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Research Article

Detection of antibiotics resistance andefflux pumps production in clinical *Acinetobacter baumannii* isolates

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Abstract
Acinetobacter baumannii is considered one of the important pathogenic bacteria and responsible for several nosocomial and community infections in humans, such as the lungs (pneumonia), the bloodstream of ICU patients, burns, surgical wounds and meningitis. During the period from September to the end of December 2022, a total of 210 blood specimens were collected inDiyala governorate Baqubah Teaching Hospital, Iraq.To investigate the prevalence of bacterial isolates among ICU patients by identifying bacteria depending on suitable media under suitable environmental conditions for growth, morphological characteristics, biochemical tests, and confirmation of identification isolates by molecular detection of *blaOXA- 51gene*, antibiotic resistance, phenotypic and molecular detection of efflux pumps *adeF* gene, and activity of ciprofloxacin andresveratrol on gene expression. The percentage positive growth of *A. baumannii* isolates from blood specimens among ICU patients was 12 %, and the highest resistance of 25 isolates against fourteen types of antibiotics was equal to cefotaxime (100 %), ticarcillin-clavulanate, and ceftriaxone (96 %), and (92 %)for each of Cefepime and Tobramycin, the lowest percentage was for doxycycline (64 %).MIC value for ciprofloxacin and doxycycline between (8- 512 mg/ml) and (4 - 128 mg/ml) respectively. Molecular studies indicate the presence of *blaOXA- 51gene* and *adeF* in all isolates under study (100 %). The treatment of resistance isolates with ciprofloxacin, resveratrol and their combination resulted in reduction of *adeF* gene. The resveratrol effect on efflux pumps gene adeF gene expression occurred for the first time in Iraq and even at the level of the Arab world.

Keywords: *Acinetobacter baumannii*, *AdeF gene*, Antibiotic, Efflux pump inhibitors

INTRODUCTION

Acinetobacter baumannii (*A .baumannii*) is a lactose-nonfermentor gram-negative coccobacillus found in abundance in nature and has become endemic in hospitals. Furthermore, *A. baumannii* has a high environmental adaptability and drug resistance. It is on the 'ESKAPE' list of the most prevalent and dangerous multidrug resistance pathogens that are the most common source of nosocomial infections in hospitals worldwide (Al-Shamiri *et al*., 2021). *A. baumannii* cause several diseases in different tissues and systems of patients, including infections in the bloodstream, urinary tract, eye, and bacteremia, especially when the patients stay for long periods combined with device use, other infections as meningitis, intra-abdominal and surgical operation, the respiratory tract, and the digestive system (Doi *et al*., 2015; Nasr, 2020).

Bacteria can stay in the intensive care unit (ICU) in hospitals for a month because the organism has several virulence factors such as biofilm production, lipopolysaccharides (LPS), vesicles and proteins, capsules possess phospholipases, proteases enzymes, porins, siderophores factor (Lee *et al*., 2018; Pakharukova *et al*., 2018). The efflux pumps are the newest bacterial resistance mechanism considered the leading cause for the evolution of antibiotic resistance of *A. baumannii, which* originated from hospital infection. Because of the low absorbency of the outer membrane, drug efflux systems have become one of the most complex mechanisms of antibiotic resistance and have played an essential role in drug resistance, especially in *A. baumannii* (Hood *et al.,* 2010). So, it would be helpful to reduce antibiotic resistance by this mechanism. Some substances (chemical or natural) can directly act on the efflux system to restore antibiotic activity and are known as efflux pump inhibitors. Due to the little research on bloodstream infections by *A. baumannii* in ICU of Baqubah Teaching Hospital, this study aimed to investigate the prevalence of bacterial

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isolates among patients in ICU, antibiotic resistance, phenotypic and molecular detection of *adeF* gene, and activity of efflux pumps inhibitors ongene expression.

MATERIALS AND METHODS

Blood specimens collection

Two hundred and ten blood specimens were collected from Baqubah Teaching Hospital from the intensive care unit (ICU) from September to December 2022. The patients' information, including date, name, age, gender, and type of infection were recorded.

Ethical approval

The study was conducted following the ethical principles. It was carried out with patients' verbal and analytical approval before subjects were recruited forthe study. The study protocol and the subject information and consent form were reviewed and approved by Diyala University, College of Sciences, Department of Biology approved this work. Research approval number 46674 in date: 5/9/2022.

Diagnosis of *Acinetobacter baumannii* isolates

Blood culture formed by the BACT\Alert 3D system was used (Rovelli 2010). Subculture was used by the direct streaking method used for inoculation on MacConkey agar.The plates were incubated for 24 hours at 37ºC. Expected colonies of *A. baumannii*were confirmed by culturing on HiChromeTM *Acinetobacter* agar to inhibit most other species of bacteria and to obtain pure isolates of *A. baumannii*. Morphological investigation of bacterial isolates depends on Gram-staining and colony characteristics, including colonies shape, texture, color, and edges (Constantiniu *et al.,* 2004). Several biochemical tests, including oxidase, urease, imvic test, growth at 44ºC, citrate utilization, and hemolysin production test, were performed according to Darweesh *et al*. (2021). Identifying *A. baumannii* isolates was confirmed from blood specimens by detection of *blaOXA-51gene*.

Susceptibility of *Acinetobacter baumannii* isolates to antibiotics

The antibiotic susceptibility of twenty-five isolates of *A. baumannii* was performed using fourteen types of antibiotics, including piperacillin-tazobactam, ticarcillin-clavulanate, cefepime, and cefotaxime. Meropenem, Imipenem, ceftriaxone, tobramycin, amikacine, tetracycline, doxycycline, ciprofloxacin, levofloxacin, trimethoprim–sulf amethoxazol. This test was achieved by the disk diffusion method, according to the Clinical Laboratories Standards Institute CLSI (2022). The turbidity of the bacteria was evaluated using the McFarland tube (0.5) after pure isolates were revived and transferred to 5 ml of normal saline. A sterile cotton swab was placed with 0.1 ml of bacterial solution on Muller Hinton agar. Then, antibiotic discs were placed in the medium using sterilized forceps (7 discs in each plate). Plates were incubated at 37ºC for 18 hours. The result was read by measuring the diameter of the inhibition zone in(mm) and isolates divided into sensitive, resistant, intermediate comparison with the National Community for Clinical Laboratory Standard (CLSI, 2022) Minimum Inhibitory Concentration (MIC) were determined 0f two antibiotics ciprofloxacin and Doxycycline for twenty-five of *A.baumannii* clinical isolates. The test was done by broth dilution method based on CLSI (2022)and used a series of microdilution tubes with concentrations (2,4,8,16 ,32,64 , 128,256, 512, and 1024 ug/ml) of each antibiotic, minimum inhibitory concentration (MIC) resveratrol was determined for three selected resistant bacteria isolates to investigate their activity to *AdeF* gene expression.

Detect of *adeF gene* belonging to the efflux pump

Phenotypic detection of efflux pumps in 25 isolates of *A.baumannii* was studied by (cartwheel pattern) agar-EtBr method. Dilutions of bacteria were prepared from isolates that reveal antibiotic resistance and can biofilm formation after incubating for 24 hours at 24°C in the (5 ml) buffer. Their concentration was adjusted by comparing to (0.5) of McFarland standard solution, then isolates inoculated on tryptic soy agar (TSA) plates containing (0.25, 0. 5, 1, 1.5, and 2 mg\ml) of ethidium bromide (EB), the plate was divided to sixteen (16) by radial lines (cartwheel pattern) and the plates incubated for 16 hours at 37 °C in the dark place then the isolates were tested under ultraviolet (UV) trans illuminator to detect the fluorescent (Martins *et al*., 2011).

Molecular study

The molecular study was used firstly to confirm of identification *A.baumannii* isolates obtained from blood specimens by detection of *blaOXA- 51gene*, and to detect *adeF* efflux pump gene, secondly, to investigate the *adeF* gene expression in resistance isolates, these isolates treated with three treatment to investigate the effect antibiotic and natural substances on the expression of *AdeF* efflux gene, treatments include first: sub mic of ciprofloxacin, second: sub mic of resveratrol, third: combination of (0.25× mic) of ciprofloxacin and (0.25× mic) resveratrol for each isolate, the experiment performed in two replications.

DNA extraction

DNA bacteria was extracted from the bacteria isolates using a genomic DNA purification Kit supplemented by a manufactured company (Promga USA) and DNA was purified and their purity was measured by using Nanodrop spectrophotometer.

Polymerase Chain Reaction (PCR)

PCR was used to identify the *blaOXA-51* gene

Table 1. Primers of blaOXA-51 and *adeF* efflux genes

Primer Name	Sequence 5`-3`	Annealing Temp. (°C)	Product size (bp)	Reference
AdeF-F	GGTGTCGACCAAGATAAACG	56	207	(Kaviani *et al*. 2020)
AdeF-R	GTGAATTTGGCATAGGGACG			
Oxa-51-like-F	TAATGCTTTGATCGGCCTT	58	342	
Oxa-51-like-R	TGGATTGCACTTCATCTTGG			

Table 2. Percentages of *Acinetobacter baumannii* among different clinical specimens

Disease	Specimens number	Isolates number	Percentage
Burns	35	4	16
surgical wounds	61	5	20
Renal Failure	36	3	12
Diabetes foot	25	6	24
Brain Bleeding	11	3	12
Respiratory tract	44	4	16
Total	210	25	100

and *adeF* efflux pump gene. Proliferation of genes with 2 primer pairs (Table 1). The amplification of DNA was performed at a total volume of 25μl, and the reaction mixture contained (5μl) PCR Premix (Promega USA), (3μl) template DNA, (1.5μlF-primer), (1.5μl R-primer) and (14μl) deionized nuclease-free water. PCR reaction of genes wasperformed in (three steps): first initial DNA denaturation at 95°C for five minutes (1 cycle), denaturation of DNA template at 95°C for 30 seconds to amplify DNA, annealing at 52°C for 30 seconds and extension at 72°C for 45 seconds (30 cycles) and final extension at 72°C for 5 minutes (1 cycle).

Gel electrophoresis

After the amplification process in thermocycler PCR apparatus, the products were run on(1.5 % agarose gel with 5μl of ethidium bromide) in 1x (TBE) buffer using DNA ladder (100-2000) bp (Bioneer, Korea) at 100 voltsfor 15 minutes. The visualization of PCR products was made under 320 nm UV light using a UV trans illuminator (Djankpa *et al*., 2021).

Real-time quantitive reverse transcription [qRT-PCR]

RNA extraction and purification from the sample were performed using the TRIzol™ reagent technique. The QuantusTM Fluorometer (Promega, USA) was used to quantify the concentration of the extracted RNA according to the supplier's manual instructions. The Gene 9600 Quantitative PCR Instrument was used for the real -time PCR. The primers were created for *adeF* (Kaviani *et al.,* 2020). The solution was put in a RT-PCR Cycler for a thermal reaction to determine the cycle threshold [CT] value. This is a method for measuring levels of gene expression. The observed (CT values*adeF*) during the heat reaction were saved to a computer, and the variations in gene expression levels were computed using the CT technique.

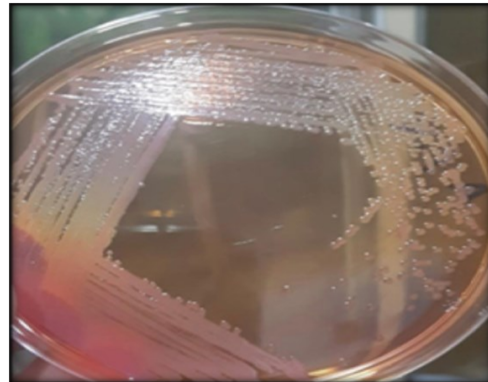

Fig. 1. *Showing A. baumannii colonies on MacConkey agar*

Real time qRT-PCR analysis

DCT (test) = CT gene of interest (target, test) – CT internal control (*adeF*) Eq. 1

DD CT=DCT (test)- DCT (calibrator), 2 -DDCt = Normalized expression ratio Eq. 2

Statistical analysis

The data in this study were analyzed using the statistical analysis application (SPSS version 23). The Chi-square test was utilized for the comparison of categorical data. The data were expressed as Means±SD and statistical significance was determined using a p-value of ≤ 0.05. These analytical techniques provided valuable insights and helped draw meaningful conclusions from the data.

RESULTS AND DISCUSSION

Growth result of blood specimen culture

The result of growth showed not all clinical specimensgave growth after culturing and incubation.Only 25 (12%) isolates of *A.baumannii* showed growth on media from 210 specimens.

Distribution of *Acinetobacter baumannii* according to the source

The percentages of *A. baumannii* among different clinical specimens distribute between 4 (16 %) forburn infections and respiratory tracts, 5 (20%) for surgical wounds, 6 (24%) for Diabetes foot (gas gangrene), and 3(12%) for Brain Bleedingand Renal failure.

Isolation and bacteriological identification

All the samples were cultured on MacConkey agar.

The colonies have appeared as pale, small, pink, and non-lactose fermenters on MacConkey agar (Havenga *et al*., 2022). Blood agar colonies showed non-pigmented creamy, convex mucoid with a smooth surface in diameter 0.5-2 mm, and the colonies productive hem lysine as shown in (Fig 1).

The results of biochemical tests reveal that all the isolates of *A. baumannii* showed positive results for the catalase test because the bacteria can produce the catalase enzyme, and all isolates gave a negative reaction for the oxidase test, Lactose fermentation, indol production, and methyl red. Voges-Proskauer. The positive results for the test appeared in simmons citrate, while the urease test gave variable results. All isolates were identified as *A.baumannii, which can* grow at 44ºC. Gram-stain showed that *A. baumannii* isolates shape differently according to the growth phase from bacilli to cocco-bacilli (Asif*etal* ., 2018).

The results of molecular identification of *A. baumannii* showed that all eighteen isolates gave a positive result for *the bla- OXA51* gene, indicating that the isolates (100%) were diagnosed as *A. baumannii* (Fig.2) . The current study is consistent with the results of studies (Bajpai *et al*., 2016; Ridha *et-al.,*2019; Kadhom and Ali, 2022).

Antibioticresistance of *Acinetobacter baumannii* isolates

The results of the current study (Fig.3) showed that high resistance to cefotaxime with a ratio (100%), ticarcillin-clavulante, and ceftriaxone with a ratio of 96% . The resistance ratio to piperacillin-tazobactam, cefepime, and tobramycin was (92%), and all these results agreed with (AL-Dahlaki, 2020). *A. baumannii* shows resistance to fluoroquinolones class (ciprofloxacin, levofloxacin) with a percentage of 72%. This resulted in an agreement with (Muhammad *et al.,*2023). The choice drug of treatment, imipenem, and meropenem, showed resistance with a percentage (72% - 76%) respectively the resulting agreement with (Kadom, 2020). But the results disagree with (AL-Dahlaki, 2020). Doxycycline showed low resistance, with a percentage of 64 % . These results were near to the study on *A.baumannii* by Subramanian *et al*., (2020). The tetracycline and trimethoprim sulfomethazol showed resistance with a ratio of 80 %. This result agreed withstudy on *A. baumannii* by AL Hadeedy and AL Jebory, 2019).

Table 3 shows the value of minimum inhibitory concentration (mic) for ciprofloxacin for all *A. baumannii* isolates ranging from 8- 512 mg/ml.This is acceptable with Muhammad *et al*. (2023) were considered highly resistant. While the MIC value of doxycycline ranged from (4 – 128 mg /ml) this result agreed with the result by (Wang *et al.,* 2023). Who reported in their study the mic value of doxycycline between (1≥ 128), while the result disagreed with (Kim *et al* ., 2022). They found the mic value of doxycycline in their study between (16 to 32 mg/ml).

Efflux pumps detection

All twenty five (100%) isolates of *A. baumannii* under study showed a positive fluorescence at the EtBr concentration (5 ,10 ,15 , 20 .25 mg /ml), except two isolates include (A .b1 and A .b 3), which showed negative fluorescence only at the lowest EtBr concentrations tested (20,25 mg /ml) because they do not have phenotypically efflux pumps that enable them to extrude EtBr.

The results of this study were acceptable to the local studies performed during different years such as Wareth *et al*. (2021),Ali and Mahmood (2022), who both reported in their studies that all isolates of *A. baumannii* have efflux pumps in the strains with percentage (99-100%) by the EtBr-agar cartwheel method, One of many reasons which made these bacteria to become resistant to almost all antibiotics, is the presence of efflux pump families (Monem *et al*., 2020). These groups of bacteria can effectively resist antibiotics, heavy metals, and various other substances. *A. baumannii* possesses multiple families of efflux pumps, with the Resistance Nodulation Cell Division (RND) family being particularly significant. This family is further categorized into three distinct types, each of which plays a crucial role in conferring resistance against specific classes of antibiotics (Salimizand *et al*., 2018). The results of molecular detection indicate that all isolates carried *adeF* genes, as shown in Fig. 4. A total of 18 bacterial isolates underwent DNA extraction in order to detect the presence of *Ade F* gene of Efflux pump in *A. baumannii* and the correlation of these genes with resistance .

The results of genes In this experiment showed that the *Ade F* gene belong to RND super family efflux pump in *A. baumannii* was distributed worldwide and was

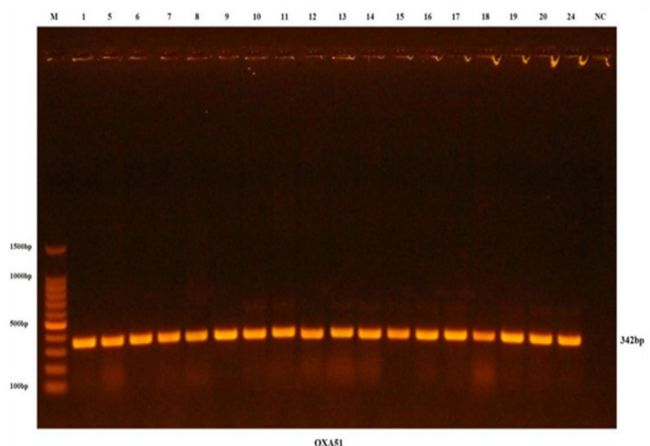

Fig. 2. *Representing results of the amplification of bla-OXA51 gene of Acinetobacter baumannii samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 1-24 resemble 342bp PCR products*

Table 3. MIC values of ciprofloxacin, and doxycycline of *A.baumannii*

Isolates Series	MIC of Cip ug/ml	MIC of Dox ug/ml	Isolates Series	MIC of Cip ug/ml	MIC of Dox ug/ml
Breakpoint	**≤1(S)/ ≥ 4(R)**	**4(S)/ ≥ 16(R)**	**Breakpoint**	**≤1(S)/ ≥ 4(R)**	**4(S)/ ≥ 16(R)**
A.b1	128	8	A.b 14	256	128
A.b2	8	32	A.b 15	256	128
A.b3	16	4	A.b 16	64	64
A.b4	16	16	A.b 17	512	4
A.b5	32	64	A.b 18	128	32
A.b6	32	16	A.b 19	64	128
A.b7	64	8	A.b 20	64	16
A.b8	128	16	A.b 21	256	64
A.b9	256	64	A.b 22	32	64
A.b10	128	128	A.b 23	16	64
A.b11	8	32	A.b 24	256	128
A.b12	64	16	A.b 25	16	64
A.b13	512	4		p.value = 0.09	

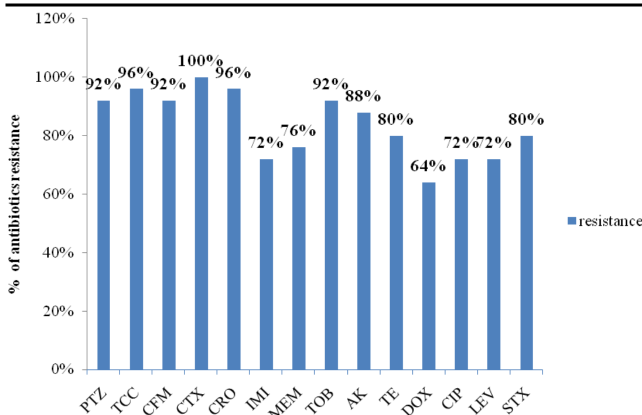

Fig. 3. *Percentage of antibiotics resistance of A. baumannii isolates (PTZ= Piperacillin- tazobactam; TCC= Ticarcillin–clavulanic ,FEP=Cefepime,CTX= Cefotaxime, CRO=Ceftriaxone , IMI=Imipenem , MEM =Meropenem , TOB = Tobramycin , AK= Amikacin , TE=Tetracycline , DOX = Doxycycline , CIP=Ciprofloxacin , LEV= Levofloxacin, STX= Trimethoprim –sulfa methoxazole*

found in approximately 100% of the clinical strains. These results are acceptable with Wareth *et al*. (2021; Ali and Mahmood (2022), who both found that superfamily RND *(adeFGH, adeIJ)* was most frequent and found in almost all isolates with percentage (100%).This result is similar likely withXu, Bilya*et al.* 2019; Kaviani *et al.2020).*

Activity of ciprofloxacin andresveratrol on*adeF*gene expression

The minimum inhibitory concentration of three MDR resistance *A. baumannii* isolates that were used in the gene expression study were ciprofloxacin (256 mg/ ml) and resveratrol (64, 32, 256 mg/ ml). The results presented in Table 4 showed that the folding mean decreased adeF gene expression after treatment with Ciprofloxacin and Resveratrol. The combination of both alone for each three resistant *A. baumannii* isolates wasequal to (0.76, 0.50, 0.93), with significant differences between the control and treatment isolates (p-value 0.045).

The system of efflux pump transporters significantly influences the process. These membrane proteins, expressed continuously, are responsible for maintaining a sufficiently low internal concentration of antibiotics.This enables bacteria to implement more sustainable defensive strategies against antibiotics, such as altering gene expression levels or acquiring mutations (Shuster *et al*., 2016). About 99% of clinical isolates possess *the AdeF* gene with the high expression is responsible forhigh levels of resistance tochloramphenicol, clindamycin, fluoroquinolones, and trimethoprim (Fernando, 2015).

The 0.93 value of expression of *the adeF* gene after treatment with the combination of two agents indicated these isolates might be induced by the presence of antibiotics, resulting in a change in expression levels that, in turn, improves the ability of these isolates to expel a large number of antibiotics, thereby allowing them to confer more resistance to ciprofloxacin. After treatment with resveratrol at level 0.50, the decreased expression was because resveratrol can increase the sensitivity of multi-drug resistant *A.baumannii* and enhance the bactericidal activity. This suppression of the synthesis of the efflux pump protein may be an essential mechanism by which resveratrol could restore the sensitivity of the bacteria to efflux pump inhibitors (Liu *et al*., 2020).

Conclusion

The present study showed twelve *A. baumannii* isolate prevalence among ICU patients percentage of antibiotic resistance in *A. baumannii*. Molecular detection showed that all isolates contained *bla OXA* -51 and *adeF* genes efflux pump. There were significant differences between the gene expression of *adeF* genes in selected isolates before and after

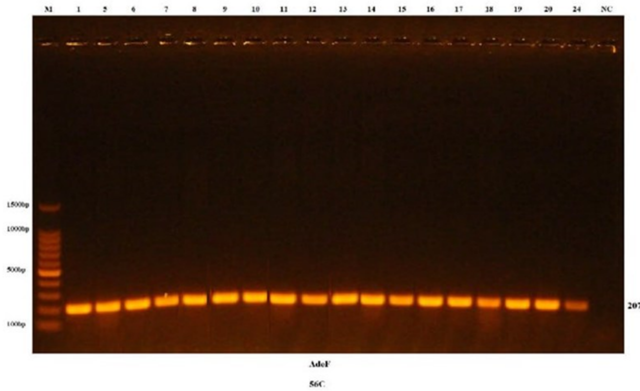

Fig.4. *Results of the amplification of AdeF gene of Acinetobacter baumannii samples were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br;M: 100bp ladder marker. Lanes 1-24 resemble 207bp PCR product*

treatment with ciprofloxacin, resveratrol, and a combination of both ciprofloxacin and resveratrol and significant effect of resveratrol as inhibitors and reduction of gene expression.

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Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

1. AL-Dahlaki, S. M. M. (2020). *Molecular detection and gene expression for hcp and blaOXA-51 genes in Acinetobacter baumannii isolated from different clinical sources* (Doctoral dissertation, MSC Thesis, College of Science, University of Diyala, Diyala, Iraq, 1-154.
2. Al-Shamiri, M. M., Zhang, S., Mi, P., Liu, Y., Xun, M., Yang, E., ... & Chen, Y. (2021). Phenotypic and genotypic characteristics of Acinetobacter baumannii enrolled in the relationship among antibiotic resistance, biofilm formation and motility, *Microbial Pathogenesis*, 155(4), 104922-104932. https://doi.org/10.1016/j.micpath.2021.104922.
3. AL Hadeedy, U. E. I., Mahdi, N. B. & AL Jebory, I. S. (2019). Isolation and Diagnosis of Acinetobacter Bumanniii Recently Isolated From Patients in Kirkuk Hospitals and Study their Antibiotics Resistance. *Kirkuk University Journal-Scientific Studies*, 14(3), 155-173. https://doi.org/10.32894/kujss.2019.14.3.12.
4. Ali, M. A. & SS Mahmood. 2022. 'Role of RND-efflux pump in levofloxacin resistance among Acinetobacter baumannii', *Iraqi Journal of Agricultural Sciences*, 53 (3),542-550.
5. Asif, M., Alvi, I. A. & Rehman, S. U. (2018). Insight into Acinetobacter baumannii: pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. *Infection and drug resistance*, 16 (9),1249-1260.
6. Bajpai, T., Bhatambare, G. S., Varma, M., & Pandey, M. (2016). Species-level identification of Acinetobacter by 16s rRNA sequencing: Necessity today, essentiality tomorrow. *New Nigerian Journal of Clinical Research*, *5*(8), 55-65.http://doi.org/ 10.4103/2250-9658.197438i.
7. Cheesman, M. J., Ilanko, A., Blonk, B. & Cock, I. E. (2017). Developing new antimicrobial therapies: are synergistic combinations of plant extracts/compounds with conventional antibiotics the solution?.*Pharmaco gnosyreviews*, 11(22), 57-67.https://doi.org/10.4103%2Fphrev.phrev_21_17
8. CLSI, (2022). Performance Standards for Antimicrobial Susceptibility Testing. 27th ed. Wayne, PA: Clinical and Laboratory Standards Institute.
9. Constantiniu, S., Romaniuc, A., Iancu, L. S., Filimon, R., & Taraşi, I. (2004). Cultural and biochemical characteristics of Acinetobacter spp. strains isolated from hospital units. *The journal of preventive medicine*, 12(34), 35-42.
10. Darweesh, A. O., Shareef, A. A. & Alriyahee, F. A. A. (2021). Isolation and Identification of Cellulytic Ruminant

Table 4. Gene expression study of *adeF* gene before and after treatment

Isolate	16srRNA	adeF	ΔCT	ΔΔCT	Folding	Mean	P –value
A .b 14	13.95	12.05	-1.91	0.00	1.00		
A .b 15	18.57	17.11	-1.47	0.00	1.00	1	
A .b 24	13.59	12. 7	-1.52	0.00	1.00		
A .b 14 (CIP)	15.73	15.67	-0.06	1.85	0.28		
A .b 15 (CIP)	18.22	16.19	-2.03	-0.57	1.48	0.76	0.045
A .b 24 (CIP)	16.62	16.03	-0.59	0.93	0.52		
A .b 14 (R)	15.60	15.35	-0.25	1.65	0.32		
A .b 15 (R)	16.02	15.48	-0.54	0.92	0.53	0.50	
A .b 24 (R)	18.72	18.23	-0.42	-0.95	0.67		
A.b14(CIP +R)	15.73	14.28	-1.45	0.46	0.73	0.93	
A.b15(CIP+ R)	18.22	16.94	-1.28	0.18	0.88		
A.b24(CIP+ R)	16.62	14.84	-1.79	-0.26	1.20		

A .b = *A. baumannii*; (CIP) = Ciprofloxacin;, R = Resveratrol; (CIP + R) = ciprofloxacin + Resveratrol

Pseudomonas Aeruginosa by Classical and Molecular Methods in Basrah Province. *Annals of the Romanian Society for Cell Biology*, 1(4),4672-4680.

11. Djankpa, F. T., Wiafe, G. A., Boateng, B. N., Tsegah, K. M., Essien-Baidoo, S., Ulanja, M. B., ... & Asante, G. (2021). Assessment of corn starch as substitute for agarose in DNA gel electrophoresis. *BMC Research Notes*, 14 (1), 1-6https://doi.org/10.1186/s13104-021-05483-1.
12. Doi, Y., Murray, G. L. & Peleg, A. Y. (2015, February). *Acinetobacter baumannii*: evolution of antimicrobial resistance-treatment options. In *Seminars in respiratory and critical care medicine,* 1(36), 85-98. http://doi.org/ 10.1055/s-0034-1398388.
13. Fernando, D. M. (2015). *Analysis of resistance nodulation division efflux pumps in Acinetobacter baumannii* (Doctoral dissertation, University of Ontario Institute of Technology) 1- 133.
14. Havenga, B., Reyneke, B., Ndlovu, T. & Khan, W. (2022). Genotypic and phenotypic comparison of clinical and environmental *Acinetobacter baumannii* strains. *Microbial Pathogenesis*, 172(14), 1049-1057.https://doi.org/10.1016/j.micpath.2022.105749
15. Hood, M. I., Jacobs, A. C., Sayood, K., Dunman, P. M. & Skaar, E. P. (2010). Acinetobacter baumannii increases tolerance to antibiotics in response to monovalent cations. *Antimicrobial agents and chemotherapy*, 54(3), 1029-1041.https://doi.org/10.1128/aac.00963-09
16. Kadhom, H. & Ali, M. (2022). Epidemiological molecular analysis of Acinetobacter baumannii isolates using a multilocus sequencing typing and global lineage. *Revis Bionatura*, *7*(1), 29.
17. Kadom, S. M. & ABID, I. N. (2020). Detection of bla OXA-51-like and bla VIM Carbapenemase Genes in Acinetobacter baumannii isolated from burn patients. *International Journal of Pharmaceutical Research*, 12(2), 23-35.
18. Kaviani, R., Pouladi, I., Niakan, M. & Mirnejad, R. (2020). Molecular detection of Adefg efflux pump genes and their contribution to antibiotic resistance in Acinetobacter baumannii clinical isolates. *Reports of Biochemistry & Molecular Biology*, 8(4), 413.
19. Kim, S. H., Yun, S., & Park, W. (2022). Constitutive Phenotypic Modification of Lipid A in Clinical Acinetobacter baumannii Isolates. *Microbiology spectrum*, 10(4), 1295-1322.https://doi.org/10.1128/spectrum.01295-22
20. Lee, H. Y., Hsu, S. Y., Hsu, J. F., Chen, C. L., Wang, Y. H. & Chiu, C. H. (2018). Risk factors and molecular epidemiology of Acinetobacter baumannii bacteremia in neonates. *Journal of microbiology, immunology and infection*, 51(3), 367-376. https://doi.org/10.1016/j.jmii.2017.07.013
21. Liu, L., Yu, J., Shen, X., Cao, X., Zhan, Q., Guo, Y.& Yu, F. (2020). Resveratrol enhances the antimicrobial effect of polymyxin B on Klebsiella pneumoniae and Escherichia coli isolates with polymyxin B resistance. *BMC microbiology*, 20 (1), 1-8.https://doi.org/10.1186/s12866-020-01995-1
22. Martins, M., Viveiros, M., Couto, I., Costa, S. S., Pacheco, T., Fanning, S., & Amaral, L. (2011). Identification of efflux pump-mediated multidrug-resistant bacteria by the ethidium bromide-agar cartwheel method. *In vivo*, 25(2), 171-178.
23. Monem, S., Furmanek-Blaszk, B., Łupkowska, A., Kuczyńska-Wiśnik, D., Stojowska-Swędrzyńska, K. & Laskowska, E. (2020). Mechanisms protecting Acinetobacter baumannii against multiple stresses triggered by the host immune response, antibiotics and outside-host environment. *International Journal of Molecular Sciences*, 21(15), 5498-5510. https://doi.org/10.3390/ijms21155498
24. Muhammad, M. J., Mubarak, K. I. & Wadi, K. D. (2023). Study of Antibacterial Activity from Alcoholic Extracts of Torilis arvensis (Huds.) Link on Biofilm formation of Acinetobacter baumannii in Diyala Province-Iraq. *Academic Science Journal*, 1(1), 60-76.https://doi.org/10.24237/ASJ.01.01.614C
25. Nasr, P. (2020). Genetics, epidemiology, and clinical manifestations of multidrug-resistant Acinetobacter baumannii. *Journal of Hospital Infection*, 104(1), 4-11. https://doi.org/10.1016/j.jhin.2019.09.021
26. PEYMAnI, A. M. I. R., FARAJnIA, S. A. F. A. R., Nahaei, M. R., Sohrabi, N., Abbasi, L. A. L. E. H., Ansarin, K. H. A. L. I. L. & Azhari, F. A. T. E. M. E. H. (2012). Prevalence of class 1 integron among multidrug-resistant Acinetobacter baumannii in Tabriz, northwest of Iran. *Polish Journal of Microbiology*, 61(1), 57-60.
27. Ridha, D. J., Ali, M. R. & Jassim, K. A. (2019). Molecular analysis of multi–drug resistant *Acinetobacter baumannii* isolated in Baghdad hospital. *Int. J. Res. Pharm. Sci*, 10 (3), 1970-1987.
28. Rovelli, S. (1998). *The role of polysaccharides in plasmodium falciparum malaria* (Doctoral dissertation), 1-185.
29. Salimizand, H., Zomorodi, A. R., Mansury, D., Khakshoor, M., Azizi, O., Khodaparast, S., & Sadeghian, H. (2018). Diversity of aminoglycoside modifying enzymes and 16S rRNA methylases in *Acinetobacter baumannii* and *Acinetobacter nosocomialis* species in Iran; wide distribution of aadA1 and armA. *Infection, Genetics and Evolution*, 66 (1), 195-199.https://doi.org/10.1016/j.meegid.2018.09.028
30. Shuster, Y., Steiner-Mordoch, S., Alon Cudkowicz, N., & Schuldiner, S. (2016). A transporter interactome is essential for the cquisition of antimicrobial resistance to antibiotics. *PLoS One*, 11(4),55-66.https://doi.org/10.1371/journal.pone.0152917
31. Subramanian Swaminathan, G. I., Arora, S., Mehta, S., Mittal, R., Mane, A. & Charugulla, S. N. (2020). Susceptibility pattern of doxycycline in comparison to azithromycin, cefuroxime and amoxicillin against common isolates: a retrospective study based on diagnostic laboratory data. *Journal of The Association of Physicians of India*, 68(1), P59.
32. Wang, Y., Su, J., Zhou, Z., Yang, J., Liu, W., Zhang, Y., ... & Li, G. (2023). Baicalein Resensitizes Multidrug-Resistant Gram-Negative Pathogens to Doxycycline. *Microbiology Spectrum*, 11(3), 4702-4722.https://doi.org/10.1128/spectrum.04702-22
33. Wareth, G., Brandt, C., Sprague, L. D., Neubauer, H. & Pletz, M. W. (2021). WGS based analysis of acquired antimicrobial resistance in human and non-human Acinetobacter baumannii isolates from a German perspective. *BMC microbiology*, 21(1), 1-13.https://doi.org/10.1186/s12866-021-02270-7
34. Xu, C., Bilya, S. R. & Xu, W. (2019). adeABC efflux gene in *Acinetobacter baumannii*. *New Microbes New Infect,* 30 (8), 100549.