ORIGINAL ARTICLE

Overexpression of *AdeABC* Efflux Pump Genes *adeR* and *adeS* in Multidrug-Resistant *Acinetobacter baumannii* Isolated from Burn Patients in Baghdad

Hanan D. Salem*, Wathiq A. Al-Draghi
Department Genetic Engineering, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq

Key words:
Acinetobacter baumannii, MDR, XDR, antibiotic resistance, efflux pumps gene

****Corresponding Author:***
Hanan Dawood Salem
Department Genetic Engineering, Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad, Baghdad, Iraq
Tel: 00964-7714833273
hanan1993d@gmail.com

ABSTRACT

Background*: Acinetobacter baumannii is a critical nosocomial pathogen with rising multidrug resistance (MDR) driven by mechanisms such as efflux pump overexpression: This study investigated antibiotic resistance profiles and the role of AdeABC efflux pump genes (adeR, adeS) in Acinetobacter baumannii isolates from burn patients in Baghdad, Iraq.* ***Methodology****: A total of 200 clinical samples were collected from burn units in Baghdad hospitals (December 2023–March 2024) The ages of the patients ranged from (14 to 81 years), both males and females, where the proportion of females being was higher than that of males. 60% for females and 40% for males, Isolates was identified via biochemical assays and the VITEK 2 system and MIC determinations were performed, followed by PCR and qPCR to detect expressione adeR, adeS, and 16S rRNA genes* ***Results****: Among 20 confirmed Acinetobacter baumannii isolates, 75% harbored adeR and 70% adeS. MDR strains exhibited significant adeR (2.71–2.75-fold; $p \leq 0.01$) and adeS overexpression (up to 40.50-fold; p=0.0001), which correlated with resistance to carbapenems (imipenem MIC >32 µg/mL), fluoroquinolones (ciprofloxacin MIC >8 µg/mL), and aminoglycosides (gentamicin MIC >16µg/mL). Mutational analysis revealed that adeS truncations (Δ95–157) and adeR substitutions were linked to efflux pump hyperactivity.* ***Conclusion****: This study revealed overexpression of efflux pump genes adeR and adeS in Acinetobacter baumannii MDR strains, indicating the need for efflux pump inhibitors in therapeutic regimens and genomic surveillance for infection control policies.*

INTRODUCTION

Acinetobacter baumannii is a gram-negative, strictly aerobic, non-fasctidious, non-fermentative, catalase-positive, and oxidase-negative rod. It is highly relevant in the healthcare environment as one of the most opportunistic pathogens, presenting growing importance and challenges [1]. *Acinetobacter baumannii* has become a prominent nosocomial pathogen in ICUs, leading to severe and often fatal infections. Moreover, this opportunistic bacterium has been notorious for having the longest survival on the surface of hospital and medical equipment, and the increasing resistance to many antibiotics [2]. Morbidity and mortality associated with *Acinetobacter baumannii* infections in ICU patients are notably high, particularly in high-risk populations, such as patients with a compromised immune system, prolonged hospitalization, or invasive medical devices [3]. Diseases induced by *Acinetobacter baumannii* cover a wide range, including, but not limited to, hospital-acquired and ventilator-associated pneumonia (HAP, VAP), urinary tract infections, and skin and wound infection [1,4]. However, *Acinetobacter baumannii* has been referred to as "Iraqibacter," because it was first identified in US military treatment centers in Iraq [5,6].

Acinetobacter baumannii is an ESKAPE organism, and ESKAPE pathogens, including **E** – *Enterococcus faecium*, **S** – *Staphylococcus aureus*, **K** – *Klebsiella pneumoniae*, **A** – *Acinetobacter baumannii*,**P** – *Pseudomonas aeruginosa*,**E** – *Enterobacter species*, represent a group of highly virulent and antibiotic-resistant bacteria that pose a significant threat to global health. These organisms have developed remarkable abilities to evade commonly used antibiotics through various mechanisms, including enzymatic inactivation, target-site modification, and biofilm formation [7,8]. Their rapid acquisition of antimicrobial resistance has led to a paradigm shift in our understanding of infectious disease pathogenesis, transmission, and treatment challenges, particularly in healthcare settings, where they are responsible for he majority of life-threatening nosocomial infections [9,10]. Among ESKAPE organisms, *Acinetobacer baumannii* is a threat to hospital settings, particularly when infecting critically ill individuals [11,12].

Notably, *Acinetobacter baumannii* is intrinsically resistant to a range of antibiotics and has the ability to acquire antibiotic resistance genes, leading to

increasingly widespread antimicrobial resistance trends[13]. This progression has led to the emergence of new and more sophisticated pathogens, which present significant challenges for clinicians [14,15]. All these features help the bacterium persist in adverse conditions and become resistant to antimicrobial therapy [16,17]. Among the various mechanisms of antibiotic resistance and virulence determinants, as well as the precise regulatory pathways of *Acinetobacter baumannii* [18]. A key component of its resistance arsenal is the AdeABC efflux pump system, which is part of the resistance-nodulation-cell division (RND) superfamily and includes the *adeA* (membrane fusion protein), *adeB* (multidrug transporter), and *adeC* (outer membrane protein) genes. These genes are regulated by a two-component system composed of *adeR* (response regulator) and *adeS* (sensor kinase) [19].

AdeABC, a chromosomal efflux pump in ~80% of *Acinetobacter baumannii* strains, drives carbapenem resistance. Antibiotic overuse upregulates AdeABC, causing multidrug resistance [20,21]. *AdeR and AdeS* are two-component systems that regulate the AdeABC efflux system. *AdeR* acts as a response regulator, whereas *AdeS* acts as a sensor kinase [22]. The adeABC operon has two copies of each gene located immediately upstream, and is transcribed in the opposite direction. Because of the increased susceptibility to infection associated with their inactivation, it has been postulated that *AdeRS* operates as a transcriptional activator of AdeABC [23]. To date, no signal has been described that directly causes adeABC expression via *AdeRS*. Antimicrobial resistance phenotypes in *Acinetobacter baumannii* clinical isolates have been linked to a range of mutations in the positive regulator, *AdeRS*, which are mediated by increased *AdeB* transporter expression [24]. A study in 2012 was the first to describe the molecular mechanism underlying the antimicrobial resistance phenotype by introducing several recombinant *adeRS* constructs into an *adeRS*-deficient *Acinetobacter baumannii* strain [25,26].

The World Health Organization (WHO) has prioritized carbapenem-resistant *Acinetobacter baumannii* (CRAB) as a critical global health threat, emphasizing the urgent need for novel antimicrobial development and targeted research [27,28]. CRAB strains frequently exhibit extensive drug resistance (XDR), rendering them resistant to β-lactams, fluoroquinolones, and aminoglycosides [29]. This study aimed to isolate, identify, and characterize *Acinetobacter baumannii* strains from burn wound infections, evaluate their antibiotic resistance profiles, and investigate the role of efflux pump genes (*adeR* and *adeS*) in multidrug resistance (MDR).

Methodology

Sample Collection: This study collected 200 clinical isolates from patients with burns admitted to two Baghdad hospitals (Medical City/Ghazi Al-Hariri for Specialized Surgeries and Burns Hospital) between December 2023 and March 2024. Samples were collected with sterile swabs, transferred to the laboratory with media transport, swab collection, and placed into sterile containers for transport media. After a 24-hour incubation period at 37°C on MacConkey and blood agar (oxidase and catalase assays) were performed manually to confirm the presence of *Acinetobacter baumannii* .*Identification* of isolates. Morphological analysis was based on Bergey's Manual [30]: Biochemical tests according to. Identification of *Acinetobacter baumannii* confirmed using the VITEK 2 compact system

Minimum inhibitory concentration (MIC)

Bacterial identification and antimicrobial susceptibility testing (AST) were performed using the VITEK 2 Compact system (bioMérieux, France), an automated system widely used for rapid and accurate microbial diagnostics.Pure bacterial colonies were selected from freshly cultured agar plates and suspended in 0.45% sterile saline. The turbidity of the suspension was adjusted to match a 0.5 McFarland standard using a densitometer (DensiCHEK). The appropriate VITEK 2 identification (ID) and AST cards were selected based on the Gram stain of the isolates. The inoculated cards were loaded into a VITEK 2 instrument, where they were automatically incubated and monitored. The system used optical sensors to record biochemical changes and bacterial growth every 15 min. Identification was determined based on biochemical reactions, while MIC values and susceptibility patterns were interpreted according to CLSI breakpoints [31]. Final results, including bacterial species and MIC-based susceptibility interpretations (susceptible, intermediate, resistant), were automatically generated by the system within 6 to 18 h, depending on the organism.

Molecular Analysis

Genomic DNA was extracted from gram-negative bacteria using an EasyPure® Bacteria Genomic DNA Kit (TRANS/China). The protocol involves bacterial lysis, enzymatic treatment, and the use of spin column technology to obtain high-quality DNA. The DNA purity was assessed using the A260/A280 ratio. PCR Amplification: Conventional PCR was performed to detect *Ade R&AdeS* primers specific to *AdeSR* were designed and validated) *adeS* F:ATGGTGACAAAGAGAGTGCA and R:CCCTTCGGCGATGATTCT (870 bp) and *adeR* F:CTGAAGGTGTACGGAAACAC R:GTTCGGCCACCTCGAATTG (322 bp) [32] frequencies were determined by PCR. Primers specific to the target genes were prepared by dissolving lyophilized primers in nuclease-free water to create a stock solution (100 pmol/µL), which was subsequently diluted to a working concentration of 10 pmol/µL. The prepared primers were stored at -23°C for long-term use, table1:

Table 1: Oligonucleotide primers used for Conventional PCR and Real- time PCR.

First name	Sequence Primer (5'-3')	SIZE bp
16sr RNA	**F:5'CAGCTCGTGTCGTGAGATGT 3'** **R:5' CGTAAGGGCCATGATGACTT3'**	150
AdeS	**F: 5'ATGGTGACAAAGAGAGTGCA3'** **R: 5' CCCTTCGGCGATGATTCTC3'**	870
AdeR	F:5' CTGAAGGTGTACGGAAACAC3' R :5' GTTCGGCCACCTCGAATTG3'	322

cDNA synthesis, RT-PCR, and quantitative RT-PCR

Total RNA was isolated using the TransZol Up Plus Kit, and RNA concentration and purity were verified spectrophotometrically (NanoDrop). Genomic DNA contamination was rigorously eliminated during RNA extraction and cDNA synthesis using EasyScript® One-Step gDNA Removal and cDNA Synthesis SuperMix. First-strand cDNA was synthesized from 0.1 ng–5 µg of total RNA using a combination of anchored oligo (dT) primers (0.5 µg/µl) and random hexamers (0.1 µg/µl) to ensure comprehensive reverse transcription of mRNA and non-polyadenylated RNA. The 20 µL reaction mixture included 10 µL of 2×ES Reaction Mix, 1 µL each of RT/RI enzyme mix and gDNA Remover, and RNase-free water. Subsequent quantitative RT-PCR analysis used this cDNA to amplify target sequences, with reaction specificity and efficiency validated through optimized primer design and stringent cycling parameters.

The expression levels of the target genes *AdeR* and *AdeS*, along with the reference gene *16sRNA*, were quantified using Real-Time Polymerase Chain Reaction (RT-PCR). For each cDNA sample, reactions were prepared in a 20 µl volume containing 10 µL of 2×EasyTaq® PCR SuperMix, 2 µL of cDNA, 2 µL of forward and reverse primer mix, and 6 µL of nuclease-free water. Amplification was performed under the following standardized thermal cycling conditions: initial denaturation at 94°C for 10 s, annealing at 54°C for 15 s, and extension at 72°C for 20 s. The *16sRNA* gene served as an endogenous control to normalize the expression data, ensuring the accurate relative quantification of *AdeR* and *AdeS* transcripts across samples. Dissociation curve analysis confirmed product specificity. Gene expression levels were quantified using the relative method with *16sRNA* as the reference gene

Statistical Analysis

The Statistical Packages of Social Sciences-SPSS (2019) program was used to detect the effect of different groups and isolates on the study parameters and gene expression (fold change). and LSD-Least significant differences were used for a significant comparison between the means. Chi-square test was used to compare the percentages (0.05 and 0.01 probability). in this study.

RESULTS

The bacterial isolates obtained with pure and predominant growth from clinical samples were identified using biomedical tests, in addition to the Vitek2 system and molecular detection. The results revealed that only n=20/200 (10 %) isolates were belonged to *Acinetobacter baumannii*, and the other isolates were n= 180 (90 %) non *Acinetobacter baumannii*. A total of 200 clinical samples were collected from burn units in Baghdad hospitals (December 2023–March 2024) the age of the patients ranged from 14 to 81 years) both males and females, with the proportion of females being was higher than that of males). The sample collection results recorded a proportion of 60% for females and 40% for males. Isolates were identified via biochemical assays, and the VITEK 2 system and MIC determinations were performed, followed by PCR and qPCR to detect expressione *adeR*, *adeS*, and 16S rRNA genes.table 2:

Table 2: show the distribution of samples for *Acinetobacter baumannii* and other organisms.

Category	Details
Total clinical samples	200 samples
Acinetobacter baumannii isolates	20 isolates (10%)
Non-Acinetobacter baumannii isolates	180 isolates (90%)
Age range of patients	14 to 81 years
Gender distribution - Females	60%
Gender distribution - Males	40%
Sample collection location	Burn units in Baghdad hospitals
Sample collection period	December 2023 – March 2024

Electrophoretic analysis of PCR-amplified products from *Acinetobacter baumannii* isolates. In Figure 1, lane M represents a DNA ladder (1500–100 bp), whereas lanes 1–7, 10–13, and 16–18 (n=14) exhibited amplification corresponding to the target sequences, indicating positive results. Conversely, lanes 8, 9, 14, 15, 19, and 20 (n=6) showed no amplification. Similarly, in Figure 2, under identical electrophoretic conditions, lanes 1–7, 9–13, and 17–19 (n=15) displayed distinct bands, confirming target amplification, whereas lanes 8, 14–16, and 20 (n=5) lacked visible bands, indicating negative outcomes. These results demonstrate variability in target presence among the tested isolates, with Figure 1, 2, and 3 differing in the proportion of positive samples (14/20 vs. 15/20, respectively).

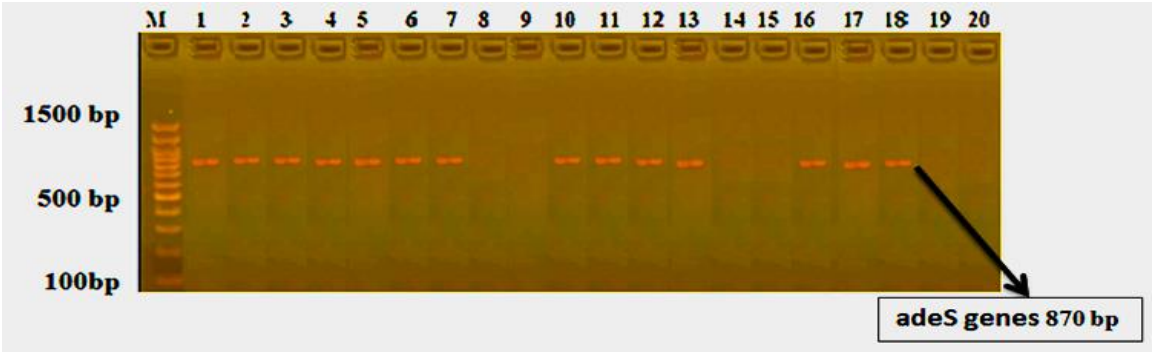

Fig. 1: Detection of ***adeS*** gene product (amplified size 870 bp) using DNA template of *Acinetobacter baumannii* isolates by PCR (agarose gel electrophoresis, 1% agarose, 70 volt for 1-2 hrs). Lane (M), DNA Ladder (1500-100 bp). Lanes (1,2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 16,17 and 18) (*n*=14) of *Acinetobacter baumannii* isolates show positive results. Lanes (8, 9, 14, 15, 19 and 20) (*n*=6) show negative results.

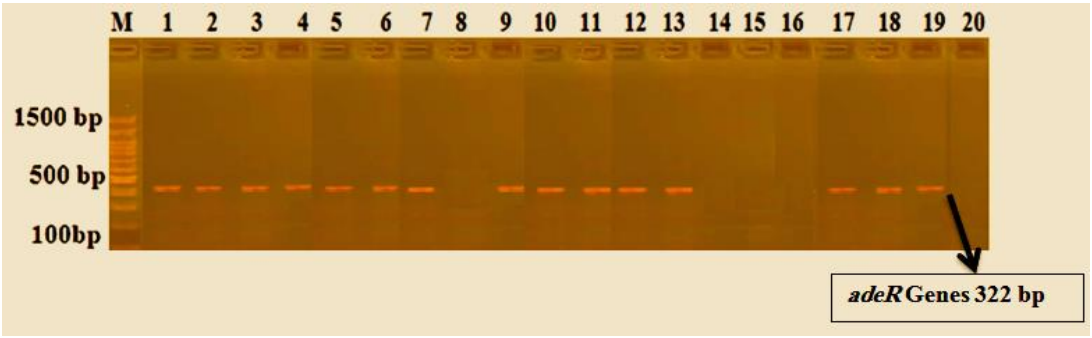

Fig. 2: Detection of ***adeR*** gene product (amplified size 322 bp) using DNA template of *Acinetobacter baumannii* isolates by PCR (agarose gel electrophoresis, 1% agarose, 70 volt for 1-2 hrs). Lane (M), DNA Ladder (1500-100 bp). Lanes (1,2 3, 4, 5, 6, 7, 9, 10, 11, 12, 13 , 17, 18 and 19) (*n*=15) of isolate *Acinetobacter baumannii* show positive results. Lanes (8, 14, 15, 16 and 20) (*n*=5) show negative results.

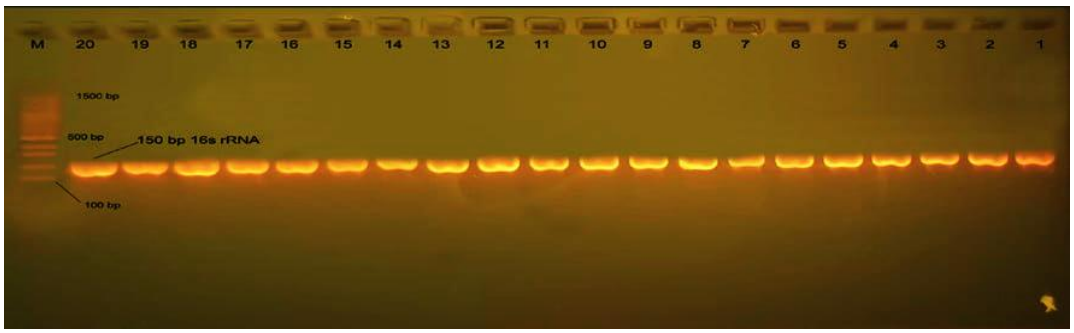

Fig. 3: Detection of ***16sRNA*** gene product (amplified size 151 bp) using DNA template of *Acinetobacter baumannii* isolates by PCR (agarose gel electrophoresis, 1% agarose, 70 volt for 1-2 hrs). Lane (M), DNA Ladder (1500-100 bp). Lanes (1,2 3, 4, 5, 6, 7,8 9, 10, 11, 12, 13 , 14,15,16,17, 18, 19and 20).

Table 3 shows the relative expression of *adeR* genes normalized to 16s rRNA using the ΔCt method and normalized expression and fold change across 14 experimental isolates and a control. isolates 8 (2.71 ±0.23-fold), 13 (2.75 ±0.21-fold), and 14 (2.11 ±0.18-fold; superscript "a") were significantly upregulated, and isolates 1 (0.47 ±0.06), 3 (0.66 ±0.08), 10 (0.43 ±0.05), and 11 (0.86 ±0.08; "b") were significantly suppressed compared with the control (1.00 ±0.00). Isolates 2, 4–7, 9, and 12 ("ab") were moderately upregulated (1.28–to 1.99-fold) with no significant differences from the extremes. Using ΔCt (4.81) and normalized expression (0.03565) of the control group as the baseline for fold-change calculations, statistical robustness was verified by LSD (1.066, $P \leq 0.01$).

The results of normalization of *AdeS* genes expression against the bacterial housekeeping gene*16s rRNA* from 14 isolates and the control are shown in table 4. There were marked differences in the expression of this fold among isolates; above average upregulation was observed in isolates 5 (25.11-fold), 11 (11.96-fold), and 13 (40.50-fold), marked with different superscripts (a, b, c), where statistically different ($P \leq 0.01$). The control isolate displayed baseline expression (1.00-fold), whereas moderate expression was observed in the majority of isolates (2.01-5.39-fold). The statistical significance of the P value (0.0001) indicates that *AdeS* expression is evidently polymorphic among isolates, which may point to differential regulatory responses or genetic engender. Normalization against *16s rRNA* allowed for comparison, and isolates 5, 11, and 13 were outliers with extremely high *adeS* activities.

Table 3: Gene expression of *AdeR* genes compared with the mean of *House-keeping gene* (16srRNA

isolates	***Means Ct of adeR***	***Means Ct of 16s***	***ΔCt (Means Ct of adeR)***	$2^{-\Delta Ct}$	***Experimental isolates/ Control isolates***	***Fold of gene expression***
1	25.28	19.37	5.91	0.01663	0.01663/0.03565	0.47 ±0.06 b
2	25.35	20.9	4.45	0.04575	0.04575/0.03565	1.28 ±0.11 ab
3	25.42	20.02	5.4	0.02368	0.02368/0.03565	0.66 ±0.08 b
4	24.81	20.89	3.92	0.06606	0.06606/0.03565	1.85 ±0.17 ab
5	25.27	20.84	4.43	0.04639	0.04639/0.03565	1.30 ±0.10 ab
6	25.44	21.45	3.99	0.06293	0.06293/0.03565	1.77 ±0.15 ab
7	24.96	20.73	4.23	0.05329	0.05329/0.03565	1.49 ±0.09 ab
8	25.02	21.65	3.37	0.09672	0.09672/0.03565	2.71 ±0.23 a
9	25.02	21.16	3.86	0.06887	0.06887/0.03565	1.93 ±1.18 ab
10	26.05	20.03	6.02	0.01541	0.01541/0.03565	0.43 ±0.05 b
11	25.84	20.82	5.02	0.03082	0.03082/0.03565	0.86 ±0.08 b
12	24.58	20.77	3.81	0.07129	0.07129/0.03565	1.99 ±0.15 ab
13	24.62	21.27	3.35	0.09807	0.09807/0.03565	2.75 ±0.21 a
14	24.9	21.17	3.73	0.07536	0.07536/0.03565	2.11 ±0.18 a
Control	24.58	19.77	4.81	0.03565	0.03565/0.03565	1.00 ±0.00 b
L.S.D. (P.value)	--	--	--	--	--	1.066 ** (0.0298)

Means having with the different letters in same column differed significantly. ** ($P \leq 0.01$).

Table4: Gene expression of *AdeS* genes compared with the mean of *House-keeping gene* (16srRNA)

Isolates	***Means Ct of ades***	***Means Ct of 16s***	***ΔCt (Means Ct of ades)***	***$2^{-\Delta Ct}$***	***experimental isolates/ Control isolates***	***Fold of gene expression***
1	28.59	19.37	9.22	0.00168	0.00168/0.00043	3.89 ±0.27 de
2	30.78	20.9	9.88	0.00106	0.00106/0.00043	2.46 ±0.19 de
3	28.77	20.02	8.75	0.00232	0.00232/0.00043	5.39 ±0.46 d
4	30.09	20.89	9.2	0.00170	0.00170/0.00043	3.94 ±0.32 de
5	27.37	20.84	6.53	0.01082	0.01082/0.00043	25.11 ±3.57 b
6	30.27	21.45	8.82	0.00221	0.00221/0.00043	5.13 ±0.41 d
7	30.42	20.73	9.69	0.00121	0.00121/0.00043	2.81 ±0.16 de
8	31.01	21.65	9.36	0.00152	0.00152/0.00043	3.53 ±0.22 de
9	30.6	21.16	9.44	0.00144	0.00144/0.00043	3.34 ±0.19 de
10	30.2	20.03	10.17	0.00087	0.00087/0.00043	2.01 ±0.14 de
11	28.42	20.82	7.6	0.00515	0.00515/0.00043	11.96 ±0.87 c
12	30.65	20.77	9.88	0.00106	0.00106/0.00043	2.46 ±0.19 de
13	27.11	21.27	5.84	0.01746	0.01746/0.00043	40.50 ±3.92 a
14	30.32	21.17	9.15	0.00176	0.00176/0.00043	4.08 ±0.37 d
Control	19.77	30.95	11.18	0.00043	0.00043/0.00043	1.00 ±0.00 e
L.S.D. (P.value)	--	--	--	--	--	3.902 ** (0.0001)
Means having with the different letters in same column differed significantly. ** (P≤0.01).						

DISCUSSION

In our study, 14 isolates showed positive results for *adeS* genes, whereas 15 isolates showed positive results for *adeR* genes (Figure 1 and 2). The prevalence of *adeR* and *adeS* varies significantly across geographical regions. For instance, a study in Iraq analyzed 32 *Acinetobacter baumannii* isolates and detected *adeR* in 31 (96.8%) and *adeS* in 22 (68.7%) [33]. Similarly, research in Türkiye reported *adeS* in 68% and *adeR* in 96% of clinical strains7, reinforcing the notion that *adeR* is more conserved than *adeS* in many populations [34]. In contrast, a study from Iran found *adeR* and *adeS* in 95% and 60% of isolates, respectively, [35]. The higher *adeS* prevalence (93.3%) in the referenced study may reflect unique environmental or clinical factors, such as prolonged exposure to specific antibiotics like fluoroquinolones or carbapenems, which are known substrates of the AdeABC efflux system [36].

Several studies have elucidated the critical role of mutations in the *AdeRS* two-component regulatory system (*adeR*; *adeS*:) in mediating overexpression of the AdeABC efflux pump, thereby contributing to antimicrobial resistance in *Acinetobacter baumannii*. Sun et al. (2012)[25] identified an insertion within the *adeS* gene that produced a truncated *AdeS* protein, resulting in the constitutive activation of the AdeABC system via *AdeR* interaction. This dysregulation was correlated with a ≥4-fold increase in tigecycline minimum inhibitory concentration (MIC), underscoring the system's direct impact on reducing antibiotic susceptibility[25]. Similarly, clinical isolates harboring double or triple mutations in *adeR* exhibited 2.71–to 2.75-fold upregulation of *adeR* expression, aligning with globally observed resistance mechanisms [37].

Further investigations have linked AdeABC overexpression with fluoroquinolone and β-lactam resistance. Lari et al. [36] reported that 36% of 50 clinical *Acinetobacter* isolates co-harbored *adeR* and *adeS* mutations, with *AdeB* overexpression (3.29–19.79-fold) directly correlating with elevated ciprofloxacin and cefepime MIC[36]. These findings suggest that amino acid substitutions in *AdeR* or *AdeS* disrupt their regulatory functions, leading to efflux pump hyperactivity. Notably, these mutations are disproportionately prevalent in carbapenem-resistant *Acinetobacter baumannii* (CRAB) and MDR strains, particularly in nosocomial environments [38]. Global surveillance data corroborates that *AdeRS* polymorphisms drive efflux-mediated resistance through quantifiable transcriptional changes.

The present study assessed the expression levels of *adeS* in clinical isolates of *Acinetobacter baumannii* compared to a susceptible control isolate using qRT-PCR. These findings highlight the complex regulatory interplay between *adeR* and *adeS*. Although both genes form a two-component regulatory system, the consistently high expression of *adeS* in resistant isolates suggests a more dominant role in efflux pump activation under antibiotic stress. This was consistent with the results reported by Xu et al. (2019)[39], who reported that overexpression of *adeS*, even in the absence of significant *adeR* activation, was sufficient to trigger multidrug efflux activity. The statistical significance of

both *adeS* and *adeR* expression among isolates supports the hypothesis that gene-specific regulation contributes to phenotypic resistance [39]. The heterogeneous expression patterns observed reflect the genomic diversity and adaptability of *Acinetobacter baumannii*, suggesting that resistance in clinical isolates may involve both the canonical and non-canonical regulatory pathways. These findings are in line with previous studies, including Ruzin et al., who reported the widespread distribution of the *AdeRS* operon in over 88% of *Acinetobacter baumannii* clinical strains [40]. Similarly, Atasoy et al. (2016) found a 100% prevalence of *adeR* and *adeS* among MDR isolates, further affirming their integral role in resistance phenotypes[41] .

CONCLUSION

This study elucidated the critical role of the *AdeRS* two-component regulatory system in mediating MDR among *Acinetobacter baumannii* isolates from burn patients and demonstrated the widespread prevalence of the efflux pump regulatory genes *adeR* and *adeS*, with significant overexpression in MDR strains, directly correlating with resistance to multiple antibiotic classes. These findings support the integration of efflux pump inhibitors into therapeutic regimens and underscore the need for genomic surveillance to guide infection control policies. Antimicrobial stewardship and novel combination therapies are critical for counteracting this escalating public health threat.

Ethical approval

This study was conducted in accordance with the ethical rules of medical research at the University of Baghdad / Institute of Genetic Engineering and Biotechnology. Before sampling, the consent of the patient or his companion was taken. The study protocol, subject Information and approval form were reviewed and approved by the Microbiology Laboratory Unit at Ghazi Hariri Hospital for Specialized Surgeries / Medical City accordance with Document No. 46111 dated (4/12/2023) to obtain this approval.

Author Contribution The authors were contributed equally in conceptualized the research, collected data, participated in data analysis and write-up, editing and review.

Competing interests The author declare that they have no competing interests

Funding No funds were received to fulfil this work.

Acknowledgements: We extend our sincere appreciation to the Institute of Genetic Engineering and Biotechnology at the University of Baghdad for their unwavering support. We are also profoundly grateful to the participants who actively contributed to this study and provided invaluable collaboration. Furthermore, we acknowledge the dedication to the staff of the Microbiology Laboratory and the administration of the Martyr Ghazi Hariri Hospital for Specialized Surgeries in the Medical City for the professional support and valuable laboratory advice that helped in the success of this work. Lastly, we recognize the significant contributions of all individuals involved in this research endeavor.

REFERENCES

1. Peleg AY, Seifert H, Paterson DL. Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev. 2008;21(3):538-582.
2. Osman AH, Darkwah S, Kotey FCN, et al. Reservoirs of Nosocomial Pathogens in Intensive Care Units: A Systematic Review. Environ Health Insights. 2024;18:11786302241243240.
3. Ambrosi C, Scribano D, Aleandri M, et al. Acinetobacter baumannii virulence traits: a comparative study of a novel sequence type with other Italian endemic international clones. Front Microbiol. 2017;8:1977.
4. Kyriakidis I, Palabougiouki M, Vasileiou E, et al. Candidemia complicating biliary atresia in an infant with hemoglobinopathy. Turkish Arch Pediatr Pediatr Arşivi. 2019;54(2):129.
5. AL-Taati HRR, Jameel ZJ, Taha RA. Study of Virulence factor of Acinetobacter baumannii and detection of bap gene. Iraqi J Biotechnol. 2018;17(1).
6. Molecular Characteristics of Multidrug Resistant Acinetobacter baumannii Isolated from Baghdad Hospitals. Iraqi J Sci. 2023;56(2B SE-Biology):1394-1399. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/10114.
7. Álvarez-Ainza ML, Fong-Coronado PA, Ruiz-Bustos E, et al. Antibiotic resistance of ESKAPE group-microorganisms in health institutions from Hermosillo and Ciudad Obregón, Sonora, México. Front Cell Infect Microbiol. 2024;14. https://www.frontiersin.org/journals/cellular-and-infection-microbiology/articles/10.3389/fcimb.2024.134809.
8. Ali S, Al-Haideri HH, Al Hishimi AM. Evaluating the Activity of Ultrasound on Biofilm Formation by Acinetobacter baumannii isolated from clinical Specimens. Baghdad Sci J. 2022;19(6 (Suppl.)):1522.
9. Santajit S, Indrawattana N. Mechanisms of antimicrobial resistance in ESKAPE pathogens. Biomed Res Int. 2016;2016.
10. basil AbdulRazzaq A, Shami AM, Ghaima KK. Detection of vanA and vanB genes Among

Vancomycin Resistant Staphylococcus aureus Isolated from Clinical Samples in Baghdad Hospitals. Iraqi J Biotechnol. 2022;21(1).

11. Tacconelli E. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development. 2017.
12. Mahmood SS. The prevalence of integron classes genes among A. Baumannii isolates. Iraqi J Sci. 2022:1955-1960.
13. Hussein N, Al-Mathkhury H, Sabbah M. Imipenem-Resistant Acinetobacter baumannii isolated from patients and hospitals environment in Baghdad. Iraqi J Sci. 2013;54(4):803-812.
14. Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of Acinetobacter baumannii virulence. Nat Rev Microbiol. 2018;16(2):91-102.
15. Skariyachan S, Taskeen N, Ganta M, Venkata Krishna B. Recent perspectives on the virulent factors and treatment options for multidrug-resistant Acinetobacter baumannii. Crit Rev Microbiol. 2019;45(3):315-333.
16. AL-Masoudi KK, AL-Saffar JM, Kendla NJ. Molecular characteristics of multidrug resistant Acinetobacter baumannii isolated from Baghdad Hospitals. Iraqi J Sci. 2015:1394-1399.
17. Hamzah AS. Phylogenetic analysis of cpn60 gene from locally isolated Acinetobacter baumannii. Iraqi J Sci. 2018:9-15.
18. Dehbanipour R, Ghalavand Z. Acinetobacter baumannii: Pathogenesis, virulence factors, novel therapeutic options and mechanisms of resistance to antimicrobial agents with emphasis on tigecycline. J Clin Pharm Ther. 2022;47(11):1875-1884. doi:10.1111/jcpt.13787
19. Wen Y, Ouyang Z, Yu Y, et al. Mechanistic insight into how multidrug resistant Acinetobacter baumannii response regulator AdeR recognizes an intercistronic region. Nucleic Acids Res. 2017;45(16):9773-9787.
20. Yoon EJ, Courvalin P, Grillot-Courvalin C. RND-type efflux pumps in multidrug-resistant clinical isolates of Acinetobacter baumannii: major role for AdeABC overexpression and AdeRS mutations. Antimicrob Agents Chemother. 2013;57(7):2989-2995.
21. Saad S, Melconian A. Cloning of copper resistance gene (copA) that presence in novel genomic island of Acinetobacter baumannii A92. Iraqi J Sci. 2014;55(2B):722-728.
22. Meyer C, Lucaβen K, Gerson S, et al. Contribution of RND-type efflux pumps in reduced susceptibility to biocides in Acinetobacter baumannii. Antibiotics. 2022;11(11):1635.
23. Schindler BD, Frempong-Manso E, DeMarco CE, et al. Analyses of multidrug efflux pump-like proteins encoded on the Staphylococcus aureus chromosome. Antimicrob Agents Chemother. 2015;59(1):747-748.
24. Chang TY, Huang BJ, Sun JR, et al. AdeR protein regulates adeABC expression by binding to a direct-repeat motif in the intercistronic spacer. Microbiol Res. 2016;183:60-67.
25. Sun JR, Perng CL, Chan MC, et al. A truncated AdeS kinase protein generated by IS Aba1 insertion correlates with tigecycline resistance in Acinetobacter baumannii. PLoS One. 2012;7(11):e49534.
26. Monaam Z, kadhum Luti KJ. Evaluation of Chitosan Activity Produced Via Biotransformation of Chitin Against Some Pathogens Associated with Burn Infection in Vitro and in Vivo. Iraqi J Sci. 2024.
27. Organization WH. Antimicrobial Resistance: Global Report on Surveillance. World Health Organization; 2014.
28. O'Neill J. Tackling Drug-Resistance Infections Globally: Final Report and Recommendations. The review on Antimicrobial Resistance Chaired by Jim O'Neill.[Online …. 2016;(May):1-84.
29. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018;18(3):318-327.
30. Sau fheu. Specification of particle movement in a spiral-screw element Isaev Yu. M., Semashkin NM, Kalenkov SA. Sustain Dev. 2015;8(3):234-241.
31. Wayne PA. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. 2011.
32. Huang J, Wang M, Ding H, et al. New Delhi metallo-β-lactamase-1 in carbapenem-resistant Salmonella strain, China. Emerg Infect Dis. 2013;19(12):2049.
33. Faraj AA. Molecular Detection of adeL , adeR and adeS Genes of Acintobacter baumannii Isolated from Different Clinical Samples. raqi J Biotechnol. 2024;2(2):56-61.
34. Terkuran M, Erginkaya Z, Konuray G, et al. Evaluation of Antibiotic Resistance and adeABC, adeR, adeS Efflux Pump Genes among Foodborne and Clinical Acinetobacter spp. in Türkiye. Iran J Public Health. 2022;51(12):2753-2763. doi:10.18502/ijph.v51i12.11466
35. Noori M, Mohsenzadeh B, Bahramian A, Shahi F, Mirzaei H, Khoshnood S. Characterization and frequency of antibiotic resistance related to

membrane porin and efflux pump genes among Acinetobacter baumannii strains obtained from burn patients in Tehran, Iran. J Acute Dis. 2019;8(2):63-66.

36. Lari AR, Ardebili A, Hashemi A. AdeR-AdeS mutations & overexpression of the AdeABC efflux system in ciprofloxacin-resistant Acinetobacter baumannii clinical isolates. Indian J Med Res. 2018;147(4):413-421. doi:10.4103/ijmr.IJMR_644_16
37. Boonsilp S, Homkaew A, Wongsuk T, Thananon K, Oonanant W. Correlation of RND efflux pump expression and AdeRS mutations in tigecycline-resistant Acinetobacter baumannii from Thai clinical isolates. Eur J Microbiol Immunol (Bp). 2024;14(3):280-288. doi:10.1556/1886.2024.00070
38. Ari H, Aydoğan O, Demirci M, Kö ksal Cakirlar F. Distribution of AdeABC efflux system genes in Acinetobacter baumannii isolated from blood cultures of hospitalized patients and their relationship with carbapenem and aminoglycoside resistance. Mediterr J Infect Microbes Antimicrob. 2019;8.
39. Xu CF, Bilya SR, Xu W. adeABC efflux gene in Acinetobacter baumannii. New Microbes New Infect. 2019;30:100549. doi:https://doi.org/10.1016/j.nmni.2019.100549
40. Ruzin A, Keeney D, Bradford PA. AdeABC multidrug efflux pump is associated with decreased susceptibility to tigecycline in Acinetobacter calcoaceticus–Acinetobacter baumannii complex. J Antimicrob Chemother. 2007;59(5):1001-1004.
41. Atasoy AR, Ciftci IH, Petek M, Terzi HA. Investigation of mutations in adeR and adeS gene regions in gentamicine resistant Acinetobacter baumannii isolates. Biotechnol Biotechnol Equip. 2016;30(2):360-367.