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ORIGINAL ARTICLE

Molecular Characterization of Aminoglycoside Resistance Genes aphA1, aphA6, and armA in Clinical Isolates of *Acinetobacter baumannii*

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Key words:
Acinetobacter baumannii, Aminoglycoside resistance, aphA1, aphA6, armA, Multidrug resistance

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ABSTRACT

Background*: Acinetobacter baumannii is a major nosocomial pathogen with increasing multidrug resistance, particularly to aminoglycosides. Aminoglycoside resistance in A. baumannii is primarily mediated by genes encoding aminoglycoside-modifying enzymes (AMEs) such as aphA1 and aphA6, as well as 16S rRNA methylases such as armA.* ***Objective*** *This study aimed to determine the prevalence and expression levels of aphA1, aphA6, and armA in A. baumannii clinical isolates and correlate these with aminoglycoside-resistant phenotypes.* ***Methodology:*** *Two hundred clinical samples were collected from diverse sources (sputum, wounds, burns, urine, and blood). Twenty A. baumannii isolates were identified and confirmed by biochemical tests, VITEK-2, and 16S rRNA PCR. Antibiotic susceptibility and minimum inhibitory concentration (MIC) were assessed using the VITEK 2 system. Gene detection was performed using PCR, and RT-qPCR was used to evaluate the expression levels normalized to 16S rRNA.* ***Results****: The isolates exhibited high resistance to aminoglycosides amikacin (90%), gentamicin, and tobramycin (85%). All isolates were multidrug-resistant (MDR), with 75% classified as extensively drug-resistant (XDR). gene prevalence was aphA1 (45%), aphA6 (65%), and armA (55%). RT-qPCR revealed significant upregulation ($P \leq 0.01$) of these genes in resistant isolates,* ***Conclusion****: This study elucidated the critical role of aminoglycoside resistance genes (aphA1, aphA6, and armA) in mediating resistance phenotypes in A. baumannii.*

INTRODUCTION

Acinetobacter baumannii is a gram-negative opportunistic pathogen that has emerged as a major cause of healthcare-associated infections (HAIs), including pneumonia, bloodstream infections, and wound infections, particularly in intensive care units (ICUs). Its ability to survive in hospital environments, resist desiccation, and acquire antibiotic resistance genes makes it a serious threat to public health [1,2].

Acinetobacter baumannii is commonly found in soil and water environments where it thrives as a natural inhabitant. It is also detected on the skin of healthy individuals, particularly healthcare personnel, and acts as a transient colonizer [3,4]. In hospital settings, *A. baumannii* is highly persistent on surfaces, medical equipment, and moist environments, such as sinks and ventilators, making it a significant cause of healthcare-associated infections. Its ability to survive under harsh conditions, including desiccation and disinfectants, contributes to its spread [5]. Although it rarely causes infections in healthy individuals, it poses a serious threat to immunocompromised patients in clinical settings [6,7]. Among the antibiotics used for treatment, aminoglycosides are potent broad-spectrum antibiotics often used in combination therapies to combat *A. baumannii* infections [8,9].

However, in recent years, these bacteria have become increasingly resistant to aminoglycosides. [10,11]. Increasing resistance to aminoglycosides in *A. baumannii* is a critical concern, particularly because multidrug-resistant (MDR) strains have spread globally [12,13]. Aminoglycosides exert their antibacterial effects by binding to 16S ribosomal RNA (rRNA) within the 30S ribosomal subunit, thereby disrupting protein synthesis. These antibiotics are commonly administered in combination with broad-spectrum β-lactam antibiotics to treat infections caused by Gram-negative bacteria [11,14]. In *Acinetobacter baumannii*, three primary resistance mechanisms against aminoglycosides have been identified: (i) enzymatic modification of aminoglycosides by aminoglycoside-modifying enzymes (AMEs), which reduce their binding affinity; (ii) structural alteration of the 16S rRNA target site via methyltransferase activity; and (iii) decreased

intracellular accumulation due to reduced membrane permeability or increased efflux pump activity [15,16].

Among these, enzymatic modification of hydroxyl (-OH) or amino (-NH_2) groups on aminoglycosides by AMEs is the most prevalent resistance mechanism. AMEs are categorized into three main classes based on their catalytic activity: aminoglycoside acetyltransferases (AAC), phosphotransferases (APH), and nucleotidyltransferases (ANT), the latter also referred to as adenylyltransferases (AAD) [17]. A distinct resistance mechanism involves the production of 16S rRNA methyltransferases encoded by genes such as *armA*. These enzymes modify the aminoglycoside-binding site of the 30S subunit, conferring high-level resistance to all clinically relevant aminoglycosides, including gentamicin, tobramycin, and amikacin. Unlike AMEs that exhibit substrate-specific activity, methyltransferases provide broad-spectrum resistance [18]. This gene is transferable via class 1 integrons and is frequently identified in carbapenem-resistant *A. baumannii* isolates [19].

In Iraq, data on the prevalence of aminoglycoside-modifying enzyme genes and 16S rRNA methyltransferases in *A. baumannii* are limited. Therefore, the objective of this study was to identify the antimicrobial non-susceptibility patterns of *A. baumannii* strains, determine the prevalence and expression levels of aminoglycoside resistance genes (*aphA1*, *aphA6*, and *armA*) in clinical isolates, and assess their correlation with phenotypic resistance to aminoglycosides.

METHODOLOGY

Sample collection and bacterial isolates

Clinical samples were collected from patients of all ages and sexes across multiple departments of Baghdad Teaching Hospitals, including the Burn Center of Medical City and Ghazi Al Hariri Hospital for Surgical Specialties. Samples were obtained from various sources, including wounds, burns, sputum, urine, and blood, between December 15, 2023, and March 15, 2024.

Antibiotic susceptibility test

The VITEK 2 system was used to determine the susceptibility of 20 Acinetobacter baumannii isolates to 15 antibiotics, selected based on the recommendations of the Clinical Laboratory Standards Institute (CLSI, 2023). Susceptibility testing revealed that the isolates were resistant to multiple antibiotics. The minimum inhibitory concentration (MIC) values (in µg/mL) and resistance interpretations were as follows: trimethoprim/sulfamethoxazole (R ≥ 320), levofloxacin (R ≥ 8), ciprofloxacin (R ≥ 4), minocycline (R ≥ 16), meropenem (R ≥ 16), imipenem (R ≥ 16), ceftazidime (R ≥ 64), cefepime (R ≥ 32), ticarcillin/clavulanic acid (R ≥128), piperacillin/tazobactam (R ≥ 128), piperacillin (R ≥ 128), tobramycin (R ≥ 16), gentamicin (R ≥ 16), and amikacin (R ≥ 64). All isolates were susceptible only to colistin, with MIC values indicating susceptibility at S ≤ 0.5 µg/mL. Based on their antimicrobial resistance profiles, the isolates were classified as multidrug-resistant (MDR), extensively drug-resistant (XDR) or pan-drug-resistant (PDR).

Molecular method DNA Extraction and PCR

Genomic DNA was extracted from the bacterial cultures using the EasyPure Bacteria Genomic DNA kit (TRANS/China), which is specifically designed for DNA isolation from diverse biological sources. The extraction process followed a protocol tailored to Gram-negative bacteria. Each gene was identified using a conventional PCR technique that uses specific primers. The bacterial *16S rRNA* and specific aminoglycoside resistance genes (*aphA1*, *aphA6*, and *armA*) were amplified. The *16S rRNA* primers (F: CAGCTCGTGTCGTGAGATGT; R: CGTAAGGGCCATGATGACTT) target a conserved region to generate a 150 bp amplicon, aiding bacterial identification [20]. *aphA1* (623 bp): F (CGAGCATCAAATGAAACTGC) and R (GCGTTGCCAATGATGTTACAG) [21]. *aphA6* (797 bp): F (ATGGAATTGCCCAATATTATTC) and R (TCAATTCAATTCATCAAGTTTTA)[22]. *armA* (315 bp): F (ATTCTGCCTATCCTAATTGG) and R (ACCTATACTTTATCGTCGTC) [11]. These primers were supplied as freeze-dried powders, dissolved in 100 picomoles/µl, and stored in a deep freezer until needed. PCR was performed using a Thermal Cycler Gradient PCR machine (Qiagen, Hilden, Germany). The amplification protocol involved an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at gene-specific temperatures (*16S rRNA*: 50°C, a*phA1*:56°C, *aphA6*:50°C, *armA*: 49°C) for 30 s each, and extension at 72°C for 30 s per cycle, with a final elongation step at 72°C for 10 min to ensure complete product synthesis.

Gene Expression by RT- qPCR (*aphA1*, *aphA6* and *armA*)

This study used *A. baumannii*, and expression of the *16SrRNA* and *armA* genes in clinical isolates of *A. baumannii* was performed using real-time PCR (Qiagen, Germany) following established protocols to ensure accuracy and consistency. Total RNA was extracted using the TransZol Up Plus Kit (TRANS, China) according to the manufacturer's instructions. RNA purity and concentration were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) for subsequent RT-qPCR analysis. The RT-qPCR protocol employed the EasyScript One-Step gDNA Removal and cDNA Synthesis SuperMix Kit to reverse-transcribe the RNA into cDNA. The primers used for RT-PCR were the same as those used for PCR, except for *aphA6* (146bp) F(TTTTCGCTTCACGAGAGACA) R(CGGAAACAGCGTTTTAGAGC) (designed).

SYBR Green, a fluorescent dye attached to amplified DNA, was used to measure the amplification level as indicated by the cycle threshold (Ct) value. For the experiment, the housekeeping gene chosen was the 16S rRNA gene due of its stable expression in the cells studied and under different conditions. Ct values for *aphA1*, *aphA6*, *armA* and reference gene (*16srRNA*), calculated ΔCt and associated 2^-ΔCt (relative expression) values, as well as fold change in gene expression by comparing the resistant isolate group and sensitive isolate as control were included.

Statistical Analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) (2019) to assess the effects of different factors on the study parameters. The LSD-Least-significant difference was used to compare means. The chi-square test was used to compare percentages (0.05 and 0.01 probability in this study) [23].

RESULTS

The distribution of A. baumannii isolates in different clinical samples was evaluated, with data collected and analyzed. A total of 200 clinical samples were collected, and 20 A. baumannii isolates were identified, accounting for 10% of the total samples. Among the 20 isolates, the majority were identified in sputum (n = 7, 35%), followed by wound swabs (n = 5, 25%), burns (n = 4, 20%), urine (n = 2, 10%), and blood (n = 2, 10%) samples. Statistical analysis revealed a significant P-value ($P \leq 0.05$) of 0.0419, indicating differences in the distribution of A. baumannii isolates across the various clinical sample types.

The Gram-Negative Susceptibility card used in the VITEK 2 Compact system was employed to test the susceptibility of 20 Acinetobacter baumannii isolates against 15 antibiotics. The results revealed high levels of resistance to most antibiotics, with resistance rates exceeding 85% for amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, levofloxacin, meropenem, piperacillin, piperacillin/tazobactam, ticarcillin/clavulanic acid, tobramycin, and trimethoprim/sulfamethoxazole.

Notably, colistin was the only antibiotic to which all isolates were fully susceptible (100%), while minocycline demonstrated intermediate efficacy, with 60% of isolates classified as sensitive. Statistical analysis showed a highly significant association between the observed resistance and sensitivity patterns ($P \leq 0.01$).

The current study revealed an alarming rate of antibiotic resistance among A. baumannii isolates, with 15 isolates (75%) classified as extensively drug-resistant (XDR) and all 20 isolates (100%) classified as multidrug-resistant (MDR). No pan-drug-resistant (PDR) isolates were detected. These resistance patterns also showed statistically significant associations ($P \leq 0.01$), emphasizing the severity of antimicrobial resistance in these clinical isolates.

The 16S rRNA gene (150 bp), a genetic marker specific to Acinetobacter baumannii, was amplified by PCR for each DNA sample. The PCR products were analyzed by gel electrophoresis to confirm the presence of the target bands. As shown in Figure 1, the PCR results for the 16S rRNA gene confirmed that all 20 clinical isolates were A. baumannii.

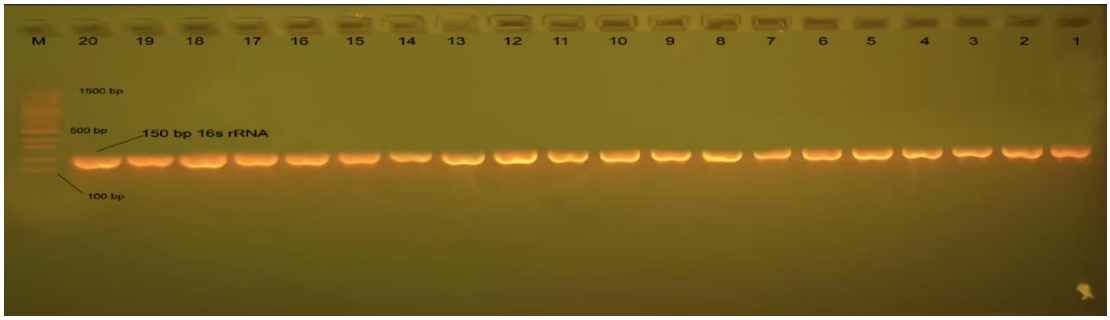

Fig. 1: electrophoresis bands for the 16S rRNA gene (agarose gel electrophoresis, 1% agarose, 70 volts for 1 hrs).

The *aphA1* gene was identified in 45% (9/20) of the MDR *A. baumannii* isolates. PCR amplification yielded a 623 bp product, which was confirmed by agarose gel electrophoresis. Positive results were observed for lanes 1, 3, 5, 6, 8, 9, 15, 18, and 20 (n=9), whereas lanes 2, 4, 7, 10–14, 16, 17, and 19 (n=11) were negative.

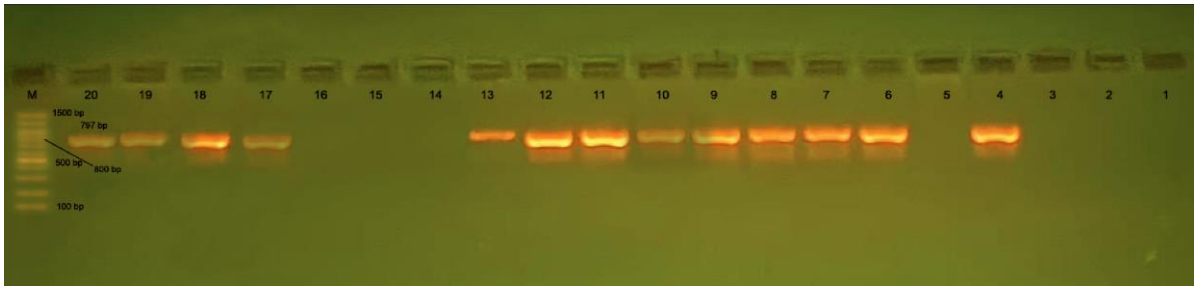

Fig. 2: Detection of aphA6 gene product (797 bp) of *Acinetobacter baumannii* isolates. (agarose gel electrophoresis, 1% agarose, 70 volt for 1 hrs) .Lane (M), DNA Ladder (1500-100 bp). Lanes (4,6,7,8,9,10,11,12,13,17,18,19 and 20) (*n*=13) of *A. baumannii* isolates show positive results. Lanes (1,2,3,5,14,15 and 16) (*n*=7) show negative results.

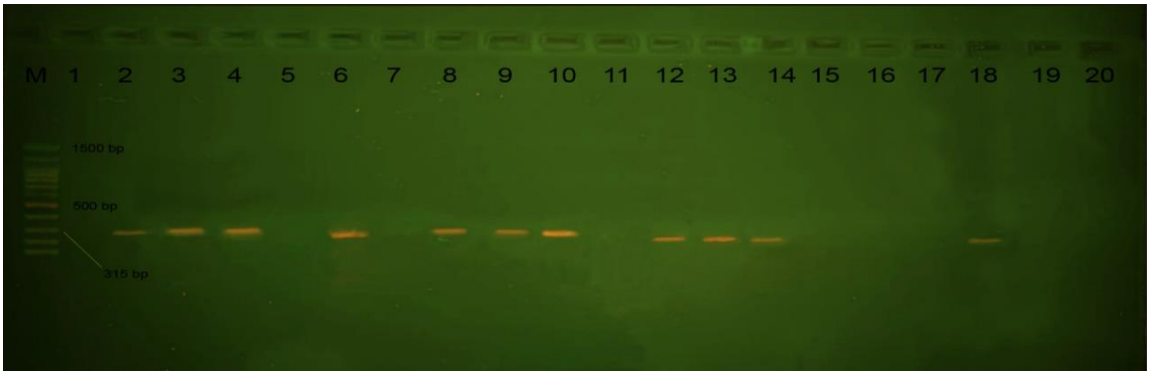

Fig. 3: Electrophoretic Analysis of armA Gene Amplification (315 bp) in *Acinetobacter baumannii* Isolates(agarose gel electrophoresis, 1% agarose, 70 volt for 1 hrs). Lane (M), DNA Ladder (1500-100 bp). Lanes (2,3,4,6,8,9,10,12,13,14,and18) (*n*=11) of *A. baumannii* isolates show positive results. Lanes (1,5,7,11,15,16,17,19 and 20) (*n*=9) show negative results.

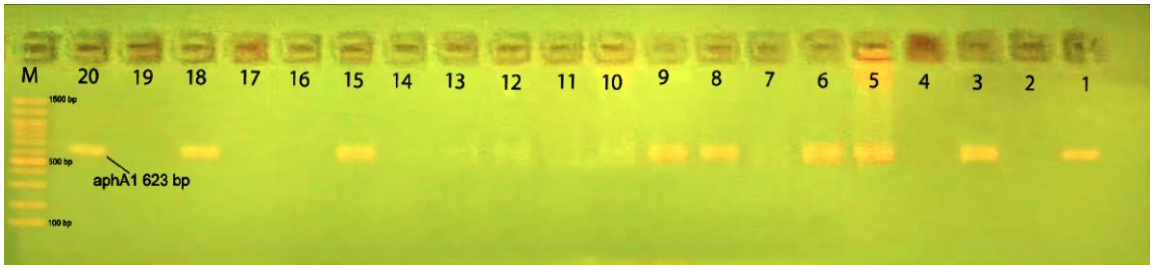

Fig. 4: Agarose Gel Electrophoresis of aphA1 Gene Amplification (623bp) in *Acinetobacter baumannii* Isolates. (agarose gel electrophoresis, 1% agarose, 70 volt for 1 hrs). Lane (M), DNA Ladder (1500-100 bp). Lanes (1,3,5,6,8,9,15,18 and20) (*n*=9) of *A. baumannii* isolates show positive results. Lanes (2,4,7,10,11,12,13,14,16,17, and19) (*n*=11) show negative results.

The expression levels of the target genes *aphA1*, *aphA6*, and *armA*, along with the reference gene *16S rRNA*, were quantified using RT-qPCR. Gene expression in aminoglycoside-resistant isolates was compared to that in sensitive isolates to evaluate the contribution of these genes to resistance. Statistical significance was determined using a threshold of $P \leq 0.01$. The results are presented in Tables 1, 2, and 3.

Table 1: Gene expression of *aphA6* gene compared with the *House-keeping gene* (*16srRNA*).

Isolates	*Ct of aphA6*	*Ct of 16s*	*ΔCt*	$2^{-\Delta Ct}$	*Fold of gene expression*
4	20.5	24.19	-3.69	12.90627	12.38 ±0.65 a
6	23.68	24.27	-0.59	1.50525	1.44 ±0.09 ef
7	20.06	23.22	-3.16	8.93830	8.57 ±0.58 b
8	20.8	22.09	-1.29	2.44528	2.34 ±0.11 de
9	19.85	22.63	-2.78	6.86852	6.59 ±0.37 c
10	23.32	21.84	1.48	0.35849	0.34 ±0.02 f
11	19.73	22.3	-2.57	5.93809	5.69 ±0.36 c
12	19.13	20.99	-1.86	3.63008	3.48 ±0.17 d
13	22.21	20.38	1.83	0.28126	0.26 ±0.03 f
17	20.81	24.64	-3.83	14.22148	13.64 ±0.78 a
19	24.17	24.27	-0.1	1.07177	1.02 ±0.06 ef
20	25.27	22.09	3.18	0.11034	0.10 ±0.02 f
Control	22.58	22.64	-0.06	1.04247	1.0 ±0.00
L.S.D. (P-value)	---	---	---	---	1.289 ** (<0.0001)
Means having with the different letters in same column differed significantly. ** (P≤0.01).					

Table 2: Gene expression of *armA* gene compared with the *House-keeping gene* (*16srRNA*).

Isolates	*Ct of armA*	*Ct of 16s*	*ΔCt*	$2^{-\Delta Ct}$	*Fold of gene expression*
2	35.33	20.84	14.49	0.000043	2.85 ±0.16 de
3	36.29	21.19	15.1	0.000028	1.87 ±0.12 ef
4	34.3	22.16	12.14	0.000222	14.52 ±0.82 a
6	35.79	22.27	13.52	0.000085	5.58 ±0.37 c
8	36.76	22.09	14.67	0.000038	2.51 ±0.13 de
9	37.56	22.63	14.93	0.000032	2.10 ±0.09 ef
10	35.63	20.03	15.6	0.000020	1.32 ±0.07 ef
12	35.6	20.99	14.61	0.000040	2.62 ±0.18 de
13	33.95	21.27	12.68	0.000152	9.99 ±0.72 b
14	35.35	21.17	14.18	0.000054	3.53 ±0.26 d
Control	38.64	22.64	16	0.000015	1.0 ±0.00 f
L.S.D. (P-value)	---	---	---	---	1.278 ** (0.0001)
Means having with the different letters in same column differed significantly. ** (P≤0.01).					

Table 3: Gene expression of *aphA1* gene compared with the *House-keeping gene* (*16srRNA*).

Isolates	*Ct of aphA1*	*Ct of 16s*	*ΔCt*	$2^{-\Delta Ct}$	*Fold of gene expression*
1	24.68	22.19	2.49	0.17801	8.94 ±0.57 b
3	25.88	21.19	4.69	0.03874	1.94 ±0.22 d
5	26.11	20.38	5.73	0.01884	0.94 ±0.08 d
6	24.75	22.27	2.48	0.17924	9.0 ±0.62 b
8	25.22	22.09	3.13	0.11423	5.74 ±0.37 c
9	25.74	22.63	3.11	0.11582	5.82 ±0.42 c
15	25.6	24.27	1.33	0.39777	19.98 ±1.05 a
20	24.61	22.09	2.52	0.17434	8.754 ±0.54 b
Control	28.29	22.64	5.65	0.01992	1.0 ±0.00 d
L.S.D. (P-value)	---	---	---	---	1.502 ** (0.0001)
Means having with the different letters in same column differed significantly. ** (P≤0.01).					

DISCUSSION

The current study revealed an alarming rate of antibiotic resistance in *Acinetobacter baumannii*, with a substantial proportion of isolates classified as extensively drug-resistant (XDR) or multidrug-resistant (MDR). However, no pan-drug-resistant (PDR) isolates were observed. Statistical analysis confirmed that these resistance patterns are significant, underscoring the severity of this public health concern. Minimum inhibitory concentration (MIC) testing showed high resistance rates to aminoglycosides. Specifically, 85% of isolates were resistant to gentamicin and tobramycin (MIC ≥ 16 µg/mL), while 90% were resistant to amikacin (MIC ≥ 64 µg/mL). Only a small proportion of isolates demonstrated susceptibility: 5% to gentamicin, 10% to tobramycin, and 5% to amikacin. These differences were statistically significant (P = 0.0001), indicating a consistent and non-random pattern of resistance.

These findings are consistent with previous reports from different regions in Iraq. In a local study by Raheem and Al-Hasnawy[24], *A. baumannii* isolates from Babylon Province showed moderate resistance to amikacin (55%), complete resistance to tobramycin (100%), and high resistance to gentamicin (80%) . Similarly, Qader[25] reported high resistance rates among clinical isolates in the Kurdistan Region, with 88% resistant to amikacin and 90% to gentamicin. In another study conducted by Ahmed et al. (2024) in Baghdad, resistance levels reached 100% for amikacin and 90% for both tobramycin and gentamicin [26]. Together, these results highlight a consistent regional trend of high-level resistance in *A. baumannii* to commonly used aminoglycoside antibiotics.

In the present study, the *aphA6* gene was identified in 65% of *A.baumannii* isolates. Comparative regional analyses show variable prevalence rates. A study conducted in Iraq reported *aphA6* in 56.25% of *A. baumannii* isolates [27]. In contrast, Egyptian investigations have revealed divergent frequencies. Rizk and Abou El-Khier [28] documented *aphA6* in 26% of clinical isolates from Egyptian hospitals, whereas a 2020 study conducted in a Cairo ICU detected the gene in 81% (64/79) of gentamicin-resistant A. baumannii isolates [29]. Similarly, a 2014 Iranian study identified *aphA6* in 60.46% (52/86) of aminoglycoside-resistant strains [22], However, a 2021 multicenter analysis of 52 *A. baumannii* isolates from diverse clinical specimens (e.g., endotracheal aspirates, blood) reported *aphA6* in only 31.4% (11/35) of resistant strains [30]. These discrepancies may be attributed to differences in local antibiotic stewardship practices, the prevalence of particular clonal lineages, or variability in horizontal gene transfer efficiency [22].

Significant variability in *aphA6* expressions was also observed among isolates in the current study, with fold changes ranging from 0.10 ± 0.02 to 13.64 ± 0.78. Isolate 17 exhibited the highest expression level (13.64 ± 0.78), whereas isolate 20 showed the lowest (0.10 ± 0.02), as presented in Table 1. This variability correlates with kanamycin resistance. Comparative genomic analysis revealed a 99.3% sequence similarity between *A. guillouiae* and *A. baumannii aphA6* proteins, suggesting horizontal gene transfer. Quantitative RT-PCR further confirmed that *A. baumannii* strains expressed *aphA6* at levels 10- to 10^6-fold higher than A. guillouiae, which corresponded to elevated kanamycin MICs (>32 µg/mL in *A. baumannii* vs. ≤8 µg/mL in *A. guillouiae*) [31].

The *armA* gene, identified in 55% of isolates in the current study, was the second most prevalent aminoglycoside resistance determinant. These findings align with those of the regional studies, highlighting the variable prevalence of *armA*. For instance, in Iraq/Babylon, 35% of *A. baumannii* isolates harbored *armA*, correlates with aminoglycoside resistance [24]. In contrast, a 2023 study in Najaf reported a lower *armA* prevalence (11%) among *A. baumannii* clinical isolates [32]. Globally, *armA* has been implicated in MDR and XDR strains, particularly in regions such as Korea, where its association with additional resistance determinants underscores its role in complicating treatment regimens [33].

In support these observations, *armA* was detected in 55% (11/20) of *A. baumannii* isolates in this study (Figure 3). Comparable prevalence rates have been documented elsewhere: 75% (15/20) of global clone 2 (GC2) *A. baumannii* isolates in Singapore carried *armA*, demonstrating resistance to carbapenems, third-generation cephalosporins, fluoroquinolones, and most aminoglycosides [34]. Similarly, studies from Iran and Egypt have reported high *armA* prevalence rates of 90.7% (88/97 GC2 isolates) and 83% (83/100 ICU-derived isolates), respectively [17,35]. The current study also found significantly elevated *armA* expression levels in *A. baumannii* isolates compared to the control group, with fold changes ranging from 1.32 ± 0.07 to 14.52 ± 0.82. Isolate 4 exhibited the highest expression (14.52 ± 0.82), while isolate 10 showed the lowest (1.32 ± 0.07), as shown in Table 2. These results suggest a strong correlation between *armA* overexpression and aminoglycoside resistance. Supporting this, clonal dissemination of sequence type 208 (ST208) and ST455 *A. baumannii* strains in Japan revealed uniform *armA* overexpression, conferring resistance to gentamicin, amikacin, and tobramycin [36]. Similarly, Singaporean isolates carrying *armA* embedded within the AbGRI3 resistance island demonstrated elevated transcript levels, particularly when the gene was associated with intact Tn6180 transposons, correlating with aminoglycoside

MICs exceeding 256 µg/mL [34]. Additionally, ST208 isolates from Tokai University Hospital exhibited a 4.5- to 64-fold increase in *armA* expression due to ISAba1 insertion upstream of the gene, resulting in amikacin MICs ≥512 µg/mL and gentamicin MICs ≥256 µg/mL [37].

Our investigation revealed that 45% (9/20) of *A. baumannii* isolates harbored the *aphA1* gene. These findings contrast with a recent pan-Indian study by Gera et al. (2024), which reported a lower *aphA1* prevalence of 18.1% among clinical isolates [38]. The 45% aphA1 prevalence observed in our study aligns with genomic analyses of multidrug-resistant (MDR) strains in high-burden settings. For example, Khurshid et al. (2020) detected *aphA1* in 28% of Pakistani A. baumannii isolates [39]. However, MDR strains from India demonstrated even higher carriage rates, with 54.7% (35/64) of isolates harboring *aphA1* [40]. Although our reported prevalence (45%, 9/20) was comparatively lower than that of the Indian cohort, the genetic context of *aphA1*, particularly its association with mobile genetic elements such as transposons and integrons, mirrors findings from global clone 2 (GC2) lineages[41]. These elements facilitate horizontal gene transfer, enabling the dissemination of *aphA1* across phylogenetically distinct clones [42].

In our study, *aphA1* displayed significant upregulation in clinical isolates, with fold-changes ranging from 0.94 ± 0.08 to 19.98 ± 1.05. Isolate 15 exhibited peak expression (19.98 ± 1.05), while isolate 5 showed minimal expression (0.94 ± 0.08) Table 3. Notably, *aphA1* is frequently located within mobile genetic elements, such as transposons, and its overexpression correlates with multidrug-resistant phenotypes in *A. baumannii* [42].

Regional disparities are evident in neighboring countries as well. For example, a Pakistani surveillance study of 143 aminoglycoside-resistant *A. baumannii* isolates identified *aphA6* (74.1%), *armA* (28%), and *aphA1* (11.2%) as the predominant resistance determinants [39], whereas Iranian isolates exhibited high frequencies of *armA* (69.8%) and *aphA6* (59.3%)[43]. This heterogeneity in the distribution of resistance genes underscores the influence of regional antimicrobial selection pressures and epidemiological factors.

CONCLUSION

The study elucidated the critical role of aminoglycoside resistance genes (*aphA1*, *aphA6*, and *armA*) in mediating resistance phenotypes in *A. baumannii*. The high prevalence of these genes and their significant upregulation in resistant isolates underscore their contribution to aminoglycoside resistance. Notably, the strong correlation between gene expression levels and elevated MIC values for amikacin, gentamicin, and tobramycin highlights the mechanistic basis of resistance. These findings underscore the critical role of the overexpression of *armA*, *aphA1*, and *aphA6* in mediating aminoglycoside resistance in *A. baumannii*. The association of these genes with mobile genetic elements and insertion sequences highlights the dynamic nature of dissemination and expression of resistance genes.

Competing interests: The author declare that they have no competing interests
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Author Contribution The authors were contributed equally in conceptualized the research, collected data, participated in data analysis and write-up, editing and review.
Ethical approval
This study an observational and cross-sectional investigation conducted in Baghdad Teaching Hospital and the Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad. The study was carried out from December 15, 2023, to March 15, 2024. Ethical approval for the study was obtained from the Scientific Committee of the Biotechnology Department at the Institute of Genetic Engineering and Biotechnology, as well as the Rusafa Health Department, Ministry of Health, Iraq. The research was authorized formally by the Ministry of Health through the Baghdad Health Department, Rusafa, as documented in official letter No. 46112 which dated on December 4, 2023.

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