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Gene Expression of Biofilm Formation *lasB* and *rblB* Genes of *Acinetobacter baumannii* Isolated from Wounds, Burns and Environmental Samples

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Abstract: Multiple-drug-resistant infections linked to medical devices are common due to A*cinetobacter baumannii*'s propensity to develop biofilm. The purpose of this work is to identify the MIC of Imipenem and Chalcon and to investigate the involvement of the *lasB* gene in formation of biofilm by *A. baumannii* in order to get better understand how these bacteria can survive l in the hospital setting. As well as comparing the effect before and after of using the antibiotic and chalcone. A 250 samples were obtained from several locations in hospitals of Baghdad/Iraq and classified to 2 groups according to their source: 100 environmental and 150 clinical samples, obtained during October 2021-March 2022. Antibiotic resistance profile of *A. baumannii* isolates was confirmed by VITEK-2 system and in vitro biofilm-forming ability was evaluated by micro titer plate methods, respectively. Isolates were tested for the presence of *lasB* gene Forty (40) isolates of *A. baumannii* were subjected to examination under microscope and to diagnosis using biochemical tests. The (40) isolates identification was confirmed by the VITEK-2 system and a molecular detection according to the *rplB* gene, which is a necessary gene located in this genus. Antibiotic susceptibility testing was carried out on all (40) identified isolates, and the results detected those 36 isolates had Multi-Drug Resistance (MDR). This work indicated that 16 isolates from these 16 isolates carrying the *rblB* gene and 2 isolates from 6 carrying *lasB* that confirmed by molecular detection methods, and 38 isolates from these 40 isolates had the ability to form biofilm in the micro titer plate method, and this study show there is down expression of *lasB* with the antibiotic while down expression of this genes with chalcone. In conclusion, the presence of the *lasB* gene was significantly correlated with A. baumannii biofilm development. Biofilm production was also higher in multidrug-resistant than in other isolates. It was concluded the role of biofilm in the isolate's resistance and the impact of the existence of *lasB* genes in formation of biofilm by *A. baumannii* strains, and *lasB* gene expression was reduced in those treated to imipenem and chalcone.

Keywords: lasB ; *Acinetobacter baumannii* ; PCR,Biofilm ; multidrug resistant.

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Introduction

Human opportunistic extracellular pathogen *Acinetobacter baumannii* is Gram-negative, non-motile coccobacilli (1). It's become a major source of nosocomial infections in patients. The bacterium *A. baumannii* has become immune to almost all existing antibiotics. Hospital-acquired infections caused by multidrug-resistant *A. baumannii* strains have been reported all over the world (2). Compared to other *Acinetobacter* species, *Acinetobacter baumannii* has been proven in multiple investigations to be more virulent to humans. In the past, *A. baumannii* infections were treated with a wide variety of conventional antibiotics, but the bacteria have become resistant to many of these drugs, including tetracycline, fluoroquinolones, carbapenems, chloramphenicol, penicillin, cephalosporins, and aminoglycosides. The rate at which resistance is developing is also

alarming. Antibiotic-resistant *Acinetobacter baumannii* outbreak strains are known for their intrinsic antibiotic resistance as well as their capacity to acquire genes encoding resistance determinants. Because of its strong resistance to such medications, there are few effective medicinal choices for treating this bacterial infection (3). To avoid death from antibiotics, bacteria can develop biofilms; this is especially true for *Acinetobacter baumannii*, the causative agent of biofilm-related medical device infection (4). Biofilm formation has been a subject of intense research as of late, with scientists isolating individual bacterial cells from biofilms and identifying genetic variables that control this intricate process. In contrast to their planktonic counterparts, the sessile phenotypes of bacteria living in biofilms are more complex and highly ordered than those of bacteria living in aqueous solutions. Biofilms are composed of a proteins and polysaccharides polymeric conglomerate with combined metabolic activities (5). Plastics, glasses, and the surfaces of various surgical tools, among other hospital surfaces, have all been found to harbor bacterial biofilms (6).

Materials and methods

Bacterial isolation and identification

A 150 clinical and 100 environmental samples, from various body's areas like blood, wound and burns of various patients, were chosen during the period from September 2021 till March 2022. These samples were selected from various patients with several clinical manifestations; such as irritation at site of infection, abscesses, pain, fevee, rapid breathing, inflammation). In addition to various sources, like instruments, floors, sinks, tables, and beds). The samples were obtained using swabs then putted in sterilized, transported medium containers. After 24 hours incubation at 37°C on CHROM, MacConkey and blood, agar, Biochemical procedures, such as the oxidase and catalase tests, were performed manually to confirm the presence of *A. baumannii*. Confirmation via biochemical tests is built into the portable VITEK2 equipment.

Antibiotic susceptibility test

Antibiotic sensitivity test number (AST- N222) cards were used in conjunction with the VITEK 2 Compact Instrument to conduct these tests, which included antimicrobial agents as follows: Amikacin (AK), Aztreonam (AZT), Cefepime (CPM), Ceftazidime(CAZ), Trimethoprim/ Sulfamethoxazole (TMP/SMX), Gentamicin (GM), Imipenem (IMI), Ticarcillin/clavu Tobramycin (TM), lanate (TIM), Rifampicin (RA), Colistin (CS), Pefloxacin(PEF), Ciprofloxacin (CIP), Meropenem (MEM), Piperacillin (PRL), Minocyclie (MNO) and Piperacillin/tazobactam (PTZ) (7).

Molecular method

DNA extraction

Genomic DNA was obtained from bacterial growth based on the technique of Wizard Genomic DNA Purification Kit. DNA isolation from a wide range of sample types is now possible with the help of this kit. DNA was extracted by this kit using bacterial protocol (for gram negative bacteria).

Conventional PCR for *rblB* and *lasB* genes

The detection of each gene was accomplished by the standard PCR process, which relied on specific

primers. Provided as a lyophilized powder, to be reconstituted with 100 Pico moles/ml final concentration. The specific primers for the *lasB* gene and *rblB* gene were used (Table 1). PCR reaction was included 20µl using nuclease free water as a final volume, containing 1µl of each primer (10 pmol/µL), 10µl of Go Taq® green master mix 2X (Promega, USA) and 3 µl DNA template (100ng/µl). After vortexing and centrifugation of the extracted DNA, it was kept at 4°C. Without DNA, negative control contained the same components of PCR reaction. PCR programs were set on Thermal cycler gradient PCR (Thermo fisher/ USA) . Also, PCR condition for *rblB* gene was as follow: 95°C for five min, then 30 cycles of 95°C for 30 sec, 50°C for 30 sec, 72°C for one min (30 cycles), followed by final extension 72°C for 7 min. The products of PCR were moved in 1.5% agarose gel.

Table (1): Primer sequences used PCR in this study.

No.	Primer name	Sequence (3'-5')	Product size	Refernce
1	*lasB F*	GGAATGAACGAAGCGTTCTC	466	(Aliramezani *et al*.,2019)
2	*lasB R*	GGTCCAGTAGTAGCCGGTTGG	466	(Aliramezani *et al*.,2019)
3	*rblB R*	CAC CAC CAC CGT GCG GGT GAT C	475	(Aliramezani *et al*.,2019)
4	*rblB F*	GTA GAG CGT ATT GAA TAC GAT CCA AAC C	475	(Aliramezani *et al*.,2019)

Biofilm formation assay

Crystal violet staining was used in a 96 well micro titer plate experiment to determine the biofilm forming potential of *A. baumannii* isolates. Each well of a 96-well flat-bottomed sterile polystyrene micro titer plate was inoculated with 1 L of 0.5-0.7 McFarland bacteria suspension, and the plates were placed in incubator for 24 hours at 37°C. Adherent cells were rinsed twice with phosphate-buffered saline (PBS), and the liquid media was discarded, and the wells were dried at 60 degrees Celsius for no more than an hour. Then, for 15 minutes, it was stained with 150 L of crystal violet. Following crystal violet staining, wells of a micro titer plate were washed twice with PBS to remove the stain. After the micro titer plate wells have been dried in the oven, the biofilm dye that coated the well walls can be re-dissolved in 150 L of 96% ethanol. After 5-10 minutes, the micro titer plate is read using spectrophotometry at 570 nm (8). Based on this method, we determined that an ODc of three standard deviations (SD) over the mean OD of the negative control would be the threshold at which further investigation would be warranted.:

ODc = average OD of negative control + (3×SD of negative control). The results were divided into four categories according to their optical densities as:

1. Strong biofilm producer (4 × ODc< OD).
2. Medium biofilm producer (2 × ODc< OD ≤ 4 × ODc).
3. Weak biofilm producer (ODc< OD ≤ 2 × ODc) Non-biofilm producer (OD ≤ ODc).

Minimum inhibitory concentration (MIC)

Fill each well of a micro titer plate with 100 ml of medium using the micropipette. Fill the wells in column A with 100 ml of the antibiotic solutions (far left of plate). Suck up and down 6-8 times with a micropipette set to 100 ml to combine the antibiotics in column A's wells. Stay away from any water.

Take 100 ml out of column A and put it into column B. Transferring 100ml from column A to column B dilutes the sample by a factor of 10. It is possible to use the same set of guidelines for the entire series of dilutions. Throw away 100 ml from column H. Fill all wells with bacteria using a micropipette calibrated to 100 l. Put the plates in a 37C incubator.

After a certain level of development is reached (24 hours).

After 30 minutes of incubation, 20 ml of resazurin dye was added to each well to check for color changes. Visual inspection of broth micro dilutions yielded the Minimum Inhibitory Concentrations by identifying the concentrations of extracts below which the resazurin broth assay showed no color change from blue to pink (9,10).

Quantitative real time-PCR

RNA extraction

The extraction of RNA was performed before and after treating with the antibiotic. Total RNA was extracted using TRIzol™Reagen.

Preparation of primers

Based on previous findings, we were able to obtain gene-specific primers (Table2).

Table (2): Primer sequences used qRT-PCR in this study.

No.	Primer	Sequence	Product size	Refrence
1	*lasB F*	5' GGAATGAACGAAGCGTTCTC '3	122	Fils *et a*l ,.2019
2	*lasB R*	5' GGTCCAGTAGTAGCCGGTTGG 3'	122	Fils *et al* ,.2019
3	*RblB F*	5'GTA GAG CGT ATT GAA TAC GAT CCA AAC C-3	136	Fils *et al* ,.2019
4	*RblB R*	CAC CAC CAC CGT GCG GGT GAT C-3’5	136	Fils *et al* ,.2019

Quantitative Real-time PCR Assay (QRT- PCR)

Amplification of mRNA fragments was conducted using the Qubit® 1-Step RT-qPCR System (Qubit®- USA) and the master amplification reaction listed in Table (3) and Table (4), respectively (4). Multiple trials were conducted to optimize cDNA synthesis and annealing temperature.

Table (3): quantitative RT-PCR Reaction Mix.

Component	20 ul Reaction
Luna Universal qPCR Master Mix	10 ul
Forward primer (10 µM)	1 ul
Reverse primer (10 µM)	1 ul
Template DNA	5 ul
Nuclease-free Water	3 ul
Total	20

Table (4): Quantitative RT-PCR Reaction Mix.

Cycle Step	Stage	Temperature	Time
1	Initial Denaturation	95 ºC	60 seconds
40-45	Denaturation Extension	95ºC 60 ºC	15 seconds 30seconds (+plate read)
1	Melt Curve	60-95 ºC	40 minutes

Delta delta Ct method

Delta delta Ct (ΔΔCt) approach requires selecting a calibrator sample and doing a direct comparison of Ct values for the target gene and the reference gene to determine relative quantification. Any sample that serves as a standard against which unknown samples can be evaluated can be used as the calibrator. In the first step, the difference in Ct (Ct) between the target gene and the reference gene is determined for each sample (unknown samples and calibrator sample) using the following equation.

ΔCt = Ct target gene - Ct reference gene

Then, the difference between the ΔCt of the unknown and the ΔCt of the calibrator is calculated, giving the ΔΔCt value.

ΔΔCt = (Ct target - Ct reference) sample - (Ct target- Ct reference) control

The normalized target amount in the sample is then equal to 2−ΔΔCt and this value can be used to compare expression levels in samples[14] .The relative changes in mRNA expression levels were determined by using a comparative threshold cycle (CT) method (2−ΔΔCt).The result was collected and analyzed by Livak formula.

Statistical analysis

To analyze the impact of experimental variables, we used SAS (2012) from Statistical Analysis System. A meaningful comparison between means was performed using the Least Significant Difference -LSD test (Analysis of Variance-ANOVA). In this analysis, the chi-square test was performed to determine whether or not a 5% and a 1% difference in outcomes was statistically significant.

Results and discussion

Identification of *A. baumannii*

74 clinical isolates were confirmed to be *A. baumannii* using the Gram-negative strain identification card that comes with the VITEK 2 system. The diagnostic and validation efficacy of biochemical tests have been demonstrated by this approach in a number of prior investigations. Antibiograms of *A. baumannii* isolates might be calculated with this automated apparatus (12).

***A. baumannii* distribution based on type of sample**

Table 5 shows that out of a total of 250 clinical and environmental samples, 29.6 percent included *A. baumannii*. Wounds, burns, sputum, and peritoneal fluid from hospitalized patients were among the sources for the collected samples (Table 5).

Table (5): Prevalence of *A. baumannii* isolates among clinical and environmental samples. No (250).

Source	No. of samples	Positive samples	Nagtive sample
Clinical samples			
Wounds	75(30%)	32 (12.8%)	43
Burns	75 (30%)	29 (11.6%)	46
Environmental samples			

Beds	25 (10%)	5 (2.0%)	20
Tables	25 (10%)	4 (1.6%)	21
Sinks	25 (10%)	3 (1.2%)	22
Loors	25 (10%)	1 (0.4%)	24
Total	**250 (100%)**	**74 (29.6%)**	

Susceptibility to antibiotic test

Using the automated VITEK 2 Compact apparatus, we checked the antibiotic resistance of all 74 *A. baumannii* isolates. Each isolate was cultured in a McFarland 0.5 standard suspension of 0.45% sodium chloride on MacCkonkey agar plates. The VITEK apparatus was loaded with liquid containing all of the isolated substances. Using the Gram-negative susceptibility card in the VITEK 2 Compact instrument, 14 antibiotics were evaluated for their ability to kill off specific bacteria (Table 6).

Table (6): Antimicrobial susceptibility test of 83 *A. baumannii* isolates to 14 antimicrobials.

Antibiotic	Resistant	Intermediate	Sensitive
Piperacillin/tazobactam(PTZ)	38 (51.30%)	2 (2.70%)	34 (45.94%)
Colistin(cs)	5 (6.75%)	9 (12.16%)	61 (82.42%)
Trimethoprim/Sulfamethoxazole (TMP/SMX)	17 (22.97%)	2 (2.70%)	55 (74.32%)
Tobramycin(TM)	24 (32.43%)	13 (17.56%)	37 (50.0%)
Piperacillin (PRL)	38 (51.35%)	0 (0.0%)	36 (48.64%)
Meropenem(MEM)	30 (40.54%)	13 (17.56%)	31 (41.89%)
Minocycline (MNO)	5 (6.75%)	4 (5.40%)	65 (87.83%)
Imipenem (IMI)	20 (27.02%)	21 (28.37%)	33 (44.59%)
Cefepime(CPM)	49 (66.21%)	0 (0.0%)	25 (33.78%)
Ceftazidime(CAZ)	70 (94.59%)	0 (0.0%)	4 (5.40%)
Ciprofloxacin(CIP)	36 (48.64%)	3 (4.05%)	35 (47.29%)
Gentamicin(GM)	30 (40.54%)	0 (0.0%)	44 (59.45%)
Ticarcillin	25 (33.78%)	13 (17.56%)	36 (48.64%)
Ticarcillin /clavulanate(TIM)	23 (31.08%)	10 (13.51%)	41 (55.40%)

Presence of *lasB* and *rblB*

Culture, biochemical, and Vitek-2 assays all agreed that all 16 (100%) A. baumannii isolates did, but PCR analysis proved it. Amplicons of this gene, which were generated during the electrophoresis method (14), ran clearly at 475 bp on agarose (Fig. 1). All 16 multidrug-resistant *A. baumannii* isolates were tested with a PCR technique to identify their virulence gene. A PCR experiment using specific primers was performed to identify a single gene. The findings showed that 2 isolates possessed the Elastase (*lasB*) as shown in Figure (2), Results were reported in a study of mousl, Iraq by The presence of *lasB* gene in their isolates were ten isolates, 52.6%, at a molecular size of 466 base pairs(15).

Biofilm Formation

In this study, determining the ability of 40 *A. baumannii* isolates to adhere and produce a slime layer (Biofilm formation) was experienced by using micro titer plates (MTP) methods. The results abstained by micro titer plates reader (GloMax® Discover Micro plate Reader), the results showed that 38 isolates (95%), possess the ability to stick and generate thin layers with varying degrees of

thickness (strong, moderate and weak), where the variation in biofilm thickness may be due to differences in isolates ability to produce biofilm whereas 2 isolates with no change in OD over the control were detected as non-biofilm former and these isolates did not have the ability to adhere and to produce slim layer the results in Table 6 shown that 24 isolate gave strong biofilm and 11 isolate gave moderate biofilm and 3 isolate gave weak biofilm (13). Used phenotypic approaches to the evaluate biofilm-forming capability of *A. baumannii* isolated from clinical samples in Baghdad hospitals and found significant differences between 83 *A. baumannii* isolates.

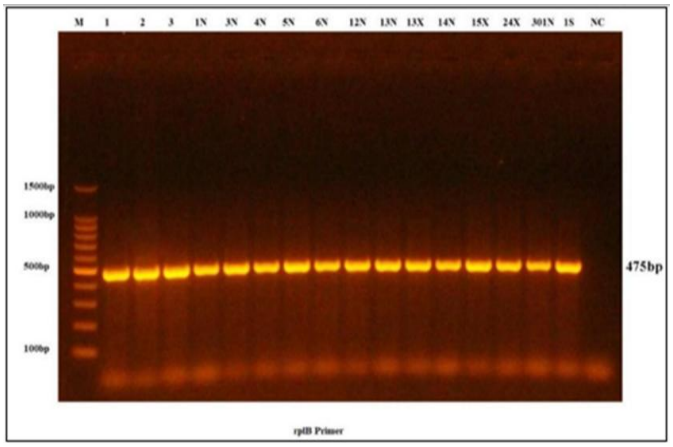

Figure (1): The results of the amplification of *rplB* gene of *Acinetobacter* baumannii samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-1S resemble 475bp PCR product.

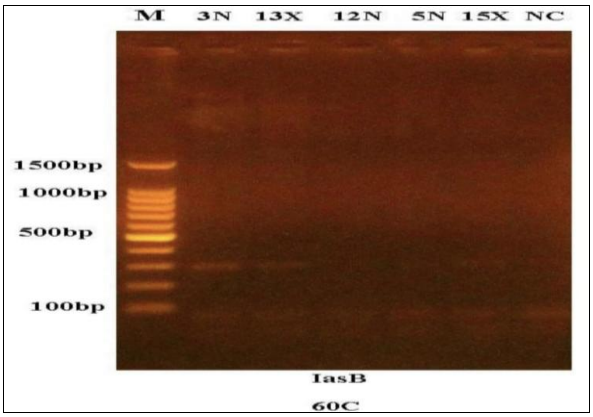

Figure (2): the results of the amplification of *lasB* gene of *Acinetobacter baumannii* samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-30N resemble 336 bp PCR product.

Results of RT-qPCR for *lasB* and gene

In this investigation, SYBR green, a fluorescent dye that binds to and intercalates with all types of double-stranded DNA (including cDNA), was used for real-time PCR quantification. A Ct value representing

the degree of amplification was recorded (cycle threshold). When the Ct value drops, more of the target is present, and vice versa (15).

This was done to determine the importance of the *rblB* and *lasB* genes in *A. baumannii* resistance by measuring their expression levels and comparing them to those of the same bacteria grown with or without the antibiotic present. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) stands out from other gene expression analysis techniques because its results are precise, sensitive, and quick. This method had already become the gold standard in analyzing gene expression. Realize that in relative quantification investigations, all experiments are often worried with comparing the expression level of a given gene between distinct samples (16).

The *rblB* gene, as the housekeeping gene, was used in the present experiment because its expression remains constant in the investigated cells under different conditions.

These isolates were chosen with sub-MIC values to antibiotic (31.25 µg/ml), two isolates treated with 125 µg/ml chalcone and two sensitive isolates as control. In the present experiment, quantitative RT- PCR assay analyzed the mRNA expression of *lasB* gene by comparing the untreated and treated group of resistant bacteria samples grown with antibiotic and chalcone.

Real time PCR quantification of *lasB* expression

Before group treatment, the lasB Ct value in isolates was (31.04-30.25). Ct values for lasB in antibiotic-treated isolates fell within the range of (31.51-29.19). Ct values for chalcone-treated isolates ranged from (29.16) to (29.92) (see Table) (7). The table 7 data shows that there was a statistically significant difference in the Ct values between the groups. Ct values in antibiotic-treated isolates were greater than those in untreated and chalcone-treated isolates; these Ct values were somewhat higher than those in untreated isolates, reflecting the presence of the genes in the mRNA's samples. These findings show that the lowest expression levels are connected with the antibiotic group, where the copy number of the target gene on mRNA is highest, and the highest expression levels are related with the chalcone treatment group, where the copy number of the target gene on mRNA is lowest.

After testing all *A. baumannii* isolates for the genes (*plc-N* and *lasB* genes), researchers found that 16 (53.33%) of the samples contained *lasB* genes and 7 (23.3%) included the *plc-N* gene. Any one of these genes can boost *A. baumannii*'s virulence and invasiveness toward the A549 cell line (17).

Table (7): Gene expression of *lasB* comparison between chalcone, antibiotic and control.

Sample	Ct *rblB*	Ct *lasB*	ΔCt	ΔΔCt	fold
1N (BT)	12.58	31.04	18.46	0	1
1X(BT)	12.02	30.25	18.23	0	1
2N (AT) chalcone	10.74	29.16	18.42	-0.04	1.028114
2X (AT) chalcone	8.86	29.92	21.06	2.83	0.140632
3N(AT) A.b	9.3	31.51	22.21	3.75	0.074325
3X(AT) A.b	9.25	29.19	19.94	1.71	0.30566
LSD	-	-	-	-	0.6874*
* (P≤0.05): Significant					

Conclusions

Nosocomial infections caused by *Acinetobacter baumannii* are a growing problem in Iraqi hospitals. In this study the *lasB* gene play role in biofilm formation, also concluded the Sub MIC of antibiotic for samples (N,X) at a concentration (31.25) while the concentration of chalcone for the same samples is 125,so the gene expression was measured Real Time-pcr it was found that the *plcN* and *bap* genes the treatment with antibiotic were higher than the Ct value of isolates before treatment and after treatment with chalcone resulting in slightly higher than those in isolates which reflects that the genes are exist in mRNAs samples. From these data, it is clear that the lowest expression of the target gene is associated with the antibiotic group, where the mRNA copy number is highest, and the highest expression of the target gene is associated with the mRNA copy number being lowest in the isolates subjected to chalcone treatment.

References

1. Chapartegui-González, I.; Lázaro-Díez, M.; Bravo, Z.; Navas, J.; Icardo, J. M. and Ramos-Vivas, J. (2018). *Acinetobacter baumannii* maintains its virulence after long-time starvation. PloS one, 13(8): e0201961.
2. Reena, A. A. A.; Subramaniyan, A. and Kanungo, R. (2017). Biofilm formation as a virulence factor of *Acinetobacter baumannii*: An emerging pathogen in critical care units. Journal of Current Research in Scientific Medicine, *3*(2): 74.
3. Kröger, C.; Kary, S. C.; Schauer, K. and Cameron, A. D. (2016). Genetic Regulation of Virulence and Antibiotic Resistance in *Acinetobacter baumannii*. Genes, 8(1): 12.
4. Singhai, M.; Malik, A.; Shahid, M.; Malik, M. A. and Goyal, R. (2012). A study on device-related infections with special reference to biofilm production and antibiotic resistance. Journal of Global Infectious Diseases, *4*(4): 193–198.
5. Stoodley, P.; Sauer, K.; Davies, D. G. and Costerton, J. W. (2002). Biofilms as complex differentiated communities. Annual Review of Microbiology, 56: 187–209.
6. Patel, S. J.; Oliveira, A. P.; Zhou, J. J.; Alba, L.; Furuya, E. Y.; Weisenberg, S. A., *et al*. (2014). Risk factors and outcomes of infections caused by extremely drug-resistant gram-negative bacilli in patients hospitalized in intensive care units. American Journal of Infection Control, 42(6): 626–631.
7. Ali, H. and Khan, E. (2017). Environmental chemistry in the twenty-first century," Environmental Chemistry Letters, 15(2): 329-346.
8. Kerkeni, L.; Ruano, P.; Delgado, L. L.; Picco, S.; Villegas, L. and Tonelli, F. (2016). We are IntechOpen. the world' s leading publisher of open Access books Built by scientists, for scientists TOP 1 %. Intech, tourism.
9. Ohikhena, F. U.; Wintola, O. A. and Afolayan, A. J. (2017). Evaluation of the Antibacterial and Antifungal Properties of *Phragmanthera capitata* (Sprengel) Balle (Loranthaceae), a Mistletoe Growing on Rubber Tree, Using the Dilution Techniques. The Scientific World Journal, 2017: 9658598.
10. AbdulRazzaq, A. B.; Shami, A. M. and Ghaima, K. K. (2022). Detection of vanA and vanB genes Among Vancomycin Resistant *Staphylococcus aureus* Isolated from Clinical Samples in Baghdad Hospitals. Iraqi Journal of Biotechnology, *21*(1).
11. Schmittgen, T. D. and Livak, K. J. (2008). Analyzing real-time PCR data by the comparative CT method. Nature Protocols, *3*(6): 1101-1108.
12. Guckan, R.; Kilinc, C.; Demir, A. D.; Capraz, A. and Yanik, K. (2015). Antimicrobial susceptibility of *Acinetobacter baumannii* complex isolated from different clinical samples in a tertiary care hospital. Journal Antibiotic Research, 1(1): 1-5.
13. AL-Mousawi, H. T.; AL-Taee, M. I. N. and AL-Hajjar, Q. N. (2018). Evaluation of Biofilm Formation Capacity of *Acinetobacter baumannii* Isolated from Clinical Samples in Baghdad Hospitals

using Phenotypic Methods. Iraqi Journal of Biotechnology, *17*(3).

14. AL-Taati, H. R., Jameel, Z. J., & Taha, R. A. (2018). Study of Virulence factor of *Acinetobacter baumannii* and detection of bap gene. Iraqi Journal of Biotechnology, 17(1):17-25.
15. Hamzah, A. S. (2018). Genetic Variations in rplB Gene Associated with Multidrug Resistance *Acinetobacter baumannii* Isolated from Different Clinical Sources. Al-Nahrain Journal of Science, 21(3): 119-125.
16. Al-Ghanimy, G. F. and Asmael, N. M. (2020, March). Estimate parking generation rates for educational sites. In *IOP Conference Series:* Materials Science and Engineering, 745(1): 012151.
17. Davis, J. L. (2018). Pharmacologic principles. Equine Internal Medicine, 4, 79-137.
18. Kapoor, G., and Saigal, S. (2017). Ashok Elongavan. Action a Resistance Mechanism of Antibiotics. Journal Anaesthesiology Clinical Pharmacology, *33*(3): 300-3005.
19. Azizi, O.; Shahcheraghi, F.; Salimizand, H.; Modarresi, F.; Shakibaie, M. R.; Mansouri, S., *et al*. (2016). Molecular analysis and expression of bap gene in biofilm-forming multi-drug-resistant *Acinetobacter baumanni*i. Reports of Biochemistry and Molecular Biology, 5(1): 62-67.