Molecular survey of aminoglycoside-resistant Acinetobacter baumannii isolated from tertiary hospitals in Qazvin, Iran

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Abstract

Aminoglycoside-modifying enzymes (AMEs) and 16S rRNA methylases (16S RMTase) are two main resistance mechanisms against aminoglycosides. This study aimed to evaluate the frequency of AMEs and 16S rRNA methylase genes among aminoglycoside non-susceptible Acinetobacter baumannii isolates and to assess their clonal relationship using repetitive extragenic palindromic-PCR (rep-PCR). In this cross-sectional study, a total of 192 A. baumannii isolates were collected from the patients hospitalized in Qazvin, Iran (January 2016 to January 2018). Identification of isolates was performed by standard laboratory methods and API 20E strips. Antimicrobial susceptibility was determined by Kirby-Bauer method followed by examination of the genes encoding the AMEs and 16S RMTase by PCR and sequencing methods. The clonal relationship of isolates was carried out by rep-PCR. In total, 98.4% of isolates were non-susceptible to aminoglycosides, 98.4%, 97.9% and 83.9% of isolates were found to be non-susceptible against gentamicin, tobramycin and amikacin, respectively. The frequencies of aph(3’)-VI, aac(6’)-Ib, aac(3)-II, aph(3’)-Ia and armA genes were 59.3%, 39.2%, 39.2%, 31.7% and 69.8%, respectively, either alone or in combination. Rep-PCR results showed that the aminoglycoside non-susceptible isolates belonged to three distinct clones: A (79.4%), B (17.5%) and C (3.2%). The findings of this study showed a high frequency for AMEs with the emergence of armA genes among the aminoglycoside non-susceptible A. baumannii isolates. Rational administration of aminoglycosides as well as using an appropriate infection control policy may reduce the presence of resistance to antibiotics in medical centres.

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Keywords: 16S rRNA methylases, Acinetobacter baumannii, aminoglycoside-modifying enzymes, repetitive extragenic palindromic-PCR

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Introduction

Acinetobacter baumannii is a clinically important Gram-negative pathogen in medical centres. This bacterium is responsible for various types of nosocomial infections including pneumonia, bacteraemia, surgical site infections, and urinary tract infections [1]. In recent years, the emergence of multidrug resistance to antibiotics has become a major clinical concern for physicians. This problem leads to serious limitations in the treatment of patients infected with these pathogens, and to increased morbidity and mortality [2,3]. Aminoglycosides are the most frequently used antibiotic agents among topically applied antibiotics in the treatment of infections caused by Gram-negative bacteria. Combining an aminoglycoside with a β-lactam is considered to be more effective treatment against infections caused by Gram-negative bacteria [4]. These antibiotics block protein synthesis in the bacterium by binding to 30S ribosome and eventually lead to bacterial death [5]. Indiscriminate use of these antibiotics increases antibiotic resistance in bacteria and makes the therapy ineffective [6]. Resistance to aminoglycosides may occur based on the following mechanisms: (a) drug inactivation using aminoglycoside-modifying enzymes (AMEs), (b) ribosomal binding site alterations, (c) reduction of antibiotic enzyme regulation by down-regulation of porin genes; and (d)
outer membrane proteins (i.e. efflux transport systems) [6,7]. Among these mechanisms, enzymatic modification is one of the most common types of aminoglycoside resistance mechanism among Gram-negative bacteria [7]. Three main classes of AMEs are known: O-phosphotransferases, which catalyse ATP-dependent phosphorylation of a hydroxyl group; N-acetyltransferases (AAC), which catalyse acetyl coenzyme A-dependent acetylation of an amino group; and O-acytetyltransferases, which catalyse ATP-dependent acetylation of hydroxyl groups [8,9]. Acetyl group transfer of acetyl coenzyme A to an amine which catalyse ATP-dependent adenylation of hydroxyl groups and transferases (AAC), which catalyse acetyl coenzyme A-dependent phosphorylation of a hydroxyl group; N-acetylation of an amino group; and transferases (AAC), which catalyse acetyl coenzyme A-dependent phosphorylation of a hydroxyl group [10].

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Materials and methods

Study design
In this cross-sectional study, a total of 192 A. baumannii isolates were obtained from different clinical specimens collected from two major hospitals of Qazvin, Qazvin province, Iran, during January 2016 to January 2018. Each clinical specimen was collected from one patient. These specimens, including respiratory secretions (sputum, trachea and bronchoalveolar lavage), urine, blood and wounds, were taken from the patients admitted to intensive care units, and to internal, infectious diseases, neurosurgery, and surgery wards. Written informed consent was obtained from all participants included in this trial. The study was approved by the ethics committee of Qazvin University of Medical Sciences (IR.QUMS.REC.1396.775).

Isolate identification
All bacterial isolates were identified by standard laboratory methods including Gram-staining, oxidase and catalase tests (Merck, Darmstadt, Germany), motility, oxidative/fermentative tests and growth ability at 37°C and 42°C [25]. Species identity was confirmed by amplification of gltA (encoding species specific citrate synthase) and bltOXA-51-like genes as described previously [26,27]. The positive control strain used in our experiments was A. baumannii American Type Culture Collection (ATCC) 19606. The A. baumannii isolates were stored at −70°C in tryptase soy broth with 20% glycerol until used for study. Isolates were subcultured before tests.

Antimicrobial susceptibility testing
Antimicrobial susceptibility testing was performed using the Kirby–Bauer disc diffusion method against the following antibiotics according to the Clinical and Laboratory Standards Institute guidelines [28]. Microbial susceptibility test was conducted using the following antimicrobials: gentamicin (10 μg), tobramycin (10 μg), amikacin (30 μg), imipenem (10 μg), meropenem (10 μg), ciprofloxacin (5 μg), cefazidime (30 μg), piperacillin/tazobactam (100/10 μg), ampicillin/sulbactam (10/10 μg) and levofloxacin (5 μg) (Mast Group Ltd., Bootle Merseyside, UK). Acinetobacter baumannii ATCC 19606 was used as the quality control strain in antimicrobial susceptibility testing.

Detection of AMEs and 16S-RMTases genes by PCR and sequencing
Isolates that were non-susceptible to at least one of the aminoglycosides were selected for PCR to detect the genes...
encoding AMEs (aac(6')-Ib, aac(3)-II, aac(3)-Ia, ant(2')-Ia, ant(4')-Ib, ant(4')-IIIa, aph(3')-IIIa, aph(3')-VIa, aph(3')-Ia) and 16S RMTase (armA, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF and rmpA) using the specific primers listed in Table 1 [12,29–37]. PCR amplification was performed in a thermocycler (Applied Biosystems, Foster City, CA, USA) as follows: first initial denaturation at 96°C for 8 minutes and then 35 cycles of 96°C for 1 minute, annealing temperature for each primer 1 minute, 72°C for 1 minute; and a final extension step at 72°C for 10 minutes. Amplification reaction was prepared in a total volume of 25 μL containing 1 U of Taq DNA polymerase 2X Master Mix with 1.5 mM MgCl2 (Ampliqon, Odense, Denmark), 0.5 μL forward primer, 0.5 μL reverse primer, 9 μL nuclease-free water and 2.5 μL DNA template (50 pg concentration). Amplification products were electrophoresed on 1% agarose gel at 100 volts for 40 minutes. The gel was stained with ethidium bromide solution and further visualized in a gel documentation system (Uvitec, Cambridge, City, CA, USA) as follows:

1. Initial denaturation at 95°C for 5 minutes followed by 30 cycles at 94°C for 1 minute, annealing at 45°C for 1 minute and an extension step at 72°C for 2 minutes. Eventually, a final extension step at 72°C for 16 minutes was applied. Amplification products were electrophoresed on 1.2% agarose gel, stained with ethidium bromide. Similar patterns of rep-PCR profile (up to two-band difference) were considered to be related to the same DNA groups [38].

**Statistical analysis**

Statistical package for the social sciences (SPSS) version 25.0 software (IBM, Armonk, NY, USA) with descriptive statistics including frequencies/cross-tabulation of the results of micro-biost tests, clinical findings and demographic characteristics were used to analyse the data.

**Results**

During the period of the study, 192 isolates were isolated as *A. baumannii* based on standard biochemical and microbiological methods and amplification of *gltA* and *bla*OXA-51-like genes. The

| Target genes | Primer sequence (5’–3’) | Annealing temperature (°C) | Product size (bp) |
|--------------|-------------------------|-----------------------------|-------------------|
| aac(6')-Ib   | TTGCGATGCTCTATGAGTGGCTA | 55                          | 482               |
| aac(6')-Ib   | CTCGAATGTCGGCGGTGT    | 57                          | 370               |
| aac(3)-II    | TGGAAACGGTACGGAGCTCTT  | 57                          | 370               |
| aac(3)-Ia    | ATGGGCACTCATATGACATGAG | 57                          | 370               |
| ant(2')-Ia   | TTAGATGCGTCATCTGGTCT  | 57                          | 370               |
| ant(2')-Ia   | TTGACGCAACAGCAGCTGC   | 56                          | 535               |
| ant(2')-Ia   | TTAGCGCGATATGCCGCCACC | 59                          | 364               |
| ant(2')-Ia   | TACCTGCGGGGCGTCGAG    | 59                          | 364               |
| ant(2')-Ia   | CACCGGCTGGAACAGCGGAA  | 57                          | 294               |
| ant(2')-Ia   | GTAAGATGACAGGATCAGAATCT  | 57                          | 294               |
| ant(2')-Ia   | ATGGAATTGCGCAATATTATT | 54                          | 780               |
| ant(2')-Ia   | TCAATTCAATCCATCAAGTCT | 53                          | 624               |
| armA-A       | ATTTGCGCTTACCTAATGG   | 50                          | 315               |
| armA-A       | ACCATATACCTAATCGCTC   | 50                          | 315               |
| armA-A       | CGAGGAATAGCAACAGCAAAAG | 50                          | 711               |
| armC-R       | ATCCGAAACATCTCCACACT  | 59                          | 635               |
| armD-F       | TTTGCTTCCATGCGCTGCC   | 59                          | 635               |
| armF-F       | CGGACCGGGATTGGAAGCG   | 55                          | 401               |
| armD-R       | CGGAAAAATGCGAGGAGG    | 50                          | 640               |
| armF-F       | GCCATTACGCGACAAATCG   | 50                          | 640               |
| armF-F       | GATATGCGGCAAAAACTAC  | 55                          | 173               |
| armF-F       | GCCTTCTCGGGGCGGTGATA  | 55                          | 173               |
| armF-F       | ATGCAATTGCCCGCTGCTAT  | 55                          | 173               |
| armF-F       | ATGAATGGATGAAATGTTGCC | 50                          | 818               |
| armF-F       | TGAATGATTCTTTTCACTTCG | 50                          | 818               |
| armF-F       | GCCATACGAAAAACGAGG    | 55                          | 589               |

| **TABLE 1. Sequences of primers used in this study** | **Annealing temperature (°C)** | **Product size (bp)** |
|---------------------------------------------------|--------------------------------|----------------------|
| **Target genes**                                  | **Primer sequence (5’–3’)** | **Annealing temperature (°C)** | **Product size (bp)** |
| aac(6')-Ib -F                                    | TTGCGATGCTCTATGAGTGGCTA     | 55                      | 482                      |
| aac(6')-Ib -R                                    | CTCGAATGTCGGCGGTGT          | 57                      | 370                      |
| aac(3)-II -F                                     | TGGAAACGGTACGGAGCTCTT       | 57                      | 370                      |
| aac(3)-Ia -F                                     | ATGGGCACTCATATGACATGAG      | 57                      | 370                      |
| ant(2')-Ia -F                                    | TTAGATGCGTCATCTGGTCT        | 57                      | 370                      |
| ant(2')-Ia -F                                    | TTGACGCAACAGCAGCTGC         | 56                      | 535                      |
| ant(2')-Ia -F                                    | TTAGCGCGATATGCCGCCACC       | 59                      | 364                      |
| ant(2')-Ia -F                                    | TACCTGCGGGGCGTCGAG          | 59                      | 364                      |
| ant(2')-Ia -F                                    | CACCGGCTGGAACAGCGGAA        | 57                      | 294                      |
| ant(2')-Ia -F                                    | GTAAGATGACAGGATCAGAATCT    | 57                      | 294                      |
| ant(2')-Ia -F                                    | ATGGAATTGCGCAATATTATT       | 54                      | 780                      |
| ant(2')-Ia -F                                    | TCAATTCAATCCATCAAGTCT       | 53                      | 624                      |
| armA-A -F                                        | ATTTGCGCTTACCTAATGG         | 50                      | 315                      |
| armA-A -F                                        | ACCATATACCTAATCGCTC         | 50                      | 711                      |
| armA-A -F                                        | CGAGGAATAGCAACAGCAAAAG      | 50                      | 711                      |
| armC -R                                          | ATCCGAAACATCTCCACACT        | 59                      | 635                      |
| armD -F                                          | TTTGCTTCCATGCGCTGCC         | 59                      | 635                      |
| armF -F                                          | CGGACCGGGATTGGAAGCG         | 55                      | 401                      |
| armD -R                                          | CGGAAAAATGCGAGGAGG          | 50                      | 640                      |
| armF -F                                          | GCCATTACGCGACAAATCG         | 50                      | 640                      |
| armF -F                                          | GATATGCGGCAAAAACTAC         | 55                      | 173                      |
| armF -F                                          | GCCTTCTCGGGGCGGTGATA        | 55                      | 173                      |
| armF -F                                          | ATGCAATTGCCCGCTGCTAT        | 55                      | 173                      |
| armF -F                                          | ATGAATGGATGAAATGTTGCC       | 50                      | 818                      |
| armF -F                                          | TGAATGATTCTTTTCACTTCG       | 50                      | 818                      |
| armF -F                                          | GCCATACGAAAAACGAGG          | 55                      | 589                      |
| armF -F                                          | ACCATGGCGCATATGCTTT         | 55                      | 589                      |

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isolates were obtained from the following samples: respiratory specimens 130 (67.7%) (including trachea 109 (56.8%), sputum 17 (8.9%) and bronchoalveolar lavage 4 (2.1%)), urine 30 (15.6%), blood 22 (11.5%) and wound 10 (5.2%). The A. baumannii isolates were separated from the patients admitted to intensive care units 114 (59.4%), and internal 32 (16.7%), infectious diseases 27 (14.1%), neurosurgery 12 (6.3%) and surgery 7 (3.6%) wards. Of all samples, 103 (53.64%) specimens were collected from infectious diseases 27 (14.1%), neurosurgery 12 (6.3%) and intensive care units 114 (59.4%), and internal 32 (16.7%), blood 22 (11.5%) and wound 10 (5.2%).

In total, the highest rates of resistance were against gentamicin (98.4%), tobramycin (97.9%) and ciprofloxacin (96.3%), respectively, whereas amikacin (16.1%) showed the highest rates of susceptibility among antibacterials tested. In total, 189 (98.4%) isolates were found to be non-susceptible against at least one of the aminoglycosides tested (Table 2).

PCR and sequencing showed that 112 (59.3%), 74 (39.2%), 74 (39.2%), 60 (31.7%) and 132 (69.8%) isolates carried the aph(3’)-VI, aac(6’)-Ib, aac(3’)-II, aph(3’)-Ia and armA genes either alone or in combination, respectively. The study isolates were negative for the presence of aac(3’)-Ia, ant(2’)-Ia, ant(4’)-Ib, ant(4’)-Ia, aph(3’)-IIIa, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF and rmtG genes. In total, 100% of isolates carrying AMEs and armA genes were non-susceptible to gentamicin and tobramycin, whereas 90.2%, 88.6%, 85%, 77% and 74.3% of those isolates found to harbour aph(3’)-VI, armA, aph(3’)-Ia, aac(3’)-II and aac(6’)-Ib genes were non-susceptible to amikacin, respectively. As shown in Table 3, aac(6’)-Ib gene was found to coexist with aac(3’)-II, aph(3’)-VI and armA genes in 14 (7.4%) isolates with a co-occurrence of aph(3’)-VI, aac(6’)-Ib and armA in 11 (5.8%) isolates. Moreover, the coexistence of armA, aph(3’)-VI and aac(3’)-Ia genes was established in 11 (5.8%) isolates.

All aminoglycoside non-susceptible isolates belonged to three distinct genotypes including A (150, 79.4%), B (33, 17.5%) and C (6, 3.2%) isolates, indicating the clonal dissemination of these resistant isolates among the target hospitals. As shown in Table 3, the armA-positive isolates mostly belonged to group A (107, 79.4%) isolates followed by 22 (17.5%) isolates related to group B and 3 (2.2%) isolates related to group C. Moreover, the co-existence of armA with aph(3’)-VI+aac(6’)-Ib+aac(3’)-II genes was shown in 14 (7.4%) isolates in which 10 (5.3%) and 4 (2.1%) isolates belonged to groups A and B, respectively.

**Discussion**

Aminoglycosides are an important category of antibacterial medications that are used against a wide range of Gram-negative bacteria such as A. baumannii [5]. Antibiotic resistance against aminoglycosides has resulted in increased health-care costs, hospital stay, morbidity and mortality of patients infected with A. baumannii [3]. Among the well-known resistance mechanisms to aminoglycosides, AMEs and 16S RMTase are the most prevalent mechanisms observed among Gram-negative bacteria, worldwide [6]. There are few reports regarding the frequency of AMEs and in particular the 16S RMTase genes in the clinical isolates of A. baumannii within the local hospital settings. We previously showed the appearance of AMEs with the emergence of armA genes among the clinical isolates of K. pneumoniae in Iran [19]. In our study, 189 (98.4%) A. baumannii strains were non-susceptible against the aminoglycosides used, among those 98.4%, 97.9% and 83.9% of isolates were non-susceptible against gentamicin, tobramycin and amikacin, respectively. These findings were similar to those reported by Khosnood et al. [39] from Iran in 2018 in which 93% and 90% of clinical isolates of A. baumannii were resistant to gentamicin and kanamycin, respectively. In another study by Shoja et al. [40] conducted in Iran in 2017, high resistance rates against tobramycin (87.5%), gentamicin (85%) and amikacin (80%) among the clinical isolates of A. baumannii were revealed. Also, Gholami et al. [41] in Iran showed that 100%, 96.36% and 90.9% of clinical isolates of A. baumannii were resistant to tobramycin, gentamicin and amikacin, respectively. According to these findings, a high rate of resistance to aminoglycosides is present in our hospital settings. Carbapenems were administered as effective antibiotics in treating the infections caused by multidrug-resistant A. baumannii; however, the excessive use of these antibacterials has led to a growing number of carbapenem-resistant A. baumannii strains. In the present study, 89.6% and 87% of A. baumannii strains were resistant to imipenem and meropenem, respectively. Other reports from Iran are indicative of high prevalence of resistance against these drugs within the hospital settings. Mortazavi et al. in a report from Ahvaz (Iran) demonstrated that 90% of their A. baumannii strains were simultaneously resistant to both gentamicin and amikacin and that 91.25% and 80% of the isolates also showed

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**Table 2. Antimicrobial susceptibility of clinical isolates of A. baumannii in Qazvin hospitals, Iran**

| Antibiotics               | R, n (%) | I, n (%) | S, n (%) | Total, n (%) |
|---------------------------|----------|----------|----------|--------------|
| Gentamicin                | 180 (93.8) | 9 (4.7) | 3 (1.6) | 192 (100)    |
| Tobramycin                | 177 (92.2) | 11 (5.7) | 4 (2.1) |              |
| Ciprofloxacin             | 179 (93.2) | 6 (3.1) | 5 (2.6) |              |
| Levofloxacin              | 180 (93.8) | 3 (1.6) | 9 (4.7) |              |
| Ampicillin/Sulbactam      | 167 (87)  | 10 (5.2) | 15 (7.8) |              |
| Imipenem                  | 172 (89.6) | 3 (1.6) | 17 (8.9) |              |
| Meropenem                 | 167 (87)  | 6 (3.1) | 19 (9.9) |              |
| Ceftazidime               | 169 (88)  | 3 (1.6) | 20 (10.4) |              |
| Piperacillin/Tazobactam   | 162 (84.4) | 6 (3.1) | 24 (12.5) |              |
| Amikacin                  | 145 (75.5) | 16 (8.3) | 31 (16.1) |              |

Abbreviations: I, intermediate resistant; R, resistant; S, susceptible.
resistance to imipenem and meropenem, respectively [42]. In another study from Iran, Fallah et al. reported that 40.7% and 80.6% of their A. baumannii isolates revealed resistance against gentamycin and amikacin, respectively [43]. The authors also found that 91.7% of the isolates were resistant to imipenem and meropenem. Finally, in a study by Ghajavand et al. carried out in Isfahan (Iran), the authors reported that 93% of their A. baumannii isolates were resistant to both imipenem and meropenem [44]. Inappropriate and extensive use of these antibiotics in our hospitals plays an important role in the emergence of these resistant isolates. Therefore, it seems that establishing a local and national antimicrobial resistance surveillance system to assess, control and prevent antimicrobial resistance in our hospital settings is necessary. Such valuable information on antibiotic resistance can be made available to physicians for planning an appropriate and effective therapy protocol for their patients. On the other hand, this seems to be the ideal and most efficient strategy in controlling the nosocomial infections.

The present study revealed that aph(3’)-VI (59.3%), aac(6’)-Ib (39.2%), aac(3’)-II (39.2%) and aac(3’)-Ia (31.7%) genes were the most common genes, either alone or in combination with other genes, among the aminoglycosides non-susceptible A. baumannii isolates. In addition, these isolates were found to be negative for the presence of aac(3)-Ia, ant(2’)-Ia, ant(4’)-Ib, ant(4’)-Ia, aph(3’)-IIIa, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF and npmA genes. Aghazadeh et al. [45], in their report from Iran in 2013, showed that aph(3’)-Va (90.6%), ant(2’)-Ia (53.33%), aph(3’)-Ia (32%), aph(3’)-IIIa, rmtA, rmtB, rmtC, rmtD, rmtE, rmtF and npmA genes were the most prevalent AMEs genes in A. baumannii. The isolates were negative for aac(3’)-Ila and rmtB genes and 26% of amikacin-resistant isolates were positive for armA methylase. In another study reported from Iran, Heidary et al. [46], in 2016, showed that 85%, 77%, 72% and 68% of their A. baumannii isolates harboured the aac(3)-Ila, aac(6’)-Ib, aadB and aadA1 genes, respectively. Altogether, these data demonstrate the important role of aph(3’)-VI, aac(6’)-Ib, aac(3’)-II and aac(3’)-Ia genes in resistance to aminoglycosides in Iran. In other countries, similar studies with findings comparable to the results of this study have been documented. Polotto et al. [47] from Brazil in 2019 reported that 55% of their A. baumannii isolates were positive for the presence of aph(3’)-VI gene, followed by aac(6’)-Ib (47%), aac(3’)-Ia (27%), and aph(3’)-Ia in 22% of the isolates. Likewise, Al-kadmy et al. [48] in 2015 in Iraq showed the presence of aac(6’)-Ib, ant(4’)-Ib, aph(3’)-VI and aac(3’) genes among aminoglycoside-resistant A. baumannii isolates. The results of the present study also revealed the co-existence of AME-encoding genes such as aph(3’)-VI+aac(6’)-Ib+aac(3’)-Ia+armA, aph(3’)-VI+aac(6’)-Ib+armA, aph(3’)-Ia+aac(3’)-II+armA and aph(3’)-VI+aph(3’)-Ia+armA. However, the co-existence of 165 RMTase and AME-encoding genes in the same clinical isolate of A. baumannii is well established and the literature includes several studies reported from different countries, worldwide [41,48].

There is no comprehensive information on the prevalence of 165 RMTase genes in the clinical isolates of A. baumannii in our region. In the current study, the armA (69.8%) gene was the most prevalent 165 RMTase gene; however, the authors failed to detect mttA, mttB, rmtC, rmtD, rmtE, rmtF and npmA genes among the target isolates. Similarly, Sheikhalizade et al. [49] in

### TABLE 3. Clonal distribution of AMEs and 16S-RMTases genes among Acinetobacter baumannii isolates

| Genes | Type A n (%) | Type B n (%) | Type C n (%) | Total n (%) |
|-------|--------------|--------------|--------------|-------------|
| amoA  | 47 (24.9)    | 4 (2.1)      | –            | 51 (27)     |
| aph(3’)-VI+aac(6’)-Ib+aac(3’)-II+armA | 10 (5.3) | 4 (2.1) | – | 14 (7.4) |
| aph(3’)-VI+aac(6’)-Ib+armA | 8 (4.2) | 3 (1.6) | – | 11 (5.8) |
| aph(3’)-VI+aac(6’)-Ib+armA | 8 (4.2) | 3 (1.6) | – | 11 (5.8) |
| aac(6’)-Ib+aac(3’)-Ia+armA | 4 (2.1) | – | 2 (1.1) | 6 (3.2) |
| aac(6’)-Ib+armA | 4 (2.1) | – | – | 4 (2.1) |
| aac(3’)-IIa | 3 (1.6) | – | 1 (0.5) | 4 (2.1) |
| aac(3’)-II | 3 (1.6) | – | – | 3 (1.6) |
| aph(3’)-VI+aac(6’)-Ib+aac(3’)-II | 1 (0.5) | – | 2 (1.1) | 4 (2.1) |
| aph(3’)-VI+aac(6’)-Ib+aac(3’)-II | 1 (0.5) | – | – | 1 (0.5) |
| aac(3’)-IIa | 3 (1.6) | – | – | 3 (1.6) |
| aac(3’)-II | 3 (1.6) | – | – | 3 (1.6) |
| aph(3’)-VI+aac(6’)-Ib+aac(3’)-II | 2 (1.1) | – | 1 (0.5) | 4 (2.1) |
| aph(3’)-VI+aac(6’)-Ib+aac(3’)-II | 2 (1.1) | – | 1 (0.5) | 3 (1.6) |
| aph(3’)-VI+aac(6’)-Ib+aac(3’)-II | 3 (1.6) | – | – | 3 (1.6) |
| aph(3’)-VI+aac(6’)-Ib+aac(3’)-II | 2 (1.1) | – | 1 (0.5) | 3 (1.6) |
| aph(3’)-VI+aac(6’)-Ib+aac(3’)-II | 3 (1.6) | – | – | 3 (1.6) |
| aac(6’)-Ib+aac(3’)-II | 6 (3.2) | – | 4 (2.1) | 10 (5.3) |
| aac(6’)-Ib+aac(3’)-II | 4 (2.1) | – | – | 4 (2.1) |
| aac(6’)-Ib+aac(3’)-II | 3 (1.6) | – | – | 3 (1.6) |
| aac(6’)-Ib+aac(3’)-II | 3 (1.6) | – | – | 3 (1.6) |
| aac(6’)-Ib+aac(3’)-II | 1 (0.5) | – | 1 (0.5) | 2 (1.1) |
| aac(6’)-Ib+aac(3’)-II | 3 (1.6) | – | – | 3 (1.6) |
| aac(6’)-Ib+aac(3’)-II | 2 (1.1) | – | 1 (0.5) | 3 (1.6) |
| aac(6’)-Ib+aac(3’)-II | 3 (1.6) | – | – | 3 (1.6) |
| aac(6’)-Ib+aac(3’)-II | 1 (0.5) | – | 1 (0.5) | 2 (1.1) |

Abbreviations: 165-RMT, 16S rRNA-methylases; AMEs, aminoglycoside-modifying enzymes.
lates of A. baumannii carried the armA gene. Similar results were obtained in the present study and none of the isolates were positive for rmtB and rmtC genes. In China, Wang et al. [50] performed a study in 2016 and showed the presence of armA in 72.0% of A. baumannii isolates with high-level resistance to aminoglycosides. Similarly, high prevalence of armA (78.1%) in pan-aminoglycoside-resistant isolates of A. baumannii was reported by Shrestha et al. [51] from Nepal in 2016. Since the 16S RMTase genes are mostly located on the mobile genetic elements, it is worth paying attention to the fact that the presence of this gene in our hospital settings should be considered as a serious clinical concern with the potential to transfer these resistant bacteria to others. The results of rep-PCR in our research revealed that all aminoglycoside non-susceptible A. baumannii isolates belonged to three distinct clones, indicating the clonal dissemination of these resistant isolates among the target hospitals. The genotype A was the most common (79.4%) type, which was strongly associated with the clonal spread of these resistant isolates and patient-to-patient transmission. The distribution and co-existence of the AMEs and 16S RMTase genes among different clones in the current study suggests the clonal spread of not only the aminoglycoside non-susceptible isolates in different wards but also the resistant genes among these bacterial strains.

In conclusion, the findings of the present study showed the emergence of high rates of aminoglycoside resistance as the result of the appearance of AMEs genes among the clinical isolates of A. baumannii within the study hospitals in which aph(3′)-VII, aac(6′)-Ib and aac(3)-II genes were the most predominant genes. Moreover, a high rate of 16S RMTase armA gene was observed in this region. Our findings also showed the clonal dissemination of these resistant isolates in target hospital settings. Based on the results of this study, there are serious clinical concerns regarding the capability of such bacteria to transfer their resistance genes to other bacteria. These results suggest that managing and controlling A. baumannii infections largely depends on adequate and appropriate antibiotic therapy, which eventually could lead to less antibiotic resistance.

Authors’ contributions

PR and MM contributed to the conception and design of the study, methodology acquisition of data and drafting the article; AP contributed to the supervision, conception and design of the study, methodology, analysis and interpretation of data, and gave final approval of the version to be submitted; AAK, RS and SH contributed to the methodology, investigation, formal analysis, acquisition of data, drafting the article, and gave final approval of the version to be submitted; DP and NH–P contributed to the conception and design of the study, analysis and interpretation of data, and to drafting the article.

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Conflict of interest

All authors declare that they have no conflict of interest.

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