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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.

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 againstinfections 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)

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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*-adenyltransferases, which catalyse ATP-dependent adenylation of hydroxyl groups [8,9]. Acetyl group transfer of acetyl coenzyme A to an amine function of aminoglycoside is carried out by AACs. Also, transfer of γ-phosphate and nucleotide monophosphate to hydroxyl groups of aminoglycosides is performed using *O*-phosphotransferases and *O*-adenyltransferases, respectively [10]. *aac(3′)-II* and *aac(6′)-Ib* are common encoding genes for AMEs in the isolates of Gram-negative bacteria [11,12]. The genes encoding for AMEs are usually found on chromosomes, plasmids and transposons [13].

Production of 16S rRNA methyl transferase (16S RMTase) is another mechanism of resistance to aminoglycoside, which is responsible for the methylation of these antibiotics [7,14]. Methylation of target sites is induced by 16S RMTase, leading to decreased affinity of 16S rRNA for aminoglycosides [15]. Plasmid-mediated 16S RMTase is identified among various Gram-negative bacterial species in different geographical regions [15–19]. To date, 11 16S RMTase genes marked as *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtD2*, *rmtE*, *rmtF*, *rmtG*, *rmtH*, *armA* and *npmA* have been detected in several species of Gram-negative bacteria with the *armA* and *rmtB* genes being the most prevalent and widely distributed in Asia [20,21]. The genes encoding 16S RMTase were first isolated from *Klebsiella pneumoniae* in France in 2003, and later named *armA* [22]. The prevalence rate of *armA* has been reported to be steadily on the rise, worldwide [19,20,23]. Horizontal gene transfer between different bacterial species can easily spread the encoding genes of these enzymes that are located on the transposon [24]. Not much information is available on AMEs and the spread of 16S RMTase genes in clinical isolates of *A. baumannii* in different part of Iran.

To better control and prevent the distribution of these bacteria, and to plan for antibiotic administration, epidemiological survey, and detection of resistant strains of these bacteria, the current study was carried out to determine the prevalence of AMEs and 16S RMTase genes among clinical isolates of *A. baumannii* strains and also to investigate their clonal relationship in the teaching hospitals of Qazvin, Iran.

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 $bla_{\text{OXA-51-like}}$ 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 trypticase 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 antibiotic discs 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), ceftazidime (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′)-IIb*, *ant(4′)-Ia*, *aph(3′)-IIIa*, *aph(3′)-VI*, *aph(3′)-Ia*) and 16S RMTase (*armA*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *rmtF* and *npmA*) 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 µM forward primer, 0.5 µM 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, UK). After these steps, the purified PCR products were sent to the Macrogen Company (Seoul, South Korea) to identify the sequence of the target genes. The online BLAST program of the National Center for Biotechnology Information (http://blast.ncbi.nlm.nih.gov/Blast) was applied for sequence alignment.

Clonal analysis by rep-PCR

Repetitive extragenic palindromic-PCR (rep-PCR) was used in a final volume of 25 µL; 2.5 µL 10X PCR buffer, 0.5 µL of dNTP Mix (10 mol), 5 µL $MgCl_2$, 25 pM primer F, 25 pM primer R, 2 U of *Taq* DNA polymerase, 3 µL extracted template DNA, and 16.1 µL distilled water. The amplification conditions included an 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 microbial tests, clinical findings and demographic characteristics were used to analyse the data.

Results

During the period of the study, 192 isolates were identified as *A. baumannii* based on standard biochemical and microbiological methods and amplification of *gltA* and $bla_{\text{OXA-51-like}}$ genes. The

TABLE 1. Sequences of primers used in this study

Target genes	Primer sequence (5′–3′)	Annealing temperature (°C)	Product size (bp)
aac(6′)-Ib -F	TTGCGATGCTCTATGAGTGGCTA	55	482
aac(6′)-Ib -R	CTCGAATGCCTGGCGTGTTT		
aac(3)-II -F	TGAAACGCTGACGGAGCCTC	57	370
aac(3)-II -R	GTCGAACAGGTAGCACTGAG		
aac(3)-Ia -F	ATGGGCATCATTCGCACATGTAGG	59	465
aac(3)-Ia -R	TTAGGTGGCGGTACTTGGGTC		
ant(2″)-Ia -F	ATGGACACAACGCAGGTCGC	56	535
ant(2″)-Ia -R	TTAGGCCGCATATCGCGACC		
ant(4′)-IIb -F	TATCTCGGCGGCGGTCGAGT	59	364
ant(4′)-IIb -R	CACGCGGGGAAACGCGAGAA		
ant(4′)-Ia -F	CAAACTGCTAAATCGGTAGAAGCC	57	294
ant(4′)-Ia -R	GGAAAGTTGACCAGACATTACGAACT		
aph(3′) – III -F	GGCTAAAATGAGAATATCACCGG	57	523
aph(3′) – III -R	CTTTAAAAAATCATACAGCTCGCG		
aph(3′)-VI -F	ATGGAATTGCCCAATATTATT	54	780
aph(3′)-VI -R	TCAATTCAATTCATCAAGTTT		
aph(3′)-Ia -F	CGAGCATCAAATGAAACTGC	53	624
aph(3′)-Ia -R	GCGTTGCCAATGATGTTACAG		
armA -F	ATTCTGCCTATCCTAATTGG	50	315
armA -R	ACCTATACTTTATCGTCGTC		
rmtC -F	CGAAGAAGTAACAGCAAAG	50	711
rmtC -R	ATCCCAACATCTCTCCCACT		
rmtA -F	CTAGCGTCCATCCTTTCTC	59	635
rmtA -R	TTGCTTCCATGCCCTTGCC		
rmtD -F	CGGCACGCGATTGGGAAGC	55	401
rmtD -R	CGGAAACGATGCGACGAT		
npmA -F	CTCAAAGGAACAAAGACGG	50	640
npmA -R	GAAACATGGCCAGAAACTC		
rmtB -F	GCTTTCTGCGCGGGCGATGTAA	55	173
rmtB -R	ATGCAATGCCGCGCTCGTAT		
rmtE -F	ATGAATATTGATGAAATGGTTGC	50	818
rmtE -R	TGATTGATTTCCTCCGTTTTG		
rmtF -F	GCGATACAGAAAACCGAAGG	55	589
rmtF -R	ACCAGTCGGCATAGTGCTTT		

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 isolated from women and 89 (46.35%) from men. The mean age of patients in this study was 55 ± 12 years (range 26–81 years).

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′)-IIb*, *ant(4′)-Ia*, *aph(3′)-IIIa*, *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *rmtF* and *npmA* 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 *aph(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 (3.2%) 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.

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.

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 Khoshnood *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 gentamycin and amikacin and that 91.25% and 80% of the isolates also showed

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* (%)
armA	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+aph(3′)-Ia+armA	8 (4.2)	3 (1.6)	–	11 (5.8)
aph(3′)-VI+aph(3′)-Ia+aac(6′)-Ib+armA	8 (4.2)	2 (1.1)	–	10 (5.3)
aph(3′)-VI+aac(3)-II+armA	6 (3.2)	1 (0.5)	1 (0.5)	8 (4.2)
aph(3′)-VI+aph(3′)-Ia+aac(3)-II+armA	4 (2.1)	–	2 (1.1)	6 (3.2)
aac(6′)-Ib+aac(3)-II+armA	4 (2.1)	2 (1.1)	–	6 (3.2)
aph(3′)-Ia+aac(6′)-Ib+aac(3)-II+armA	5 (2.6)	–	–	5 (2.6)
aph(3′)-VI+aac(3)-II	3 (1.6)	2 (1.1)	–	5 (2.6)
aph(3′)-Ia+aac(6′)-Ib+aac(3)-II+aph(3′)-VI+armA	4 (2.1)	–	–	4 (2.1)
aph(3′)-Ia+aac(3)-II+armA	3 (1.6)	1 (0.5)	–	4 (2.1)
aac(6′)-Ib+aac(3)-II	1 (0.5)	1 (0.5)	2 (1.1)	4 (2.1)
aph(3′)-Ia+aac(6′)-Ib+aac(3)-II+aph(3′)-VI	2 (1.1)	1 (0.5)	–	3 (1.6)
aph(3′)-Ia+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+aph(3′)-Ia	3 (1.6)	–	–	3 (1.6)
aph(3′)-VI+aac(6′)-Ib	2 (1.1)	1 (0.5)	–	3 (1.6)
aph(3′)-Ia+aac(6′)-Ib+armA	–	2 (1.1)	–	2 (1.1)
aph(3′)-Ia+aac(3)-II	–	–	1 (0.5)	1 (0.5)
aph(3′)-VI+aph(3′)-Ia+aac(3)-II	–	1 (0.5)	–	1 (0.5)
No AMEs and 16S-RMTases genes	27 (14.3)	4 (2.1)	–	31 (16.4)
Total	150 (79.4)	33 (17.5)	6 (3.2)	189 (100)

Abbreviations: 16S-RMT, 16S rRNA-methylases; AMES, aminoglycoside-modifying enzymes.

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 *aph(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′)-IIb*, *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′)-VIa* (90.6%), *ant(2″)-Ia* (53.33%), *aph(3′)-Ia* (32%), *aph(3″)-Ia* (17.33%), *aph(3′)-Ia* (17.33%) and *aac(6′)-Ib* (8%) were the most prevalent AMEs genes in *A. baumannii*. The isolates were negative for *aac(3′)-IIa* 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)-IIa*, *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 *aph(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′)-IIb*, *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)-II+armA*, *aph(3′)-VI+aac(6′)-Ib+armA*, *aph(3′)-Ia+aac(3)-II+armA* and *aph(3′)-VI+aph(3′)-Ia+aac(3)-II*. However, the co-existence of 16S 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 16S 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 16S RMTase gene; however, the authors failed to detect *rmtA*, *rmtB*, *rmtC*, *rmtD*, *rmtE*, *rmtF* and *npmA* genes among the target isolates. Similarly, Sheikhalizade *et al.*, [49] in

2017 in a study from Iran, reported that 64.2% of clinical isolates 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 gene among the clinical isolates of *A. baumannii* within the study hospitals in which *aph(3′)-VI*, *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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References

[1] Almasaudi SB. *Acinetobacter* spp. as nosocomial pathogens: epidemiology and resistance features. Saudi J Biol Sci 2018;25:586–96.
[2] Shoja S, Moosavian M, Rostami S, Abbasi F, Tabatabaiefar MA, Peymani A. Characterization of oxacillinase and metallo-β-lactamas genes and molecular typing of clinical isolates of *Acinetobacter baumannii* in Ahvaz, South-West of Iran. Jundishapur J Microbiol 2016;9:e32388.
[3] Hanlon GW. The emergence of multidrug resistant *Acinetobacter* species: a major concern in the hospital setting. Lett Appl Microbiol 2005;41:375–8.
[4] Tamma PD, Cosgrove SE, Maragakis LL. Combination therapy for treatment of infections with gram-negative bacteria. Clin Microbiol Rev 2012;25:450–70.
[5] Poulikakos P, Falagas ME. Aminoglycoside therapy in infectious diseases. Expert Opin Pharmacother 2013;14:1585–97.
[6] Touati A. Aminoglycoside resistance mechanism inference algorithm: implication for underlying resistance mechanisms to aminoglycosides. EBioMedicine 2019;46:8.
[7] Garneau-Tsodikova S, Labby KJ. Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. Medchemcomm 2016;7:11–27.
[8] Busch-Sørensen C, Sönmezoglu M, Frimodt-Møller N, Højbjerg T, Miller GH, Espersen F. Aminoglycoside resistance mechanisms in Enterobacteriaceae and *Pseudomonas* spp. from two Danish hospitals: correlation with type of aminoglycoside used. APMIS 1996;104:763–8.
[9] Kim JY, Park YJ, Kwon HJ, Han K, Kang MW, Woo GJ. Occurrence and mechanisms of amikacin resistance and its association with beta-lactamases in *Pseudomonas aeruginosa*: a Korean nationwide study. J Antimicrob Chemother 2008;62:479–83.

[10] Ramirez MS, Tolmasky ME. Aminoglycoside modifying enzymes. Drug Resist Updat 2010;13:151–71.
[11] Ramirez MS, Nikolaidis N, Tolmasky ME. Rise and dissemination of aminoglycoside resistance: the *aac (6')-Ib* paradigm. Front Microbiol 2013;4:121.
[12] Haldorsen BC, Simonsen GS, Sundsfjord A, Samuelsen Ø. Increased prevalence of aminoglycoside resistance in clinical isolates of *Escherichia coli* and *Klebsiella* spp. in Norway is associated with the acquisition of AAC(3)-II and AAC(6')-Ib. Diagn Microbiol Infect Dis 2014;78:66–9.
[13] Zárate SG, De la Cruz Claure ML, Benito-Arenas R, Revuelta J, Santana AG, Bastida A. Overcoming aminoglycoside enzymatic resistance: design of novel antibiotics and inhibitors. Molecules 2018;23:E284.
[14] Salimizand H, Zomorodi AR, Mansury D, Khakshoor M, Azizi O, Khodaparast S, et al. Diversity of aminoglycoside modifying enzymes and 16S rRNA methylases in *Acinetobacter baumannii* and *Acinetobacter nosocomialis* species in Iran; wide distribution of *aadA1* and *armA*. Infect Genet Evol 2018;66:195–9.
[15] Lioy VS, Goussard S, Guerineau V, Yoon EJ, Courvalin P, Galimand M, Grillot-Courvalin C. Aminoglycoside resistance 16S rRNA methyltransferases block endogenous methylation, affect translation efficiency and fitness of the host. RNA 2014;20:382–91.
[16] Taylor E, Sriskandan S, Woodford N, Hopkins KL. High prevalence of 16S rRNA methyltransferases among carbapenemase-producing *Enterobacteriaceae* in the UK and Ireland. Int J Antimicrob Agents 2018;52:278–82.
[17] Vaziri F, Najar Peerayeh S, Behzadian Nejad Q, Farhadian A. The prevalence of aminoglycoside-modifying enzyme genes (*aac (6')-I, aac (6')-II, ant (2")-I, aph (3')-VI*) in *Pseudomonas aeruginosa*. Clinics (Sao Paulo) 2011;66:1519–22.
[18] Galani I, Souli M, Panagea T, Panagea T, Poulakou G, Kanellakopoulou K, Giamarellou H. Prevalence of 16S rRNA methylase genes in *Enterobacteriaceae* isolates from a Greek university hospital. Clin Microbiol Infect 2012;18:E52–4.
[19] Nasiri G, Peymani A, Farivar TN, Hosseini P. Molecular epidemiology of aminoglycoside resistance in clinical isolates of *Klebsiella pneumoniae* collected from Qazvin and Tehran provinces, Iran. Infect Genet Evol 2018;64:219–24.
[20] Ayad A, Drissi M, de Curraize C, Dupont C, Hartmann A, Solanas S, et al. Occurence of *ArmA* and *RmtB* aminoglycoside resistance 16S rRNA methylases in extended-spectrum β-lactamases producing *Escherichia coli* in Algerian hospitals. Front Microbiol 2016;7:1409.
[21] Al Sheikh YA, Marie MA, John J, Krishnappa LG, Dabwab KH. Prevalence of 16S rRNA methylase genes among β-lactamase-producing *Enterobacteriaceae* clinical isolates in Saudi Arabia. Libyan J Med 2014;9:24432.
[22] Galimand M, Courvalin P, Lambert T. Plasmid-mediated high-level resistance to aminoglycosides in Enterobacteriaceae due to 16S rRNA methylation. Antimicrob Agents Chemother 2003;47:2565–71.
[23] Lee TH, Hwang JH, Lee WK, Shin MK, Woo HR, Chung KM, et al. *ArmA* and *RmtB* were the predominant 16S RMTase genes responsible for aminoglycoside-resistant isolates in Korea. J Korean Med Sci 2018;33:e262.
[24] Zacharczuk K, Piekarska K, Szych J, Jagielski M, Hidalgo L, San Millán Á, et al. Plasmid-borne 16S rRNA methylase *ArmA* in aminoglycoside-resistant *Klebsiella pneumoniae* in Poland. J Med Microbiol 2011;60:1306–11.
[25] Mahon CR, Lehman DC, Manuselis G. Textbook of diagnostic microbiology. 4th ed. Maryland Heights: Mo. Saunders/Elsevier; 2011.
[26] Abdulzahra AT, Khalil MAF, Elkhatib WF. First report of colistin resistance among carbapenem-resistant *Acinetobacter baumannii* isolates recovered from hospitalized patients in Egypt. New Microbes New Infect 2018;26:53–8.
[27] Wen H, Wang K, Liu Y, Tay M, Lauro FM, Huang H, et al. Population dynamics of an *Acinetobacter baumannii* clonal complex during colonization of patients. J Clin Microbiol 2014;52:3200–8.
[28] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-Fifth informational supplement M100-S25. Wayne, PA: CLSI; 2015.
[29] Park CH, Robicsek A, Jacoby GA, Sahm D, Hooper DC. Prevalence in the United States of *aac(6')-Ib-cr* encoding a ciprofloxacin-modifying enzyme. Antimicrob Agents Chemother 2006;50:3953–5.
[30] Jensen VF, Jakobsen L, Emborg HD, Seyfarth AM, Hammerum AM. Correlation between apramycin and gentamicin use in pigs and an increasing reservoir of gentamicin-resistant *Escherichia coli*. J Antimicrob Chemother 2006;58:101–7.
[31] Hujer KM, Hujer AM, Hulten EA, Bajaksouzian S, Adams JM, Donskey CJ, et al. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* sp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. Antimicrob Agents Chemother 2006;50:4114–23.
[32] Emaneini M, Taherikalani M, Eslampour MA, Sedaghat H, Aligholi M, Jabalameli F, et al. Phenotypic and genotypic evaluation of aminoglycoside resistance in clinical isolates of *Staphylococci* in Tehran, Iran. Microb Drug Resist 2009;15:129–32.
[33] El-Badawy MF, Tawakol WM, El-Far SW, Maghrabi IA, Al-Ghamdi SA, Mansy MS, et al. Molecular identification of aminoglycoside-modifying enzymes and plasmid-mediated quinolone resistance genes among *Klebsiella pneumoniae* clinical isolates recovered from Egyptian. Int J Microbiol 2017;2017:8050432.
[34] Almaghrabi R, Clancy CJ, Doi Y, Hao B, Chen L, Shields RK, et al. Carbapenem-resistant *Klebsiella pneumoniae* strains exhibit diversity in aminoglycoside-modifying enzymes, which exert differing effects on plazomicin and other agents. Antimicrob Agents Chemother 2014;58:4443–51.
[35] Doi Y, Arakawa Y. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin Infect Dis 2007;45:88–94.
[36] Davis MA, Baker KN, Orfe LH, Shah DH, Besser TE, Call DR. Discovery of a gene conferring multiple-aminoglycoside resistance in *Escherichia coli*. Antimicrob Agents Chemother 2010;54:2666–9.
[37] Hidalgo L, Hopkins KL, Gutierrez B, Ovejero CM, Shukla S, Douthwaite S, et al. Association of the novel aminoglycoside resistance determinant RmtF with NDM carbapenemase in Enterobacteriaceae isolated in India and the UK. J Antimicrob Chemother 2013;68:1543–50.
[38] Chen CH, Huang CC. Molecular epidemiological study of clinical *Acinetobacter baumannii* isolates: phenotype switching of antibiotic resistance. Ann Clin Microbiol Antimicrob 2013;12:21.
[39] Khoshnood S, Eslami G, Hashemi A, Bahramian A, Heidary M, Yousefi N, et al. Distribution of aminoglycoside resistance genes among *Acinetobacter baumannii* strains isolated from burn patients in Tehran, Iran. Arch Pediatr Infect Dis 2017;5:e57263.
[40] Shoja S, Moosavian M, Rostami S, et al. Dissemination of carbapenem-resistant *Acinetobacter baumannii* in patients with burn injuries. J Chin Med Assoc 2017;80:245–52.
[41] Gholami M, Haghshenas M, Moshiri M, Razavi S, Pournajaf A, Irajian G, et al. Frequency of 16S rRNA methylase and aminoglycoside-modifying enzyme genes among clinical isolates of *Acinetobacter baumannii* in Iran. Iran J Pathol 2017;12:329–38.
[42] Mortazavi SM, Farshadzadeh Z, Janabadi S, Musavi M, Shahi F, Moradi M, et al. Evaluating the frequency of carbapenem and aminoglycoside resistance genes among clinical isolates of *Acinetobacter baumannii* from Ahvaz, south-west Iran. New Microbes New Infect 2020;38:100779.
[43] Fallah F, Noori M, Hashemi A, Goudarzi H, Karimi A, Erfanimanesh S, et al. Prevalence of bla_{NDM}, bla_{PER}, bla_{VEB}, bla_{IMP}, and bla_{VIM} genes

among *Acinetobacter baumannii* isolated from two hospitals of Tehran, Iran. Scientifica (Cairo) 2014;2014:245162.

[44] Ghajavand H, Esfahani BN, Havaei SA, Moghim S, Fazeli H. Molecular identification of *Acinetobacter baumannii* isolated from intensive care units and their antimicrobial resistance patterns. Adv Biomed Res 2015;4:110.

[45] Aghazadeh M, Rezaee MA, Nahaei MR, Nahaei MR, Mahdian R, Pajand O, et al. Dissemination of aminoglycoside-modifying enzymes and 16S rRNA methylases among *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates. Microb Drug Resist 2013;19:282–8.

[46] Heidary M, Salimi Chirani A, Khoshnood S, Eslami G, Atyabi SM, Nazem H, et al. Molecular detection of aminoglycoside-modifying enzyme genes in *Acinetobacter baumannii* clinical isolates. Acta Microbiol Immunol Hung 2017;64:143–50.

[47] Polotto M, Casella T, Modesto Tolentino F, Mataruco MM, Porto NK, Binhardi MF, et al. Investigation of carbapenemases and aminoglycoside modifying enzymes of *Acinetobacter baumannii* isolates recovered from patients admitted to intensive care units in a tertiary-care hospital in Brazil. Rev Soc Bras Med Trop 2020;53:e20190044.

[48] Al-kadmy IMS, Al-Kaabi MH, Musafer HK. Detection of aminoglycoside modifying enzymes and β-lactamases in *Acinetobacter baumannii* isolated from hospitalized Iraqi patients. Greener J Biol Sci 2015;5:10–9.

[49] Sheikhalizadeh V, Hasani A, Ahangarzadeh Rezaee M, Rahmati-Yamchi M, Hasani A, Ghotaslou R, et al. Comprehensive study to investigate the role of various aminoglycoside resistance mechanisms in clinical isolates of *Acinetobacter baumannii*. J Infect Chemother 2017;23:74–9.

[50] Wang Y, Shen M, Yang J, Dai M, Chang Y, Zhang C, et al. Prevalence of carbapenemases among high-level aminoglycoside-resistant *Acinetobacter baumannii* isolates in a university hospital in China. Exp Ther Med 2016;12:3642–52.

[51] Shrestha S, Tada T, Shrestha B, Kirikae T, Ohara H, Rijal BP, et al. Emergence of aminoglycoside resistance due to *armA* methylase in multi-drug resistant *Acinetobacter baumannii* isolates in a university hospital in Nepal. J Nepal Health Res Counc J 2016;14:72–6.