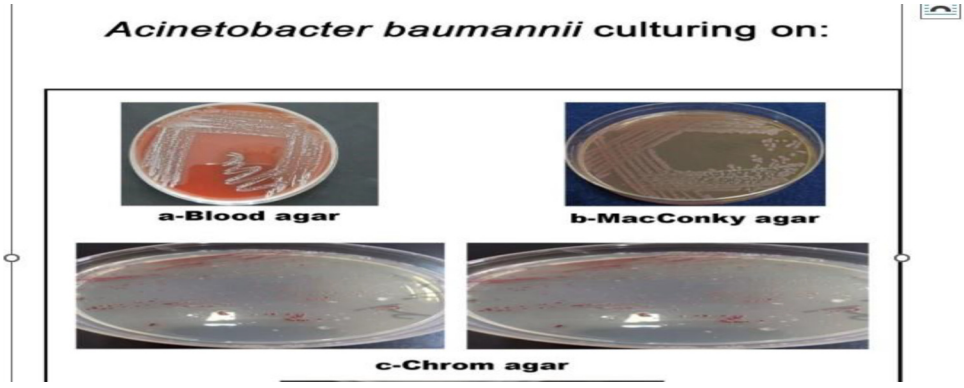


Fig. 2. A. baumannii culture characteristics on blood, MacConky, chromagar media.

product of (353) bp showed Fig. 3 results to *blaOXA-51* in 20 *A. baumannii* isolates 20(100%)

3.4. DNA-sequencing

DNA sequencing was conducted to analyze genetic changes in the *blaOXA-51* gene of a local A. *baumannii* isolate, comparing it with NCBI-GenBank *A. baumannii* strains.The Identities % and Accession of sequencing results for *blaoxa-51* gene as in Table 3. phylogenetic Trees are illustrated in Fig. 4.

3.5. Registration of blaoxa-51gene in GenBank

All five isolates were registered in NCBI under accession numbers (**LC600868 and LC600872**)-AAHH1,AAHH2,AAHH3,AAHH4,AAHH5 as in Figs. 5 and 6, and Alignment *of blaoxa-51* gene in (Fig. 7).

4. Discussion

Acinetobacter baumannii was identified based on cultural characteristics, microscopic examination, and VITEK 2 system for identification and AST for *A. baumannii.*

In present study showed highly resistant rate for Amikacin 100%, Cefepime100%, Ceftazidime100%, and Ciprofloxacin 100%, agreement with the study by [8] which reported high resistance for B-lactam class. Study [9] found a resistance rate for imipenem and meropenem 100% and present study showed Colistin 33.33%, Imipenem93.3%, Meropenem 86.67%, Piperacillin100%, Ticarcillin100%, Ticarcillin/Clavulanic Acid100%, and Tobramycin 80%.

In this study and others, variations in resistance ratios may be due to the diversity of isolated sources and the acquisition of resistant genes in *A. baumannii* isolates. In the present study, the blaOXA-51 gene was detected in all 20 *A. baumannii* isolates (100%). This contrasts with another study, which found *blaOXA-51* in 45 out of 61 carbapenem-resistant *A. baumannii*

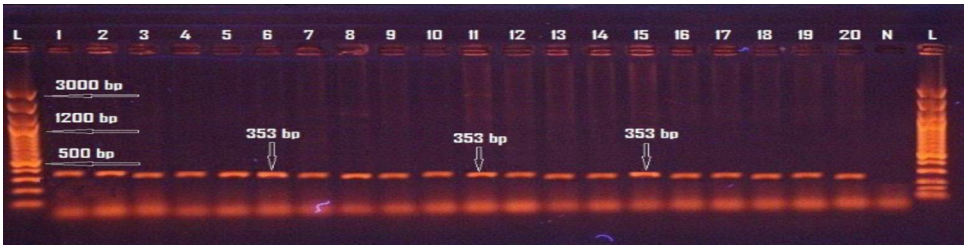


Fig. 3. Gel electrophoresis (1.5% agarose, 70 volt for 60–120 min) of blaOXA-51 gene product (amplified size 353 bp) using A. baumannii isolate DNA template. Lane (L), molecular size marker of DNA (100-bp Ladder). All lines (1–20) exhibit positive results.Lane (N), Negative control.

Table 3. Identities % and Accession of sequencing results for blaoxa-51 gene in A. baumannii isolates

Gene name	Strains. N O	Identities %	Accession	Gaps	Score
Blaoxa-51	AAHH-1-F	312/315(99%)	LC600868	3/315(0%)	562 (304)
	AAHH-1-R	272/296(92%)	LC600868	1/296(0%)	412 (223)
	AAHH-2-F	310/315(98%)	LC600869	3/315(0%)	551(298)
	AAHH-2-R	310/315(98%)	LC600869	3/315(0%)	551(298)
	AAHH-3-F	318/321(99%)	LC600870	2/321(0%)	575(311)
	AAHH-3-R	275/299(92%)	LC600870	3/299(1%)	416(225)
	AAHH-4-F	312/314(99%)	LC600871	1/314(0%)	568(307)
	AAHH-4-R	269/304(99%)	LC600871	4/304(1%)	364(197)
	AAHH-5-F	307/314(98%)	LC600872	4/314(1%)	538(291)
	AAHH-5-R	286/308(93%)	LC600872	3/308(0%)	444(240)

isolates (73.77%). Similarly, a study conducted in Babylon hospitals reported a 100% detection rate of *blaOXA-51* in *A. baumannii* isolates.

However, another study reported a much lower detection rate of 13%. In this study, the *blaOXA-51* gene was genetically screened genetically using

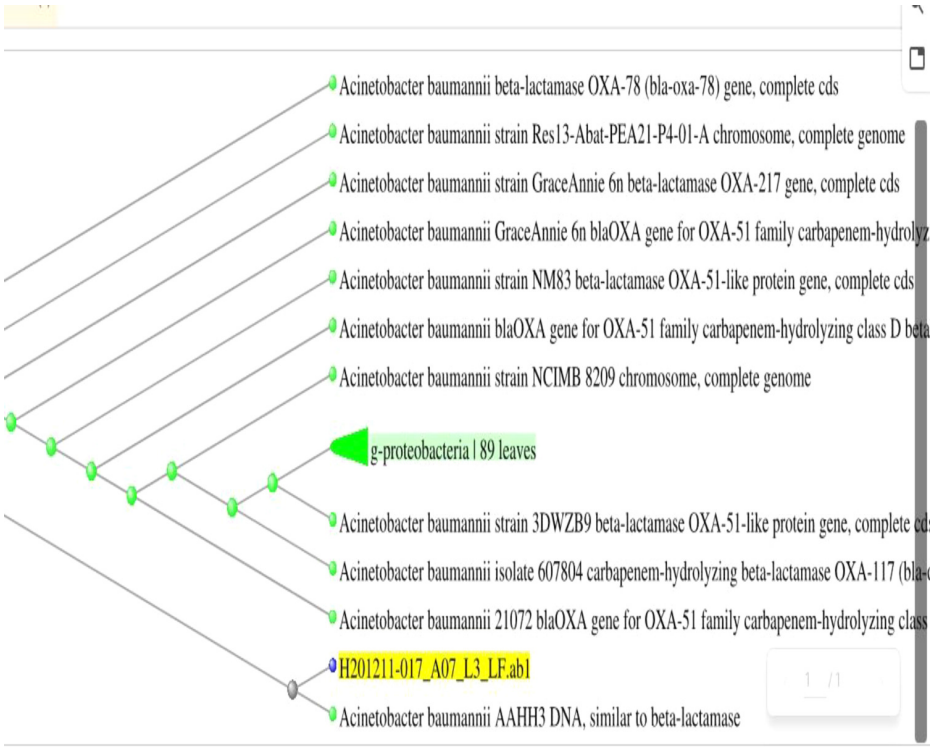
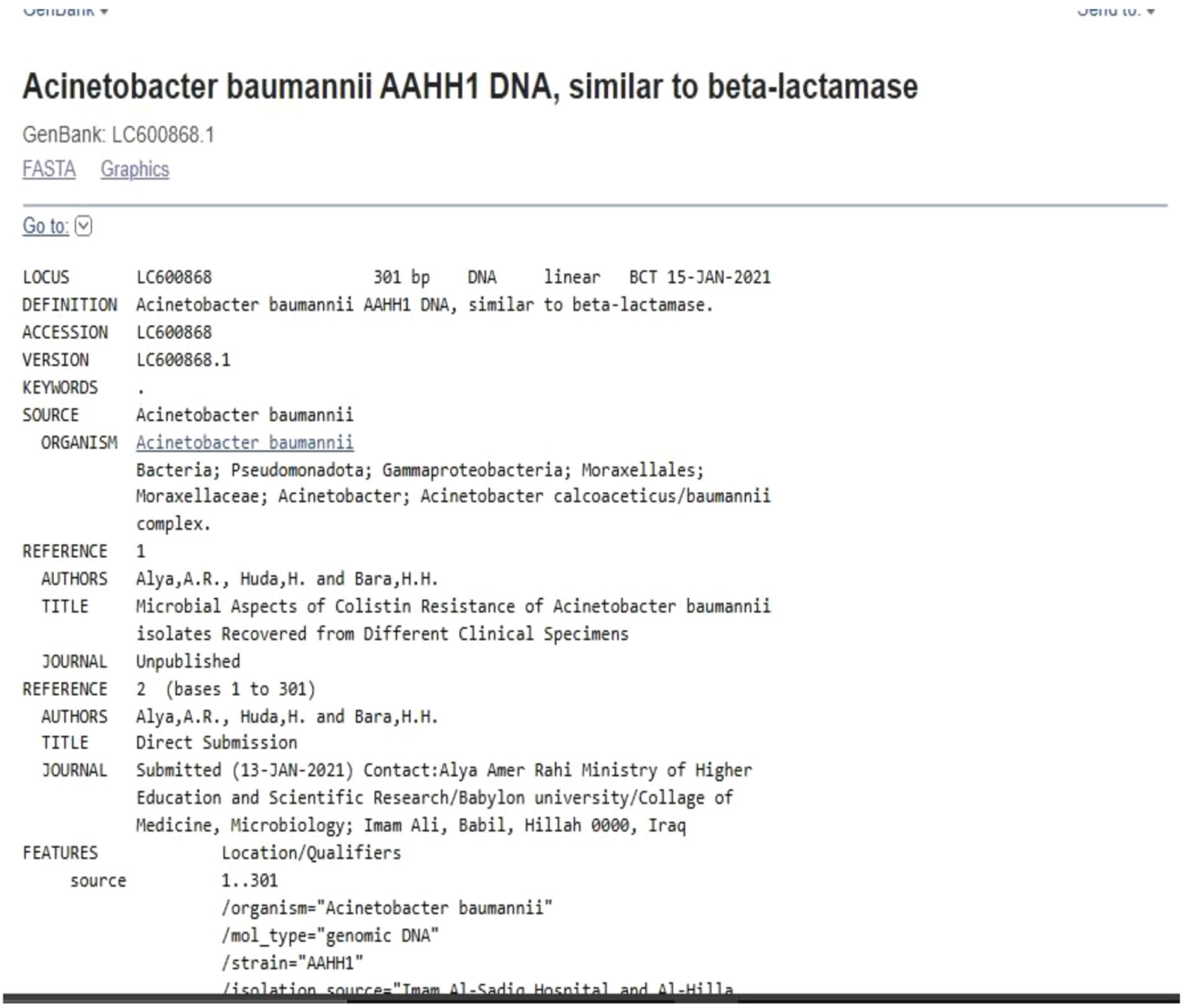


Fig. 4. Phylogenetic Trees based on aspecific blaoxa-51 gene for A. baumannii isolates from different clinical specimens.



Acinetobacter baumannii AAHH1 DNA, similar to beta-lactamase

GenBank: LC600868.1

FASTA Graphics

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  JOURNAL   Unpublished
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  AUTHORS   Alya,A.R., Huda,H. and Bara,H.H.
  TITLE     Direct Submission
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Fig. 5. Registration of the blaoxa-51gene (AAHH-1) in GenBank.

conventional PCR, followed by sequencing. PCR was utilized as a specific diagnostic tool to identify *A. baumannii* isolates from various specimens. Five isolates (AAHH1, AAHH2, AAHH3, AAHH4, AAHH5) were tested along with a positive control. The PCR amplification product size for detecting the *blaOXA-51* gene was 353 bp for all 20 isolates, as shown in (Fig. 3).

After PCR, the amplification product size for detecting the *blaoxa-51* gene was 353 bp for 20 isolates with *A. baumanni* infection .To determine the nucleotide sequences of the *blaOXA-51* gene from the PCR products, 50 μL of the PCR product for each sample, along with the *blaOXA-51* gene primer, was sent to Macrogen in South Korea. After receiving the sequencing results, they were compared with sequences of globally registered strains using MEGA [6] software. The sequences were matched against the original sequences of each gene.

The results of the current study revealed the sequences of the PCR products for five subjects with *A. baumannii* infection (Table 3), with the results for the five isolates showing sequence identity percentages of 92%, 99.08%, and 98%, respectively. The DNA sequencing results were first examined to confirm the nucleotide sequences and their close relationships with other global strains. This verification was conducted using the NCBI BLAST-query nucleotide online tool, which provided precise identity percentages ranging from 90% to 99% for the *blaOXA-51* gene.

The nucleotide sequences of the *Acinetobact*er *blaOXA-51* gene were determined for the five *A. baumannii* isolates. The BioEdit software was used to analyze the DNA sequences. Gene was known in

Acinetobacter baumannii AAHH5 DNA, similar to beta-lactamase

GenBank: LC600872.1
FASTA Graphics

Go to:

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REFERENCE   1
  AUTHORS   Alya,A.R., Huda,H. and Bara,H.H.
  TITLE     Microbial Aspects of Colistin Resistance of Acinetobacter baumannii
            isolates Recovered from Different Clinical Specimens
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 299)
  AUTHORS   Alya,A.R., Huda,H. and Bara,H.H.
  TITLE     Direct Submission
  JOURNAL   Submitted (13-JAN-2021) Contact:Alya Amer Rahi Ministry of Higher
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//
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Fig. 6. Registration of the blaoxa-51gene (AAHH-5) in GenBank.

[5] *A. baumannii* isolates. The program (Bio-Edited) was used in DNA sequences. The results of DNA sequencing should be firstly examined to confirm the nucleotide sequences and closed relationships with other worldwide strains. The test used to confirm this was through using NCBI- Blast-query nucleotide –online, it was a perfect program and provided exact results of identity percentage with other global strains, ranging they were ranged from (90%–99%) for $bla_{\text{OXA-51}}$ gene. The sequence of the nucleotide of the *Acinetobacter* $bla_{\text{OXA-51}}$ gene was known in [5] *A. baumannii* isolates.

The program (Bio-Edited) was used for DNA sequences. In the phylogenetic analysis with five isolates retrieved from the Gen Bank [19, 20]. genomic sequencing has been used to develop new diagnostic tests to identify *A. baumannii*. Moreover, genome sequencing helps identify new targets for diagnosis. Therefore, the purpose of conducting the sequences in our study is to find the correlation of the isolates with the previous studies conducte in the countries of the world to show the extent of similarity and difference. Additionally, knowing the genetic specifications genetically will benefit us in the future, especially the Ministry of Health, by following the protocol through case management, treatment, and rapid identification through *blaoxa-51*. Thus, it was a perfect program when examining the results of the current study, which was compared with other strains proven in the gene bank through the use of NCBI-BLAST-query nucleotide online. It gave the exact results of identifying percentages with other worldwide strains, ranging from (90%–99%) for *blaoxa-51* gene. The *blaoxa-51* gene sequences were

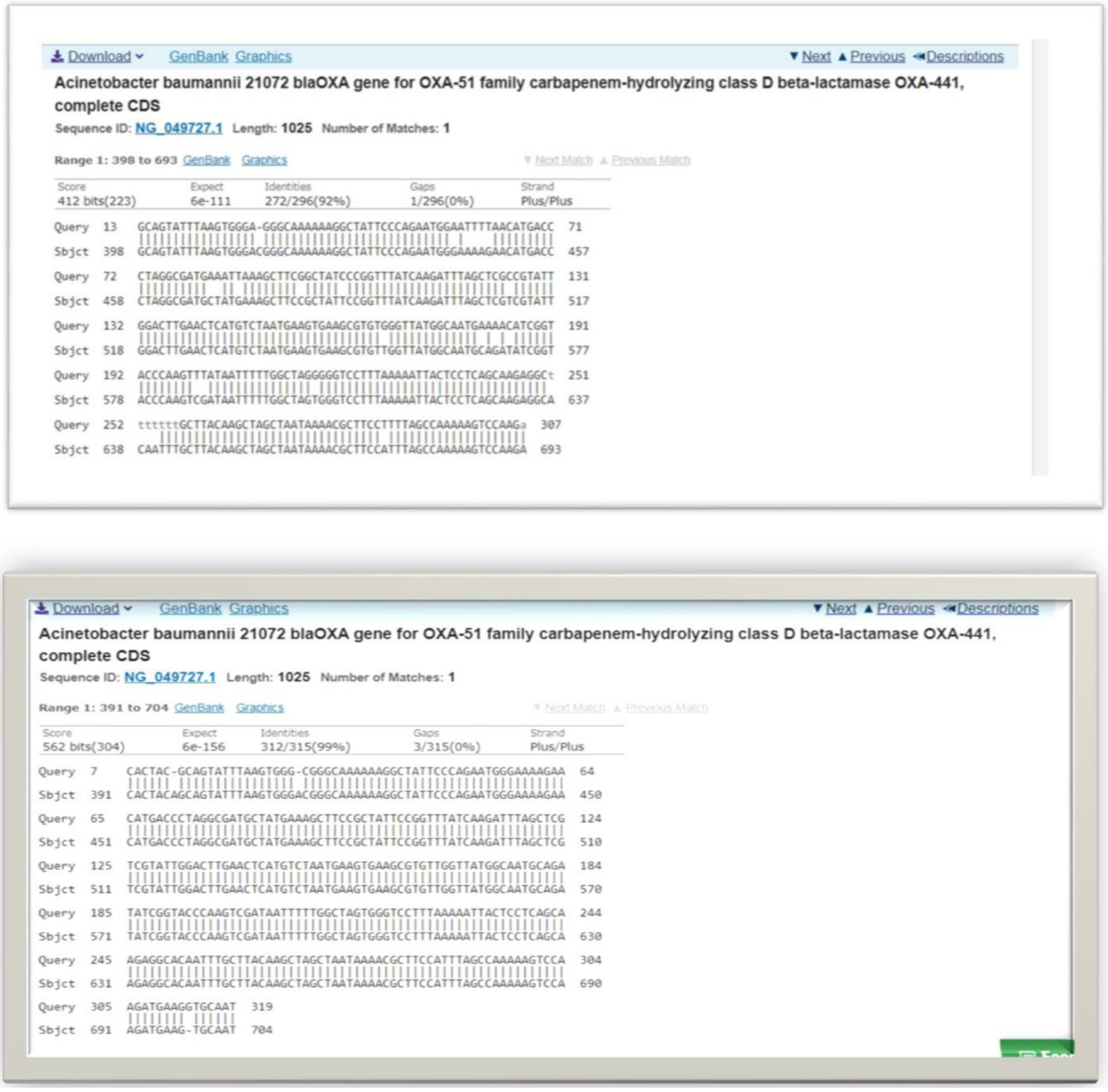

Fig. 7. Local basic alignment of blaoxa-51 gene isolate with similarity NCBI-BLAST A. baumannii carried blaoxa-51 gene.

submitted to Genbank-bank under direct submission through contact Alya Amer Rahi as in (Figs. 5 and 6). However, these procedures also apply best methods for identification of *A. baumannii* isolates. Sequencing of $bla_{\text{OXA-51}}$ PCR product, revealed that the amplicons belonged to *A. baumannii* and can be used as a diagnostic marker for this bacterium.

5. Conclusion

blaOXA-51 is a key factor in the antibiotic resistance profile of *Acinetobacter baumannii*. Its intrinsic presence and potential for upregulation underscore the need for continuous surveillance, innovative treatment strategies, and stringent infection control practices to combat the challenges posed by this formidable pathogen.

Acknowledgments

The authors express their gratitude to all patients who contributed samples, enabling the completion of this research.

Conflict of interest

The authors declare no conflicts of interest.

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