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ORIGINAL ARTICLE

# Coexistence of genes encoding aminoglycoside modifying enzymes among clinical *Acinetobacter baumannii* isolates in Ahvaz, Southwest Iran

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**ABSTRACT**

Aminoglycosides are widely recommended for treatment of *Acinetobacter baumannii* infections in combination with β-lactams or quinolones. This cross-sectional study was aimed to investigate the coexistence of aminoglycoside modifying enzyme (AME) genes among *A. baumannii* isolates from clinical samples in Ahvaz, Iran. A total of 85 clinical *A. baumannii* isolates typed by ERIC-PCR were investigated for the presence of AME genes, including *ant(3″)-Ia*, *aac(6′)-Ib*, *aac(3′)-Ia*, *ant(2″)-Ia*, and *aph(3′)-VIa* by PCR. The resistance rates to aminoglycoside agents were evaluated by disk diffusion. In this study, 84 out of 85 *A. baumannii* isolates were resistant to at least one of the aminoglycosides and harbored at least one AME gene. The most common gene encoding AMEs was *aph (3′)VIa*, followed by *aac(3′)-Ia*, *ant(3″)-Ia*, *ant (2″)-Ia*, and *aac(6′)-Ib*. The aminoglycoside-resistant genotypes were completely matched to resistant phenotypes to each one of the aminoglycoside agents. There was a clear association between AME gene types and the phenotype of resistance to aminoglycosides with their ERIC-PCR types. Our findings highlight the coexistence of AME genes and clonal dissemination of multiresistant *A. baumannii* in hospital setting.

**KEYWORDS**

aminoglycoside, aminoglycoside modifying enzymes, *Acinetobacter baumannii*

## INTRODUCTION

*Acinetobacter baumannii* is a common nosocomial opportunistic pathogen that can cause severe infections including pneumonia, meningitis, bacteremia, urinary tract infections, surgical wounds, as well as soft tissue infections [1]. In the past two decades, according to some reports, the relatively high prevalence of this microorganism has been reported, especially from intensive care units where patients are treated with broad-spectrum antimicrobial agents [2]. The extensive use of antibiotics in hospitals has been associated with the increasing emergence and dissemination of multidrug-resistant (MDR) *A. baumannii* isolates [3].

Moreover, *A. baumannii* is resistant to most commonly used antibiotics, including aminoglycosides [4]. Aminoglycosides are widely recommended in combination with β-lactams or quinolones for the treatment of infections caused by Gram-negative bacilli, such as *A. baumannii*. Despite side effects and increasing resistance of *A. baumannii* isolates to

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aminoglycoside agents, they yet retain as the valuable therapeutic choices because of their bactericidal activity and their synergy with β-lactams [5]. The most common mechanism of resistance to aminoglycosides in *A. baumannii* strains is enzymatic modifications. Moreover, aminoglycoside modifying enzymes (AMEs) catalyze the modification at −OH or −NH2 groups of the 2-deoxystreptamine nucleus or the sugar moieties and thereby can be acetyltransferases, nucleotidyltranferases, or phosphotransferases. These genes encoding AMEs can be transferred at the molecular level as part of gene cassettes harbored on integrons and at the cellular level through conjugation [6]. Other mechanisms conferring resistance to aminoglycosides are efflux pumps, 16s RNA methylases, substitution of ribosomal proteins, and mutation of 16S rRNA [7].

Previous studies in Iran indicated a relatively high prevalence of AME genes among *A. baumannii* strains [8–11]. However, already, the distribution of these genes is not evaluated in our region, Ahvaz, Iran; hence, in this present work, we investigated the distribution of genes encoding AMEs among *A. baumannii* strains from clinical samples of hospitalized patients in two teaching hospitals in Ahvaz, Iran.

## MATERIALS AND METHODS

### Study design and bacterial isolates

This cross-sectional study was performed on 85 non-duplicate clinical *A. baumannii* isolates. These isolates were obtained from our previous study on different clinical specimens (including blood, urine, trachea, and wound) of hospitalized patients in Taleghani and Imam Khomeini Hospitals in Ahvaz, Iran. The phenotypic identification of these isolates was performed using biochemical tests in our previous study [12]. *In addition*, the molecular identification of *A. baumannii* isolates was performed by the amplification of $bla_{\text{OXA-51-like}}$ gene using the previously described primers by Turton et al. [13]. The *A.* baumannii ATCC19606 was used as the reference strain.

### Antibiotic susceptibility testing

The antibiotic susceptibility of these isolates was determined by disk diffusion method according to the Clinical and Laboratory Standards Institute guidelines [14] in our previous study [12]. In addition, in this study, antibiotic susceptibility to tobramycin was evaluated by disk diffusion method. Briefly, the bacterial suspensions were prepared by suspending isolated colonies from fresh overnight culture plates in sterile normal saline and adjusted to a 0.5 McFarland standard. The following antibiotic impregnated disks were used: imipenem (10 μg), meropenem (10 μg), ceftazidime (30 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), gentamicin (10 μg), amikacin (30 μg), tetracycline (30 μg), piperacillin (100 μg), cefepime (30 μg), piperacillin/tazobactam (100/10 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), colistin (10 μg), ampicillin/sulbactam (10/10 μg), tobramycin (10 μg), ceftriaxone (30 μg), and polymyxin B (300 U). Then, the plates were incubated at 37 °C for 18–24 h, and the diameters of the inhibition zones were measured in millimeters.

### DNA extraction

Total DNA extraction was performed using boiling method. Briefly, the colonies suspected to be *A. baumannii* were suspended in 500 μl of TE buffer (10 mM Tris-HCl and 0.1 mM EDTA, pH 8.0), boiled at 95 °C for 10 min, and centrifuged at 14,000 × *g* for 5 min. The supernatants were collected as DNA templates and were stored at −20 °C for the polymerase chain reaction (PCR) assay [15].

### Enterobacterial repetitive intergenic consensus (ERIC)-PCR typing

Genetic relatedness of *A. baumannii* isolates was evaluated in our previous study [12] using the ERIC-PCR with primers ERIC-F (5′-ATGTAAGCTCCTGGGGATTCAC-3′) and ERIC-R (5′-AAGTAAGTGACTGGGGTGA GCG-3′) [16]. Briefly, the PCR reaction was performed in the final volume of 25 μl containing 1U Taq DNA polymerase, 1.5 mM of $MgCl_2$, 200 μM of dNTPs, 0.5 μM of each primer, 10× PCR buffer, 6.5 μl of template DNA, and distilled water up to a final volume of 25 μl. The amplification process was performed in Mastercycler Nexus Thermal Cycler Gradient (Eppendorf, Hamburg, Germany) with one cycle of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 60 s, annealing at 57 °C for 60 s, extension at 72 °C for 80 s, and a cycle of final extension at 72 °C for 10 min. The amplified products were visualized on 1.5% agarose gel, stained with safe stain. The data analyses were performed using the Gel Compare II software version 6.6 (Applied Math, Sint-Martens-Latem, Belgium). The similarity pattern was calculated using the unweighted-pair group method/the Dice similarity coefficient with a position tolerance of 1%. Isolates with more than 90% similarity were considered as a clonal type.

### Amplification of AME genes by PCR

All isolates without respect to their resistance levels to aminoglycoside agents were subjected to the detection of the genes encoding AMEs [*aac(6′)-Ib+aac(3′)-Ia+ant(2″)-Ia, ant(3″)-Ia+aph(3′)-VIa*] using the specific primers listed in Table I [9, 17]. The amplification reactions were prepared in a final volume of 20 μl containing 1 U of Taq DNA polymerase, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.4 μM of each primer (forward and reverse), 10× PCR buffer, 1.5 μl of template DNA (100 pg concentration) and nuclease free water up to a final 20 μl. The amplification reactions were performed in a thermocycler (Applied Biosystems, USA) with one cycle initial denaturation at 95 °C for 3 min, 35 cycles with a denaturation temperature of 95 °C for 45 s,

**Table I.** Primer sets used in this study

| Gene | Primer (5′–3′) | Amplicon size (bp) | Annealing temperature (°C) | Ref. |
|---|---|---|---|---|
| *Aph(3′)*-F | ATGGAATTGCCCAATATTATTC | 797 | 55 | [9] |
| *aphA(3′)*-R | TCAATTCAATTCATCAAGTTTTA | | | |
| *ant(3″)*-F | ATGAGGGAAGCGGTGATCG | 792 | 52 | [9] |
| *ant(3″)*-R | TTATTTGCCGACTACCTTGGTG | | | |
| *ant(2″)*-F | ATGGACACAACGCAGGTCGC | 534 | 55 | [9] |
| *ant(2″)*-R | TTAGGCCGCATATCGCGACC | | | |
| *aac(3′)-Ia*-F | ATGGGCATCATTCGCACATGTAGG | 456 | 52 | [9] |
| *aac(3′)-Ia*-R | TTAGGTGGCGGTACTTGGGTC | | | |
| *aac(6′)-Ib* | ATGACTGAGCATGACCTTG | 524 | 52 | [17] |
| *aac(6′)-Ib* | AAG GGT TAG GCA ACA CTG | | | |

annealing temperature of 52 °C for *ant(3″)-Ia*, *aac(6′)-Ib* and *aac(3′)-Ia* and 55 °C for *ant(2″)-Ia* and *aph(3′)-VIa* for 45 s, extension at 72 °C for 30 s, and a final extension step at 72 °C for 5 min. PCR products were electrophoresed on 1% agarose gel at 100 V for 60 min and were staining with safe stain.

## RESULTS

### Determination of antibiotic susceptibility

In this study, among 85 *A. baumannii* isolates, resistance to amikacin, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, cefotaxime, gentamicin, tobramycin, imipenem, meropenem, piperacillin/tazobactam, piperacillin, ampicillin/sulbactam, trimethoprim/sulfamethoxazole, and tetracycline was seen in 76 (89.41%), 76 (89.41%), 77 (90.58%), 76 (89.41%), 76 (89.41%), 77 (90.58%), 78(91.8%), 51 (60%), 71 (83.52%), 74 (87.05%), 77 (90.58%), 76 (89.41%), 44 (51.76%), 75 (88.23%), and 49 (57.64%) isolates, respectively. All strains were sensitive to polymyxin B and only two strains were resistant to colistin. Altogether 77 out of these 85 (90.58%) *A. baumannii* isolates were identified as MDR, as reported in our previous study.

### ERIC-PCR analysis

In this study, 85 *A. baumanni* isolates were classified into 21 clone types (CT) and 23 single types (ST) of ERIC-PCR, as reported in our previous study [12]; among which CT16 with 6 isolates was as the most common clone type obtained from this analysis. Table II shows the resistance to aminoglycoside agents, including gentamicin, amikacin, and tobramycin among these isolates with respect to their ERIC-PCR types. According to these results, there was a significant association ($p < 0.05$) between the clone types and the resistance to these aminoglycoside agents.

### Frequency rate of genes encoding AMEs

In this study, 84 out of 85 *A. baumannii* isolates were resistant to at least one of the aminoglycoside agents and these isolates harbored at least one of genes encoding AMEs. The most common gene encoding AMEs was *aph (3′)VIa* (50 isolates, 58.82%), followed by *aac(3′)-Ia* (45 isolates, 52.9%), *ant(3″)-Ia* (38 isolates, 44.7%), *ant (2″)-Ia* (28 isolates, 32.9%), and *aac(6′)-Ib* (27 *i*solates, 31.76%). According to our results, the coexistence of two or more than two gene encoding AMEs was found in 55 (64.70 %) isolates. The distribution patterns of genes encoding AMEs among *A. baumannii* isolates with respect to their substrates are shown in Table II. According to the data, we indicated 18 coexistence pattern of genes encoding AMEs, among which *aph(3′)-VIa*+*aac(3′)-Ia* was the most common in the distribution pattern of genes encoding AMEs, followed by *ant(3″)-Ia*+*aac(6′)-Ib*+*aac(3′)-Ia* (12 isolates), *aph(3′) VIa* (12 isolates), *ant(2″)-Ia* (7 isolates), *ant(2″)-Ia*+*aph(3′)-VIa*+*aac(3′)-Ia* (7 isolates), and *ant-(2″)-Ia*+*ant(3″)-Ia*+*aac(6′)-Ib* (6 isolates).

### Association of between presence of AME genes and ERIC-PCR types

Table III describes the pattern of genes encoding AMEs and the phenotype of resistance to aminoglycoside agents with respect to their ERIC-PCR types. According to our findings, there was a significant association ($p < 0.05$) between each one of genes encoding AMEs and the phenotype of resistance to aminoglycosides with their ERIC-PCR types.

**Table II.** The distribution profile of AMEs among *A. baumannii* isolates with respect to their substrate

| Profile of AME genes | Substrate | Type | *N* (%) |
|---|---|---|---|
| *ant(2″)-Ia+aph(3′)-VIa+aac(3′)-Ia* | G-A-T | CT01 and CT11 | 7 (8.23) |
| *ant(3″)-Ia+aac(6′)-Ib+aac(3′)-Ia* | G-A-T | CT02, CT12, CT13, and CT14 | 12 (14.11) |
| *ant(2″)-Ia+ant(3″)Ia* | G-T | CT03 | 2 (2.35) |
| *aph(3′)-VIa* | A-G | CT04, CT08, CT18, ST02, ST07, ST21, ST22, and ST23 | 12 (14.11) |
| *ant(3″)-Ia* | NOT | ST04 | 1 (1.17) |
| *aac(6′)-Ib+aac(3′)-Ia* | G-A-T | CT05 and ST09 | 4 (4.7) |
| *aph(3′)-VIa+aac(3)-Ia* | A-G | CT06, CT10, ST05, ST06, ST16, ST17, ST18, ST19, and ST20 | 13 (15.29) |
| *ant(2″)-Ia+ant(3″)-Ia+aac(6′)-Ib* | G-A-T | CT07, CT09, and ST08 | 6 (7.05) |
| *ant(2″)-Ia+ant(3″)-Ia+aac(3′)-Ia* | G-A | CT19 | 3 (3.52) |
| *ant(2″)-Ia+aph(3′)-VIa* | G-A-T | CT15 | 5 (5.88) |
| *aac(3′)-Ia* | G | ST10 | 1 (1.17) |
| *ant(3″)-Ia+aac(6′)-Ib* | A-T | CT16, | 5 (5.88) |
| *ant(2″)Ia+ant(3″)-Ia+aph(3′)-VIa* | G-A-T | CT17 | 4 (4.7) |
| *ant(2″)-Ia* | G-T | CT20, ST01, ST11, ST12, ST13, and ST15 | 7 (8.23) |
| *ant(3″)-Ia+aph(3′)-VIa* | G-A | CT21 and ST14 | 3 (3.52) |
| *ant(3″)-Ia+aph(3′)-VIa+aac(6′)-Ib* | G-A-T | ST03 | 1 (1.17) |
| *ant(3′)-Ia* | NOT | ST04 | 1 (1.17) |
| *aac(3′)-Ia* | G | ST11 | 1 (1.17) |

*Note:* AME: aminoglycoside modifying enzyme; G: gentamicin; A: amikacin; T: tobramycin; F: forward; R: reverse.

## DISCUSSION

Multidrug resistance in *A. baumannii* is a global challenge due to the lack of effective treatment options [18]. Aminoglycoside agents have been considered as one of the most important treatment options of infections caused by *A. baumannii* [19]. However, the antibiotic resistance rates to aminoglycoside agents are increasing among *A. baumannii* isolates that resulted in the reduction of the efficacy of these agents [20]. In this study, the rate of antibiotic resistance to tobramycin was lower than amikacin and gentamicin. Hence, it seems that tobramycin is the most effective aminoglycoside agent for the treatment of *A. baumannii* infections. In agreement with our results, several studies also confirmed the higher susceptibility of tobramycin rather than two other aminoglycoside agents in *A. baumannii* isolates [9, 21, 22].

In this study, we focused on the detection of genes encoding AMEs as the most common mechanism of resistance to aminoglycosides [6]. In addition, we indicated that all aminoglycoside-resistant isolates harbored at least one AME gene.

Moreover, the most common gene encoding AMEs was *aph (3′)VIa*. Similar to our studies, there are many researches that have reported the distribution of genes encoding AMEs among *A. baumannii* [9, 22–24]. In addition, our findings are similar to the studies of Aghazadeh et al. [22] and Moniri et al. [20] who reported the *aph (3′)VIa* gene as the most predominant gene encoding AMEs in the majority of aminoglycoside-resistant *A. baumannii* strains. However, some other researchers indicated the higher prevalence of other genes encoding AMEs rather than *aph (3′)VIa* [9, 11, 23–25]. These differences in the distribution patterns of AMEs may be due to the diversity in the dissemination of one or more clonal types in a region.

In this study, the association of each AME gene with resistance to a special aminoglycoside agent was not evaluated by statistical analysis because an *A. baumannii* strain may harbor simultaneously several genes encoding AMEs for

**Table III.** Pattern of genes encoding AMEs and the phenotype of resistance to aminoglycoside agents with respect to their ERIC-PCR types

| Strain | Type | G | A | T | Genes |
|---|---|---|---|---|---|
| SF01 | ST01 | S | R | R | *ant(3″)-Ia* and *aph(3′)-VIa* |
| SF02 | CT01 | R | R | R | *ant(2″)-Ia*, *aph(3″)-VIa*, and *aac(3′)-Ia* |
| SF03 | CT01 | R | R | R | *ant(2″)-Ia*, *aph(3′)-VIa*, and *aac(3′)-Ia* |
| SF04 | ST02 | R | R | S | *aph(3′)-VIa* |
| SF05 | ST03 | R | R | R | *ant(3″)-Ia*, *aph(3)-VIa*, and *aac(6)-Ib* |
| SF06 | CT02 | R | R | R | *ant(3″)-Ia*, *aac(3)-Ia*, and *aac(6)-Ib* |
| SF07 | CT02 | R | R | R | *ant(3″)-Ia*, *aac(3)-Ia*, and *aac(6)-Ib* |
| SF08 | CT02 | R | R | R | *ant(3″)-Ia*, *aac(3)-Ia*, and *aac(6)-Ib* |
| SF09 | ST04 | S | S | S | *ant(3″)-Ia* |
| SF10 | CT03 | R | S | R | *ant(2)-Ia* and *ant(3″)-Ia* |
| SF11 | CT03 | R | S | R | *ant(2)-Ia* and *ant(3″)-Ia* |
| SF12 | ST05 | R | R | S | *aph(3)-VIa*, and *aac(3)-Ia* |
| SF13 | ST06 | R | R | S | *aph(3)-VIa*, and *aac(3)-Ia* |
| SF14 | CT04 | R | R | S | *aph(3)-VIa* |
| SF15 | CT04 | R | R | S | *aph(3)-VIa* |
| SF16 | CT05 | R | R | R | *aac(3)-Ia* and *aac(6)-Ib* |
| SF17 | CT05 | R | R | R | *aac(3)-Ia* and *aac(6)-Ib* |
| SF18 | CT05 | R | R | R | *aac(3)-Ia* and *aac(6)-Ib* |
| SF19 | CT06 | R | R | S | *aph(3)-VIa* and *aac(3)-Ia* |
| SF20 | CT06 | R | R | S | *aph(3)-VIa* and *aac(3)-Ia* |
| SF21 | CT06 | R | R | S | *aph(3)-VIa* and *aac(3)-Ia* |
| SF22 | CT07 | R | R | R | *ant(2)-Ia*, *ant(3″)-Ia*, and *aac(6)-Ib* |
| SF23 | CT07 | R | R | R | *ant(2″)-Ia*, *ant(3″)-Ia*, and *aac(6)-Ib* |
| SF24 | CT08 | R | R | S | *aph(3′)-VIa* |
| SF25 | CT08 | R | R | S | *aph(3′)-VIa* |
| SF26 | ST07 | R | R | S | *aph(3′)-VIa* |
| SF27 | CT09 | R | R | R | *ant(3″)-Ia*, *ant(2″)-Ia*, and *aac(6′)-Ib* |
| SF28 | CT09 | R | R | R | *ant(3″)-Ia*, *ant(2″)-Ia*, and *aac(6′)-Ib* |
| SF29 | ST08 | R | R | R | *ant(3″)-Ia*, *ant(2″)-Ia*, and *aac(6′)-Ib* |
| SF30 | ST09 | R | R | R | *aac(3′)-Ia* and *aac(6′)I-b* |
| SF31 | CT10 | R | R | S | *aph(3′)-VIa* and *aac(3′)-Ia* |
| SF32 | CT10 | R | R | S | *aph(3′)-VIa* and *aac(3′)-Ia* |
| SF33 | ST10 | R | R | S | *aph(3′)-VIa* and *aac(3′)-Ia* |

*(Continued)*

**Table III.** Pattern of genes encoding AMEs and the phenotype of resistance to aminoglycoside agents with respect to their ERIC-PCR types *(Continued)*

| Strain | Type | G | A | T | Genes |
|---|---|---|---|---|---|
| SF34 | ST11 | R | S | S | *aac(3′)-Ia* |
| SF35 | ST12 | R | S | R | *ant(2″)-Ia* |
| SF36 | CT11 | R | R | R | *ant(2″)-Ia, aph(3′)-VIa,* and *aac(3′)-Ia* |
| SF37 | CT11 | R | R | R | *ant(2″)-Ia, aph(3′)-VIa,* and *aac(3′)-Ia* |
| SF38 | CT11 | R | R | R | *ant(2″)-Ia, aph(3′)-VIa,* and *aac(3)-Ia* |
| SF39 | CT11 | R | R | R | *ant(2″)-Ia, aph(3′)-VIa,* and *aac(3′)-Ia* |
| SF40 | CT11 | R | R | R | *ant(2″)-Ia , aph(3′)-VIa,* and *aac(3′)-Ia* |
| SF41 | CT12 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF42 | CT12 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF43 | CT13 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF44 | CT13 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF45 | CT14 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF46 | CT14 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF47 | CT14 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF48 | CT14 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF49 | CT14 | R | R | R | *aac(3′)-Ia, aac(6′)-Ib,* and *ant(3″)-Ia* |
| SF50 | ST13 | R | R | R | *ant(2″)-Ia* |
| SF51 | ST14 | R | R | S | *ant(3″)-Ia* and *aph(3′)-VIa* |
| SF52 | ST15 | R | S | R | *ant(2″)-Ia* |
| SF53 | CT15 | R | R | R | *ant(2″)-Ia* and *aph(3′)-VIa* |
| SF54 | CT15 | R | R | R | *ant(2″)-Ia* and *aph(3′)-VIa* |
| SF55 | CT15 | R | R | R | *ant(2″)-Ia* and *aph(3′)-VIa* |
| SF56 | CT15 | R | R | R | *ant(2″)-Ia* and *aph(3′)-VIa* |
| SF57 | CT16 | R | R | R | *ant(2″)-Ia* and *aph(3′)-VIa* |
| SF58 | CT16 | S | R | R | *ant(3″)-Ia* and *aac(6′)-Ib* |
| SF59 | CT16 | S | R | R | *ant(3″)-Ia* and *aac(6′)-Ib* |
| SF60 | CT16 | S | R | R | *ant(3″)-Ia* and *aac(6′)-Ib* |
| SF61 | CT16 | S | R | R | *ant(3″)-Ia* and *aac(6′)-Ib* |
| SF62 | CT16 | S | R | R | *ant(3″)-Ia* and *aac(6′)-Ib* |
| SF63 | CT17 | R | R | R | *ant(2″)-Ia, aph(3′)-VIa, ant(3″)-Ia,* and *aac(3′)-Ia* |
| SF64 | CT17 | R | R | R | *ant(2″)-Ia, aph(3′)-VIa, ant(3″)-Ia,* and *aac(3′)-Ia* |
| SF65 | CT17 | R | R | R | *ant(2″)-Ia, aph(3′)-VIa, ant(3″)-Ia,* and *aac(3′)-Ia* |

**Table III.** Pattern of genes encoding AMEs and the phenotype of resistance to aminoglycoside agents with respect to their ERIC-PCR types *(Continued)*

| Strain | Type | G | A | T | Genes |
|---|---|---|---|---|---|
| SF66 | CT17 | R | R | R | *ant(2″) -Ia, aph(3′)-VIa, ant(3″)-Ia,* and *aac(3′)-Ia* |
| SF67 | ST16 | R | R | S | *aph(3′)-VIa* and *aac(3′)-Ia* |
| SF68 | CT18 | R | R | S | *aph(3′)-VIa* |
| SF69 | CT18 | R | R | S | *aph(3′)-VIa* |
| SF70 | CT18 | R | R | S | *aph(3′)-VIa* |
| SF71 | CT19 | R | R | S | *aph(3′)-VIa, ant(3″)-Ia,* and *aac(3′)-Ia* |
| SF72 | CT19 | R | R | S | *aph(3′)-VIa, ant(3″)-Ia,* and *aac(3′)-Ia* |
| SF73 | CT19 | R | R | S | *aph(3′)-VIa, ant(3″)-Ia,* and *aac(3′)-Ia* |
| SF74 | CT19 | R | R | S | *aph(3′)-VIa, ant(3″)-Ia,* and *aac(3′)-Ia* |
| SF75 | ST17 | R | R | S | *aph(3′)-VIa* and *aac(3′)-Ia* |
| SF76 | ST18 | R | R | S | *aph(3′)-VIa* and *aac(3′)-Ia* |
| SF77 | ST19 | R | R | S | *aph(3′)-VIa* and *aac(3′)-Ia* |
| SF78 | ST20 | R | R | S | *aph(3′)-VIa* and *aac(3′)-Ia* |
| SF79 | CT20 | R | S | R | *ant(2″)-Ia* |
| SF80 | CT20 | R | S | R | *ant(2″)-Ia* |
| SF81 | CT21 | R | R | S | *aph(3′)-VIa* and *ant(3″)-Ia* |
| SF82 | CT21 | R | R | S | *aph(3′)-VIa* and *ant(3″)-Ia* |
| SF83 | ST21 | R | R | S | *aph(3′)-Via* |
| SF84 | ST22 | R | R | S | *aph(3′)-Via* |
| SF85 | ST23 | R | R | S | *aph(3′)-VIa* |

*Note:* G: gentamicin; A: amikacin; T: tobramycin; ERIC-PCR: enterobacterial repetitive intergenic consensus–polymerase chain reaction; AME: aminoglycoside modifying enzyme; R: resistant; S: susceptible.

the modification of one special aminoglycoside agent. Hence, it is not clear which one of these genes encoding AMEs had modified the special aminoglycoside agent.

On the other hand, according to our results, the aminoglycoside-resistant genotypes were completely matched to resistant phenotypes to each one of these aminoglycoside agents in all *A. baumannii* isolates. The findings are similar to those reported by Aghazadeh et al. [22], indicating that the AMEs had the effective role in conferring resistance to aminoglycosides.

According to the results obtained from this study, the coexistence of two or more than two genes encoding AMEs was found among 64.70% of isolates, resulting in the modification of several aminoglycoside agents simultaneously. Moreover, around 50% of *A. baumannii* isolates harbored one of the gene profiles of *aph(3′)-Via+aac(3′)-Ia+ant(3″)-Ia+aac(6′)-Ib+aac(3′)-Ia* or *aph(3″)-VIa*. In parallel with our findings, Aghazadeh et al. [22], Wang et al. [23], and Nie et al. [24] also indicated the coexistence of genes encoding AMEs in *A. baumannii* isolates. Moreover, Wang et al. [23] and Nie et al. [24] indicated that the gene profile of *ant(2″)-Ia+aac(6′)-Ib+aac(3)-Ia* and *aac(3)-I+aac(6′)-Ib+ant(3″)-I+armA* was the most predominant gene profile, respectively.

In the past few decades, molecular typing methods have become the beneficial tools for characterizing taxonomic and phylogenetic properties of infectious agents in epidemiological studies [26]. ERIC-PCR is a rapid and plausible typing method for the differentiation of the genetic variations and the identification of the clonal relatedness of the bacterial isolates [27]. According to the results obtained from ERIC-PCR typing of *A. baumannii* isolates, we found that the isolates belonging to a same clone type had similar patterns

in genes encoding AMEs and aminoglycoside resistance phenotype, indicating the clonal dissemination of genes encoding AMEs among *A. baumannii* isolates.

## CONCLUSIONS

Our results indicated the high prevalence of resistance to aminoglycoside agents, especially gentamicin. All aminoglycoside-resistant *A. baumannii* isolates harbored at least one AME genes that were completely matched with resistant phenotypes to each one of these aminoglycoside agents. These findings highlight that the coexistence of genes encoding AMEs had the effective role in conferring resistance to aminoglycosides and their clonal dissemination can be a serious concern in hospital setting.

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All authors contributed to interpretation of the data, critically revised the article, and approved the final version of the manuscript.

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