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DETECTION THE ROLE OF PROTEIN AND GENES RESISTANCE MECHANISMS IN ACINETOBACTER BAUMANNII ISOLATES RECOVERED FROM DIFFERENT CLINICAL SPECIMENS IN BABYLON PROVINCE

jwan Ahmed Ali[(1)], Huda H. Al-Hasnawy[(2)],Lamees A.Abdul-Lateef [(3)], AbeerThaher Naji AL-Hasnawi [(4)], Hayam K Almasoudi [(5)]

(1) University of Babylon, College of Medicine, Department of Microbiology.
Email: Jwanalhamawandi@gmail .com

(2) University of Babylon, College of Medicine, Department of Microbiology.
Email: Hudashmm@gmail.com

(3) University of Babylon, College of Medicine, Department of Microbiology.
Email:dr.lamees.razzak@gmail.com

(4) University of Karbala, College of Medicine, Department of Microbiology.
Email: abeer.zahir@uokerbala.edu.iq

(5) University of Babylon, College of Medicine, Department of Microbiology.
Email: med.hayam@ uobabylon.edu.iq

Abstract:

the Search included 300 different clinical specimens from patients at Al-Hilla General Teaching Hospital were collected for the study From September to December 2022. These specimens comprised blood, urine, burns, and wounds. 40(13.3%) *A.baumannii* out of 300 isolates. The focus of the study is to characterize the CarO protein,Int-2 and bla_{oxa-51} resistance mechanisms in Acinetobacter baumannii isolates recovered from different specimens. The samples were taken from various clinical specimens, which were then dispersed as follows: 40 isolates were tested for antibiotic susceptibility using the (Antibiotic Susceptibility Test-AST) on 15 (37.5%) swabs from burns, 10 (25%) swabs from wounds, 10 (25%) from urine, and 5 (12.5%) from blood samples. All isolates were found to be antibiotic-resistant, with the exception of polymyxin B, which had a 30% sensitivity rate and a 70% resistance rateThe gene Caro was found in 10 (25%) of the 40 isolates genetically analyzed using the PCR method, the gene Int-2 was found in 8 (20%) of the 40 specimens, and the gene blaOXA-51 was found in 19 (47.5%) of the 40 isolates. These results show that the gene Caro is present in proteins that encode the enzyme Carbapenemase. The findings of this investigation confirmed previous findings that bacteria have substantial polymyxin B resistance and are resistant to the majority

of antibiotics. For the diagnosis of this bacteria, the blaOXA-51 gene was regarded as a diagnostic marker (A baumannii). In Conclusion Polymerase chain reaction technique was found to be simple and useful tool for detection of outer membrane protiens *car*O. Class 2-Integron was found to be carried by *A. baumannii* isolates and antibiotic resistance genes were distributed on this integron.

Keywords: *bla*$_{OXA-51}$ gene, CarO- protein, AST, ESKAPE,I.C.P,Int-2 *Acinetobacter baumannii*

Introduction:

Acinetobacter baumannii have characteristics that distinguish them, namely, they are aerobic, immobile, polymorphic, bacillus, opportunistic and Gram-negative.Immunocompromised individuals, particularly those who spend a lot of time in hospitals, are susceptible to it and it spreads among them. It typically colonizes illnesses of the skin, lungs, and oropharynx. Due to its widespread antibiotic resistance, it is regarded as a red alert bacteria.. *Acinetobacter baumannii* causes R.I, pneumonia and UTI infections. Since they can be found in almost all samples of soil and surface water, the Acinetobacter genus of organisms is frequently recognized as being widespread in nature. As said by Shirin (2018), this species is often produced from the sputum or respiratory secretions, wounds, and urine of hospitalized patients. Acinetobacter commonly colonizes irrigation solutions and intravenous solutions in a hospital setting (Tiwari *et al*., 2015). These bacteria are considered a multi-dangerous enemy and have the potential to acquire antibiotic resistance because they have the ability to acquire drug-resistant genes and the ability to form a biofilm that enables them to resist treatments. Sequence similarity and phylogenetic analyses confirmed that most of the resistance genes found in the Acinetobacter strain had been recently acquired from bacteria of the genera Pseudomonas, Salmonella or Escherichia (Howard et al., 2012). Immunocompromised people frequently contract A. Baumannii, especially if they've had a lengthy (>90 day) hospital stay. Numerous infectious illnesses, including pneumonia, bloodstream infections (bacteremia and sepsis), meningitis, necrotizing fasciitis, and urinary tract infections, can be brought on by Acinetobacter (Castro et al., 2015). In 2017, according to the Centers for Disease Control and Prevention, carbapenem-resistant Acinetobacter is thought to have been the cause of 700 estimated fatalities and 8,500 hospitalized patient infections in the United States. Antibiotics imipenem (9 % vs. 10%), cefepime (14 % vs. 16 %), ceftazidime (18 % vs. 20 %), piperacillin-tazobactam (15 % vs. 21 %), and ciprofloxacin all showed increasing resistance patterns (24 % vs. 26 %) (Chua & Alejandria, 2008).

Acinetobacter baumannii is one of the ESKAPE Pathogens are a major cause of hospital infection all over the world. They are isolates of multiple resistance to treatments, which are considered one of the most serious problems facing humanity because of the inability to find alternative treatments to eliminate them. Current antibiotics used against Acinetobacter infections such as Carbapenems causes side effects like nausea, vomiting, diarrhea, and seizure (Choudhary et al., 2017). The efficacy of such antibiotics is also decreasing due to the continuous increase of resistance ability of A. baumannii. Hence, there is a need to search new infection fighting medicines to control

microbial infections, more specifically, *Acinetobacter baumannii*'s. This study is conducted to provide additional option to plant-derived medicines. Bermuda grass *(Cynodon dactylon)* had shown antibacterial effects on Gram-positive and Gram-negative bacteria. This implies that this plant is a promising candidate for the formulation of new medicine that can fight Acinetobacter infections(Lee et al., 2010).

The study's main objective:

The study's objective is to identify the function of the genes blaoxa-51 and INT-2, as well as the protein carO, in Acinetobacter baumannii isolates recovered from various clinical specimens in hospitals. **Materials and Methodology**

Out of 300 specimens obtained from swabs of burns, wounds, blood, and urine, with respective percentages of 37.5%, 25%, 12.5%, and 25%, the research focused on 40 specimens of A.baumannii. The findings of an antibiotic sensitivity test for 40 isolates using 8 antibiotics (Amikacin 30 mg, Piperacillin/Tazobactam 10 mg, Cefepime 30 mg, Cefotataxime 30 mg, Ciprofloxacin 5 mg, Polymyxin B 25 mg, Imipenem 10 mg, and Tetracycline 30 mg) were recorded based on CLSI,2022. **Primers**The following primers were used in this research to identify the target genes in A. baumannii isolates as listed in Table (1).

Table (1) Primers used in this study.

Primer Name	Sequence(5'_3')	Product size (bp)	References
BlaOXA51	F5'-TAATGCTTTGATCGGCCTTG -3 '	353	(Turton *etal*., 2006)
	R5'-TGGATGCACTTCATCTTGG -3 '		
CarO	F5'- ATGAAAGTATTACGTGTTTTAGTGACAAC 3	730	(Zhu,2019)
	R5'-TTACCAGTAGAATTCTACACCAACT -3'		
Int-2	F5'-CACGGATATGCGACAAAAAGGT-3'	320	Peymani *etal.,* 2012)
	R5'-GTAGCAAACGAGTGACGAAATG-3'		

Results and Discussions:

Acinetobacter *baumannii* Recognition and Isolation *:i*

Using biochemical testing and visual characteristics, isolates were identified. To isolate A. baumannii, a total of 40 samples from urine, swab of burns, and swab of wounds from patients at

several hospitals in Hilla City were gathered. The attending physicians and clinical microbiologists decided what was clinically relevant. The samples were first infected on MacConkey agar and chromagar, and they were then incubated for 24 hours at 37 °C. (300) clinical samples from individuals with various illnesses were used in the current investigation. Out of 300 samples, 40 isolates (or 13.3%) were identified as A. baumannii. (The samples consisted of 10 wounds, 15 burns, 10 pee, and 5 blood.) However, 30 of the 300 isolates (10%) were classified as different bacterial species. While there has been no growth(10%).

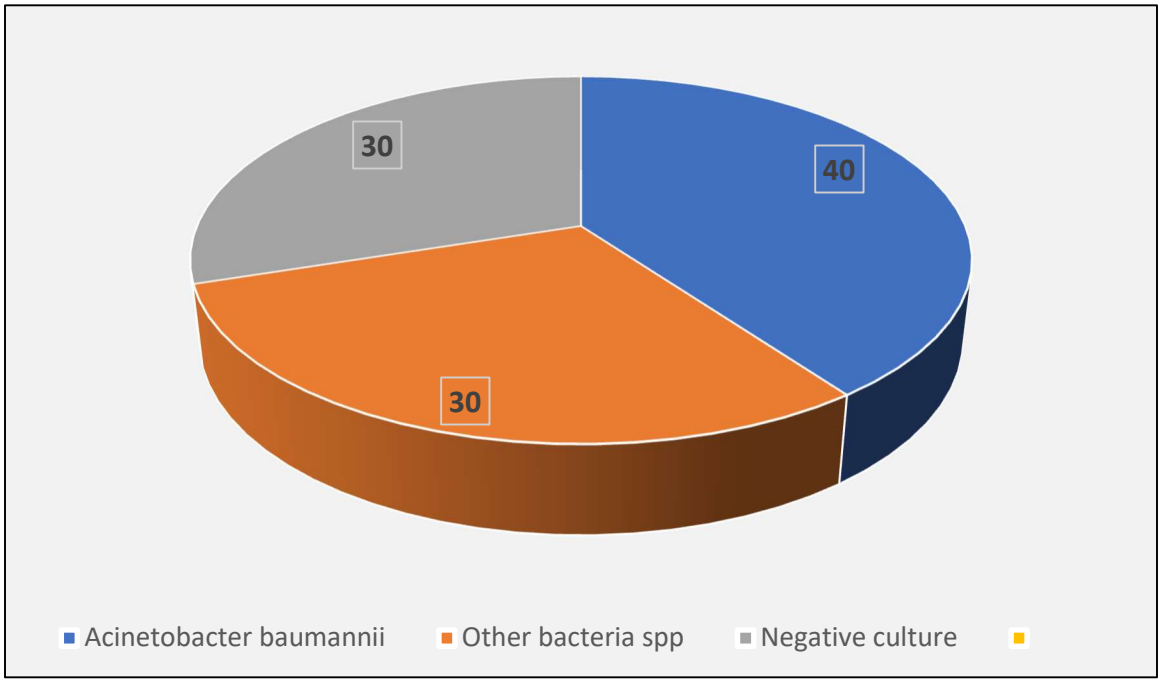

Figure (1) Percentage of Isolation rate in *A.baumannii* from Different Clinical specimens.

In biochemical identification , each isolates gave negative results for indole production , oxidase negative,gas negative,non fastidious,hemolysis negative,nitrate reduction, Vogus Proskaur , motility and urease test while the positive result appear in catalase, simmons' citrate and the results in Kliglar Iron Agar (KIA) developed an alkaline slant,no change bottom,H2S negative without gas production .All isolated of *A. baumannii* have ability to grow at 37 °C.

Distribution of *Acinetobacter baumannii* isolates Among Clinical Specimens

Acinetobacter baumannii isolates had been mended with various percentages from medical specimens, as shown in table (2). Distribution of *A. baumanni* isolates among clinical specimens .

Table (2) Distribution of *A.baumannii* isolates among clinical specimens.

Type of specimens	No. of specimens	No. of Isolates	Percentage
Burn swab	104	15	37.5%
Wound swab	132	10	25 %
Urine	30	10	25 %
blood	34	5	12.5%
Total	300	40	100%

According to the study's findings, out of 300 samples, 40 (13.3%) isolates were found to be A. baumannii, and they were distributed among burn swabs (15.5%), wound swabs (10.5%), urine (10.5%), and blood (5.5%). According to Rahi and Raheem's investigations (Raheem, 2020), The greatest finding from this investigation was the rate of bacteria isolates in Hilla , which was 3.33 percent. The isolation rate of an A. baumannii isolate was identified as being 21.5% by Raheem (2020), Al-Harmoosh (2015), Al-Kadmy et al. (2019), Al-Baroody and AL-Ghnimi (2020), and Al-Kadmy et al. (2019). Al-Masoudi (2014) discovered that the isolation rate (15%) of the current study was comparable to that of other studies. The isolation rate of A. baumannii was reported to be 20 (9.23%) in a study by Al-Zubaibi that was published in 2020; however, studies by Hamza and Hadi (2020) came to the conclusion that the isolation rate was 20%.

AST of bacterial isolates:

Using the Kirby-Bauer method, the antibiotic susceptibility profiles of 40 isolates of A. baumannii against eight different types of antibiotics were assessed (DDT). Clinical and Laboratory Standard Institute's disk diffusion method for determining antibiotic susceptibility on Muller Hinton Agar .The resulting reading according to CLSI,2022.InstituteCLSI breakpoints standard value (2022).

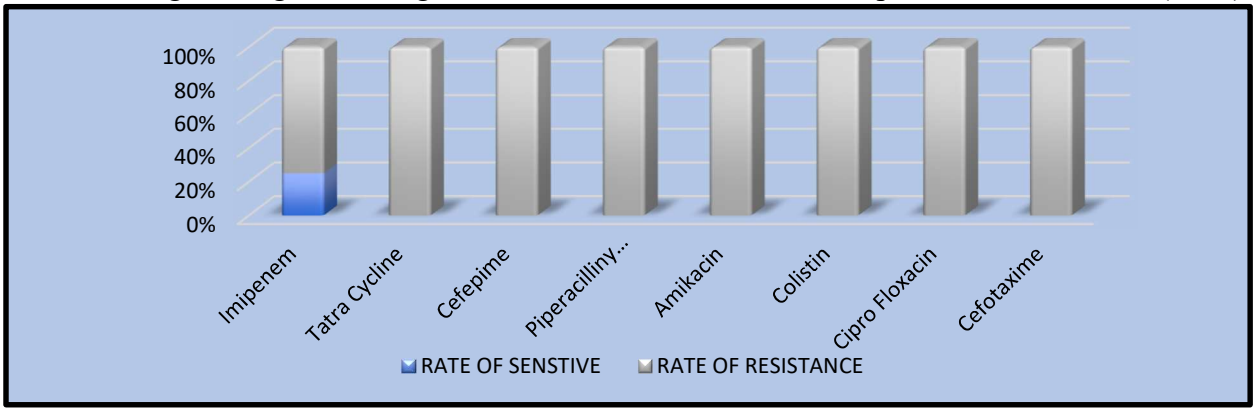

Figure (2) Percentage of Antibiotics Susceptibility Profile of *Acinetobacter baumannii* Isolates Detected by DDT (n=40)

Figure (2), demonstrated that all A. baumannii isolates tested in this investigation had the highest level of resistance to most antibiotics. All of the isolates tested positive for resistance to Piperacillin/Tazobactam, which is consistent with research by a local Babylon province study by Al-Warid (2014). Research on the carbapenems group, Imipenem, revealed resistance rates of 100% in 15 isolates, which varied between hospitals in Thailand. Thirapanmethee et al. (2020) discovered that A. baumannii isolates were resistant, in contrast to Rahi (2021) who discovered (100%) imipenem resistance rate, and Mshachal et al. (2017), who revealed (50%) polymyxin B resistance rate, sensitive 30%, and resistant 70% in AST. .

Detection of *bla*$_{\text{OXA-51}}$-like Intrinsic Carbapenemase Gene:

The result of the polymerase chain reaction (PCR) used to detect the blaOXA-51 gene as in Figure(3): included 353 bp (Agarose 1%, for 10 minutes at 100 volts, and then decreased to 70 volts, for 60 minutes). visualized under ultraviolet lighting following ethidium bromide staining. Lane L: DNA ladder (100–1500 bp). Lanes 1–15 and 21, 26, 34, 36 indicated bacterial DNA isolates with positive results. Lanes 16–20, 37, 38, 39, and 22–25 represented Negative control is represented by results lane N. the results for 40 A in blaOXA-51. baumannii isolates 19(85%) , Compared with another study conducted in Baghdad, (Abdul-Hussein *et al.,* 2019), recorded that blaOXA-51 was detected in 45 (73.77%) isolates among 61 carbapenem -resistant *A.baumannii* isolates. and studies by Al-Hindawi, (2018),blaOXA-51 was detected in *A.baumannii* isolates in Babylon hospitals of(100%)and comparable observations were made and other studies by (Al-Hasnawy ,2018),), recorded that blaOXA-51 was detected in 13 (13%) *A. baumannii* isolate. Also studies inTailand by (Thirapanmethee *et al* .,2020), was detected in all clinical isolates 183. While in (Al- Masoudi,2015) , blaOXA-51 was detected in bacterial isolates in percentage (80%) (twelve isolates out of fifteen isolates of bacteria and another study by (Al –Baroody,2020), recorded that blaOXA-51 was detected in all isolates of *A. baumanii* 15 isolates (100%). But another study by(Mekkey *et al* ., 2020) showed the presence of the blaoxa-51-like gene 33 (66%) in out of 50 isolates and, a study by (Anane *et al* ., 2020), was detected (15%) blaOXA-51 gene in *A.baumannii* isolates and study by (Rao *et al*.,2020), recorded that the blaOXA-51 was detected in all isolates of 13 isolates (100%). Further more *blaOXA-51* was considered a major factor in the resistance to carbapenem in *A.baumannii* (Al-Harmoosh, 2015), and another studies by (Mohamed *et al* ., 2020) the results revealed that all isolates carried *blaOXA-51* gene was detected in 82.3% (14/17) in Eygpt . In present results *for* detection of *bla*$_{\text{OXA-51}}$ gene by molecular methods such as PCR is a gold standard method for confirmation of *A.baumannii* isolates.

Detection of *INT*-2 Gene by polymerase chain reaction :

ThePCR amplification of integron genes showed in figure (**4**). **(***int-2* gene) showed 320bp. Lanes 38-39and 31,30,29,28) represented positive results of *bacterial DNA* isolates, Lanes1-25,26,27,28) represented Negative results lane N represent negative control

that 8 isolates (20%) harbored class- 2 integron, of.Comparable with another study by (Xu *et al* ., 2020) , who recorded that no ClassII integron was found in *A. baumannii* which is not greement with the current study, and another study by (Halaji *et al* .,2018) , who mentioned that Class II integrons were detected in 78.2% of the bacteriaisolates. And a study by (Salehi *et al* ., 2017), who showed class II integrons that 88.6% of the *A.baumannii* isolates carried the intII genes.And a study by (Zeighami *et al* ., 2019), who recorded that class II (10%) out of 100 bacteria isolates. And a study by (Ardeshiri *et al* ., 2017), that recorded the class 2 integrons Was 53.8%, out of bacterial isolates. The establishment of MDR A. baumannii isolates is significantly influenced by the existence of integrons as a key source of antimicrobial resistance genes within microbial populations. Integrons are genetic elements that are capable of capturing and mobilizing gene cassettes that contain antibiotic resistance genes. The integron-2 (int-2) gene is a component of class 1 integrons, which are the most commonly found integrons associated with antibiotic resistance. Polymerase chain reaction (PCR) is a widely used technique for the detection of specific DNA sequences, including the int-2 gene. In this article, we will discuss the detection of the int-2 gene by PCR.PCR is a highly sensitive and specific method for amplifying specific DNA sequences. The basic principles of PCR involve the use of a thermostable DNA polymerase enzyme, DNA primers, and deoxyribonucleotide triphosphates (dNTPs) to amplify the target sequence. The process consists of three main steps: denaturation, annealing, and extension. In the denaturation step, the double-stranded DNA is separated into two single strands by heating to a high temperature. In the annealing step, the temperature is lowered to allow the primers to bind to their complementary sequences on the single-stranded DNA. In the extension step, the temperature is raised again to allow the DNA polymerase to extend the primers and synthesize new complementary strands.To detect the int-2 gene by PCR, specific primers are designed to anneal to the target sequence. These primers should be complementary to the unique regions flanking the int-2 gene, ensuring that only the int-2 gene is amplified. The PCR reaction is set up by mixing the DNA template (containing the int-2 gene), the primers, the DNA polymerase enzyme, and dNTPs. The reaction is then subjected to a series of temperature changes to allow denaturation, annealing, and extension to occur.After the PCR reaction is complete, the products are analyzed using gel electrophoresis. Gel electrophoresis is a technique that separates DNA fragments based on their size. The PCR products are loaded onto an agarose gel and subjected to an electric field. The DNA fragments migrate through the gel, with smaller fragments traveling faster and further than larger ones. The gel is then stained with a DNA-binding dye, and the PCR products can be visualized under UV light.The detection of the int-2 gene by PCR is an important tool in the study of antibiotic resistance. By amplifying and detecting the int-2 gene, researchers can study the prevalence of class 1 integrons in bacterial populations and determine the likelihood of antibiotic resistance. Additionally, the detection of the int-2 gene can be used as a diagnostic tool for antibiotic-resistant infections, allowing for more targeted and effective treatment. PCR-based detection of the int-2 gene is a powerful technique for the detection and study of antibiotic resistance genes and their associated integrons.

Detection of *CarO* protien by polymerase chain reaction:

For molecular detection of outer membrane protein (OMPs) **.** 40 isolates were analayzed and found in figure **(5).** (*CarO*gene) showed 730bp. that 10 out of 40 were positive for *car*O gene (25%), , in comparison with study of Zhu *et al*., (2019) who presented that all isolates were observed were carrying *car*O gene.With the increased number of resistant Acinetobacter baumannii isolates,.Furthermore Catel-ferreira at el., (2011), determined the The first proof that the specificities of carO channels depend on their fundamental structure and that they have an imipenem (but not meropenem) binding site. Thus, any reduction in carO expression would lessen A. baumannii's resistance to this antibiotic. Also, Uppalapati at el .,(2020) proved that decreased expression of the OMPs was significantly associated with carbapenem resistance. The collection of outer membrane proteins (OMPs) is one such arm of A. baumannii. When it comes to aiding bacterial adaptation to antibiotic- and host-induced stressors, OMPs in A. baumannii play unique functions. OMPs are important allergenic proteins that give bacteria host-fitness benefits such degranulation, stress tolerant, and antibiotic and antibacterial sensitivity. (Uppalapati at el .,2020).

CarO (Carbapenem-Resistant Organism Outer membrane porin) is a type of protein that is commonly found in carbapenem-resistant Gram-negative bacteria. Carbapenems are a class of beta-lactam antibiotics that are often used as a last resort for the treatment of infections caused by multidrug-resistant bacteria. However, the emergence of carbapenem-resistant bacteria has become a major public health concern in recent years, and the detection of CarO protein has become an important tool for identifying these organisms.The polymerase chain reaction (PCR) is a widely used molecular biology technique that can be used to detect the presence of specific DNA sequences in a sample. In the case of CarO protein detection, PCR is used to amplify the DNA sequence that encodes for the CarO protein. This amplified DNA sequence can then be detected using various methods, such as gel electrophoresis or fluorescent labeling.The PCR process involves several steps. First, the DNA sample containing the target sequence is denatured, or separated into single strands, by heating it to a high temperature. Then, a pair of primers specific to the target sequence are added to the sample. These primers are short pieces of DNA that bind to the complementary sequence on either side of the target sequence. The primers serve as the starting point for the synthesis of new DNA strands.Next, a DNA polymerase enzyme is added to the sample. This enzyme extends the primers by adding nucleotides to create new strands of DNA that are complementary to the target sequence. The polymerase enzyme is heat-stable, which allows the PCR process to be carried out at a high temperature without the enzyme being denatured.The PCR process is typically repeated for multiple cycles, with each cycle consisting of a denaturation step, an annealing step where the primers bind to the template DNA, and an extension step where the polymerase enzyme synthesizes new DNA strands. The number of cycles required depends on the initial amount of target DNA present in the sample and the sensitivity of the detection method.Once the PCR process is complete, the amplified DNA can be detected using various methods. Gel electrophoresis can be used to separate the DNA fragments by size, allowing the presence of the target sequence to be visualized as a distinct band

on the gel. Alternatively, fluorescent labels can be added to the PCR products, allowing the amplified DNA to be detected using fluorescence-based methods.In conclusion, PCR is a powerful tool for detecting the CarO protein in carbapenem-resistant Gram-negative bacteria. By amplifying the DNA sequence that encodes for the CarO protein, PCR allows for rapid and accurate detection of these organisms, which is critical for effective treatment and infection control.

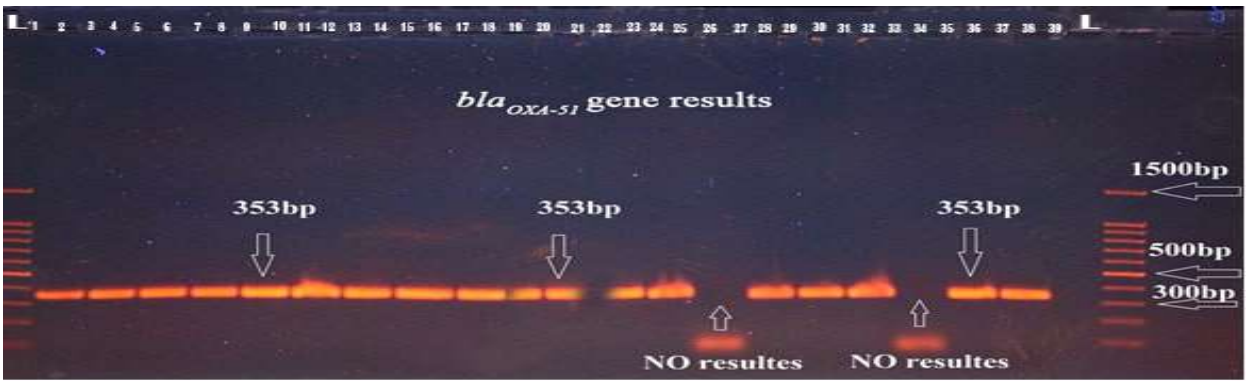

Figure (3) :- Gel electrophoresis for PCR product (*blaoxa-51* gene) showed 353bp.

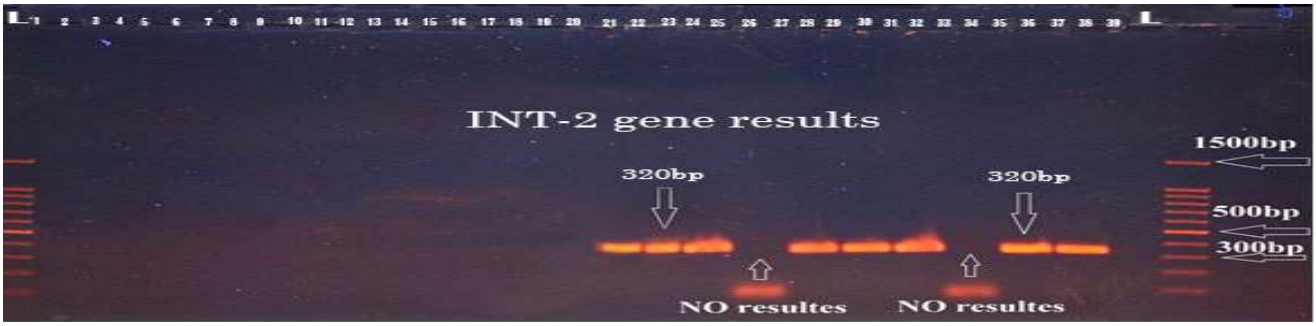

Figure (4) :- Gel electrophoresis for PCR product (*int-2* gene) showed 320bp.

Figure (5) :- Gel electrophoresis for PCR product (*CarO*gene) showed 730bp.

Conclusion:

Acinetobacter baumannii is considered a threat to humanity as a result of its ability to acquire resistance to treatments, so it is necessary to seek to find modern treatments that suit the developments of this bacteria, and the gene bla_{oxa-51} is considered a diagnostic indicator for bacteria

and It is considered blaoxa-51 gene is essential to identify *Acinetobacter* species as *A. baumannii* .Class 2,Integron was found to be carried by *A. baumannii* isolates and antibiotic resistance genes were distributed on this integron.All *A. baumannii* isolates were found to be MDR in present study.and High prevalence of Carbapenemase resistance genes *A. baumannii* isolates detected in this study. The detection of CarO and BlaOxa proteins and the presence of integrons in bacteria are all important tools in the fight against antimicrobial resistance. Carbapenem-resistant organisms, which produce the CarO and BlaOxa proteins, pose a serious threat to public health, as these organisms are often resistant to multiple classes of antibiotics. Integrons, on the other hand, are genetic elements that can facilitate the spread of antibiotic resistance genes among bacteria.The detection of CarO and BlaOxa-51 proteins is typically performed using molecular biology techniques such as polymerase chain reaction (PCR). By amplifying the DNA sequences that encode for these proteins, it is possible to detect their presence in bacterial samples. This information is critical for identifying antibiotic-resistant organisms and selecting appropriate treatment options.Integrons-1 are genetic elements that can capture and rearrange genes, including those that confer antibiotic resistance. The detection of integrons in bacteria is important because it indicates that the organism has the potential to rapidly acquire antibiotic resistance genes from other bacteria in the environment. The detection of integrons is typically performed using PCR or other molecular biology techniques.In conclusion, the detection of CarO and BlaOxa -51proteins and the presence of integrons are all important tools in the fight against antibiotic resistance. These techniques allow for the rapid and accurate identification of antibiotic-resistant organisms and the potential for the spread of antibiotic resistance genes. This information is critical for selecting appropriate treatment options and implementing effective infection control measures. Continued research and development of these detection techniques will be essential in the ongoing battle against antibiotic resistance.

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