Archives of Razi Institute, Vol. 77, No. 3 (2022) 959-966

DOI: 10.22092/ari.2022.357271.2010

Original Article

Detection of the *aadA1* and *aac (3)-1V* resistance genes in *Acinetobacter baumannii*

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Received 27 December 2021; Accepted 16 January 2022
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Abstract

Acinetobacter baumannii is a gram-negative aerobic bacterium that can be found in different environments, such as food, containing vegetables, meat, and fish; moreover, it can be present in soil and freshwater. *A. baumannii* has globally considered an opportunistic nosocomial bacterium in the healthcare setting contributing to increased morbidity and mortality. The current study aimed to detect the aminoglycoside genes in *A. baumannii* isolated from different clinical causes. In total, 20 isolates of *A. baumannii* were obtained from different clinical cases. Bacterial isolate DNA was extracted using a DNA extraction kit. Quantus Fluorometer was used to detect the concentration of the extracted DNA in order to detect the goodness of samples. 1 µl of DNA and 199 µl of diluted QuantiFlour Dye were mixed. After 5 min incubation at room temperature, DNA concentration values were evaluated, and following the initial amplification of the *A. baumannii aada1* gene, 20 µl of PCR product with F and R primers were sent to Sanger sequencing. The results of the antimicrobial susceptibility revealed that *A. baumannii* isolates were resistant to Gentamicin (95%), Amikacin (90%), and Tobramycin (60%). Molecular investigation of the *aadA1* and *aac (3)-IV* genes exhibited that the *aadA1* gene was detected in 15% of the isolates. However, *the aac (3)-IV* gene was not detected in any of the isolates. The gel electrophoresis revealed that the molecular weight of the *aadA1* gene was 490bp. The DNA sequence of the *aadA1 gene* was conducted in this study, and the results exhibited no mutations in all isolates.

Keywords: *aac (3)-1V*, Acinetobacter baumannii, *aadA1*, Resistance genes in bacteria

1. Introduction

Acinetobacter baumannii is a gram-negative aerobic bacterium that can be found in different environments, such as food containing vegetables, meat, and fish; moreover, it can be present in soil and freshwater (1). *A. baumannii* is an emerging cause of diseases, such as pneumonia, meningitis, urinary tract infections, bacteremia, soft-tissue (2) wound, and bone infections (3). *A. baumannii* belongs to a group of ESKAPE pathogens that include *Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, Enterococcus faecium,* and *A. baumannii*. Due to their ability to effectively resist antibiotic treatments (4), they are among the most common opportunistic pathogens; in addition, they can colonize the hospital setting and survive longer in the hospital environment (5). *A. baumannii* became resistant to many antibiotics, and treatment is almost impossible in some cases (6). Antibiotic overuse causes the development of multidrug-resistant bacteria, resulting in treatment failure and exacerbating the complications (5). Monem, Furmanek-Blaszk (4) mentioned that a major issue is not only multidrug resistance but also the bacterial use of various virulence factors to avoid host defense and survive in harsh environmental conditions.

A. baumannii infections have been increased during the past decades, and cases were mainly reported from countries in tropical or sub-tropical regions (7). *A. baumannii* has been isolated from blood, sputum, skin, pleural fluid and urine (8), oropharyngeal and pulmonary secretions of infected patients, as well as the central nervous system (9). Furthermore, it was the third predominant bacterial pathogen with 14.83% prevalence from samples isolated from the wounds of burnt patients in a Burn Intensive Care Unit (BICU) in Eastern India (5). The World Health Organization (WHO) has assigned *A. baumannii* as a great threat to human health (9). It was reported that nearly 1,000,000 people are infected with *A. baumannii* every year (10). Additionally, in a tertiary care center in Lebanon, and according to a recent survey, the most common site of infections was the respiratory tract (80.8%), followed by skin colonization (12.4%) (11).

A. baumannii have different virulence factors including an exopolysaccharide capsule, the outer membrane (OM), and lipopolysaccharide (12). OM proteins, such as Omp A porin, are responsible for adhesion and invasion of the bacteria into human epithelial cells and apoptosis (13), outer membrane vesicles, biofilm formation, and cytotoxicity. Nemec, Dolzani (14) in 2004 reported the aminoglycoside resistance *aadA1* genes in 95% of the bacterial isolates. The aminoglycoside 3-N-acetyltransferase IV gene (*aac (3)-IV*) confirmed resistance to Gentamycin, Tobramycin, and Paromomycin (15). The current study aimed to detect the aminoglycoside genes in *A. baumannii* isolated from different clinical causes.

2. Materials and Methods

2.1. Collection of Samples and Identification of Bacterial Isolates

A total of 20 samples of *A. baumannii* were kindly provided by Professor Dr. Rana Mujahid Abdullah, Department of Biology, College of Education for Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.

2.2. Extraction of DNA

DNA from bacterial isolates was extracted using a DNA extraction kit (Wizard Genomic DNA Purification Kit, Promega) following the manufacturer's instructions.

2.3. Quantitation of DNA

A Quantus Fluorometer was used to detect the concentration of extracted DNA in order to detect the goodness of samples for downstream applications. 1 µl of DNA and 199 µl of diluted Quanty Flour Dye were mixed. After 5 min incubation at room temperature, DNA concentration values were detected.

2.4. Preparation of Primers

The stock solution of primers was prepared according to the instructions of the manufacturer (Alpha DNA, USA) using nuclease-free water to obtain a concentration of 100 pmol/µl as a stock solution. The working solution of each initiator was prepared separately at 10 pmol/µl by adding 10µl from each stock solution to 90 µl of nuclease-free water and mixed well with vortex, keeping the stock solutions at -20˚C until used (Table 1).

Table 1. Primers used for the gene amplification by PCR

Gene	Primer Sequence (5`-3`)	Size of product (bp)	References
aadA1-F aadA1-R	5`-TATCCAGCTAAGCGCGAACT-3` 5`-ATTTGCCGACTACCTTGGTC-3`	449	Askari, Momtaz (16)
aac (3)-1V-F aac (3)-1V-R	5`-TCATCTCGTTCTCCGCTCAT-3` 5`-CTTCAGGATGGCAAGTTGGT-3`	286	Askari, Momtaz (16)

2.5. Preparation of PCR Mixture

The reaction mixture consisted of 5µl GO Taq Green Master Mix Bioneer (Korea), 2 µl of F-Primer, 2 µl of R-Primer, 5 µl of DNA template, and 6 µl of nuclease-free water. The optimum conditions for the reaction were: one cycle for 5 min at 95˚C for initiation, 30 cycles for 30 sec at 95˚C for DNA denaturation, 30 sec at 55˚C for annealing, 30 sec at 72˚C for elongation, and finally, one cycle for 7 min at 72˚C for final elongation.

2.6. Agarose Gel Electrophoresis

After PCR amplification, Agarose Gel Electrophoresis was performed to confirm the presence of amplicon by loading 10 µl of the PCR products to the wells directly and running at 100 volt for 75 min. Following that, the bands were visualized using the gel imaging system (Gel Imaging System Major Science, Taiwan) (17).

2.7. DNA Sequencing Analysis

After initial amplification of the *A. baumannii aadA1* gene, 20 µl of PCR product with F and R primers were sent to Sanger sequencing using ABI3730XL (Macrogen Corporation, South Korea). DNA sequence data were analyzed using National Center for Biotechnology Information database and BioEdit program (V.7.2.5) (18).

3. Results and Discussion

A total of 20 isolates of *A. baumannii* were included in this study. The isolates showed resistance to Gentamicin (95%), Amikacin (90%), and Tobramycin (60%), as shown in table 2. The current result was in agreement with that reported by Boone, Whitehead (19), who mentioned that 11 (64.7%) of 17 clinical isolates of *A. baumannii* were selected from different anatomical and disease origins of patients from Nashville, Tennessee were resistant to three or more categories of antibiotics; therefore, they were considered to be a multi-drug resistant (MDR). In addition, Zeighami, Valadkhani (20) state that 100% of *A. baumannii* clinical isolates from immunocompromised patients in the ICU were MDR. Moreover, Vahdani, Yaghoubi (21) recorded that the frequency of MDR *A. baumannii* isolates ranged from 32.7% to 93%. The infections caused by *A. baumannii* are difficult to treat and complicated in many countries, such as India, Turkey, and Iran (22), which is a serious problem in healthcare settings due to the extensive misuse of antimicrobial agents.

Table 2. Antibiotic susceptibility distribution of *Acinetobacter baumannii* to Amikacin, Gentamicin, and Tobramycin

No.	AM	GN	TOP
1	R	R	S
2	R	R	R
3	R	R	R
4	S	R	R
5	R	R	R
6	R	R	R
7	R	R	S
8	R	R	R
9	R	S	R
10	R	R	S
11	R	R	S
12	R	R	R
13	R	R	R
14	R	R	R
15	R	R	S
16	R	R	S
17	R	R	S
18	R	R	R
19	R	R	R
20	S	R	S
% resistance	90%	95%	60%

Duarte, Ferreira (23) exhibited that the *A. baumannii* strains resistance to Tobramycin and Gentamicin were positive for biofilm formation. Furthermore, Thummeepak, Kongthai (24) mentioned that the isolates that have the ability to form biofilms were more frequently resistant to Gentamicin. This indicated a relationship between virulence genes and antibiotic susceptibility patterns in *A. baumannii* strains. Gurung, Khyriem (25), as well as Sanchez, Mende (26) exhibited that the MDR phenotype of *A. baumannii* was linked to biofilm production. A study performed by Duarte, Ferreira (23) found that the isolates resistant

to Gentamicin and Tobramycin were more frequently able to form biofilms (74% and 73.3%, respectively). It is suggested that this correlation may be due to biofilm-associated and gentamicin resistance determinants located on the same genomic island or plasmid.

A. baumannii is still considered a serious nosocomial pathogen. Bulens, Sarah (27) observed that among 621 clinical isolates, 56.9% were susceptible to Tobramycin. This result was relatively close to the present study which showed that 8 out of 20 (٤٠%) isolates were sensitive to Tobramycin. Eichenberger and Thaden (28) state that *A. baumannii* usually develop acquired resistance to several antibiotic classes and subclasses through a wide variety of mechanisms, including efflux pumps, antibiotic target mutations, porin expression, antibiotic target mutations, and drug-inactivating enzymes. Due to the continuous exposure to significant selective pressure in the hospital environment, high prevalence rates of MDR clinical isolates have been reported. Asaad, Ansari (29) referred to the importance of the length of hospital stay, and they found that the clinical isolated resistance to Amikacin, Gentamicin, and Tobramycin were 77.7%, 89.4%, and 96.8%, respectively.

The results of the current study showed that three (15%) isolates, including 1, 3, 16 of *A. baumannii* only carried the *aadA1* gene (490bp); however, no band appeared at *aac (3)-IV* gene in all isolate of *A. baumannii*, as shown in figures 1 and 2.

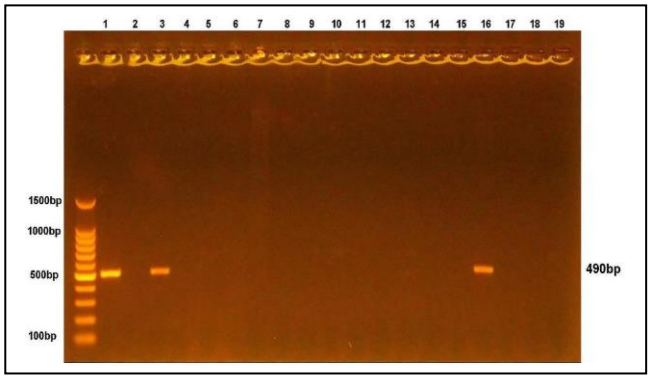

Figure 1. Gel electrophoresis of the *aadA1* gene in *A. baumannii* (490 bp) at 100 volt for 75 min M (Ladder 100-1500bp) (1-3-16) are the positive samples carrying the *aadA1 gene*

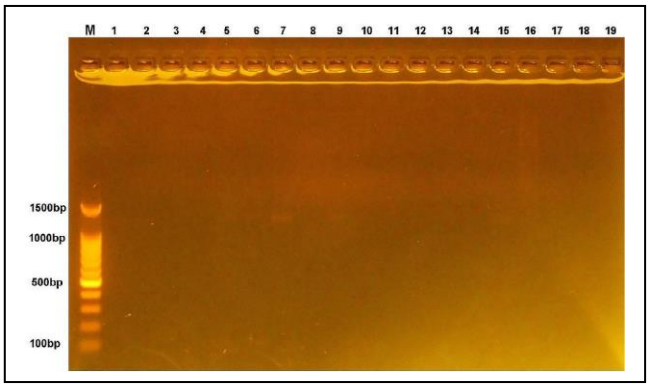

Figure 2. Gel electrophoresis of the *aac (3)-IV* gene (286bp) in *A. baumannii* at 100 volt for 75 min M (Ladder 100-1500bp) (1-19) negative results

The *aadA1* gene was detected in three samples in our study. This is in agreement with previous research conducted by Asaad, Ansari (29) on *A. baumannii* in northeast Iran. Additionally, our findings are compatible with the results of a study performed by Kishk, Soliman (30), who reported that the *aadA1* gene was detected in five (14.2%) isolates out of 35 aminoglycoside resistant strains collected from the ICU patients in Egypt. In addition, current results were compatible with the findings of an Iranian study that exhibited that the *aadA1* gene was detected in 27% of the isolates (31). Aliakbarzade, Farajnia (32) reported that 9 out of 80 (11.25%) clinical isolates harbored *aadA1*. Another study was incompatible with other investigators, such as a study by Salimizand, Zomorodi (33) who reported that *aadA1* was the predominant gene amongst nosocomial *A. baumannii* strains from Iranian patients (34). Furthermore, Salimizand, Zomorodi (33) assessed aminoglycoside resistance genes in 95% of the isolates in which the *aadA1* was prevalent. Another study reported the presence of the *aadA1* gene in 41.7% of the strains (14). Tavakol, Momtaz (35) mentioned the high distribution of the *aadA1* gene (45.45%) of the *A. baumannii* strains isolated from meat specimens of different animals. Furthermore, El-Sheridy, El-Moslemany (36) reported that the *aadA1* gene was detected in 34% of *A. baumannii* clinical isolates from patients in different ICUs in Alexandria, Egypt.

In terms of the above differences, it appeared that the aminoglycoside resistance genes were distributed among

various genotypically groups of *A. baumannii* isolates. Therefore, these data may reflect a widespread occurrence in *A. baumannii* strains in a different milieu. *A. baumannii* can cause severe healthcare-associated infections of the skin, wound infections, pneumonia and urinary tract infections, as well as bloodstream infections (35). Moreover, it may develop antibiotic resistance rapidly (1). The resistance mechanisms to the aminoglycoside of *A. baumannii* isolates are various and essentially include the production of aminoglycoside-modifying enzymes. The genes of those enzymes are carried on plasmids and transferred easily among *A. baumannii* communities (37). In this study, the DNA sequence of the *aadA1 gene* was detected in *A. baumannii*, and the results showed no mutations in all isolates, as shown in table 3, as well as figures 3, 4, and 5.

Table 3. Analysis sequences of the *aadA1* gene of isolates 1, 3, and 16 of *A. baumannii* using the Bio Edit Sequence Alignment Editor Software

No. of isolate	Sequence
1	ATGACATTCTTGCAGGTATCTTCGAGCCAGCCACGATCGACATTGATCTGGCTATCTTGCTGACAAAAGC AAGAGAACATAGCGTTGCCTTGGTAGGTCCAGCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGA TCTATTTGAGGCGCTAAATGAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGGCGATGAGCG AAATGTAGTGCTTACGTTGTCCCGCATTTGGTACAGCGCAGTAACCGGCAAAATCGCGCCGAAGGATGT CGCTGCCGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGAAGCTAGACAGGC TTATCTTGGACAAGAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGT GAAAGGCGAGATGACCAAGGTAG
3	ATGACATTCTTGCAGGTATCTTCGAGCCAGCCACGATCGACATTGATCTGGCTATCTTGCTGACAAAAGC AAGAGAACATAGCGTTGCCTTGGTAGGTCCAGCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGA TCTATTTGAGGCGCTAAATGAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGGCGATGAGCG AAATGTAGTGCTTACGTTGTCCCGCATTTGGTACAGCGCAGTAACCGGCAAAATCGCGCCGAAGGATGT CGCTGCCGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGAAGCTAGACAGGC TTATCTTGGACAAGAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGT GAAAGGCGAGATGACCAAGGTAG
16	ATGACATTCTTGCAGGTATCTTCGAGCCAGCCACGATCGACATTGATCTGGCTATCTTGCTGACAAAAGC AAGAGAACATAGCGTTGCCTTGGTAGGTCCAGCGGCGGAGGAACTCTTTGATCCGGTTCCTGAACAGGA TCTATTTGAGGCGCTAAATGAAACCTTAACGCTATGGAACTCGCCGCCCGACTGGGCTGGCGATGAGCG AAATGTAGTGCTTACGTTGTCCCGCATTTGGTACAGCGCAGTAACCGGCAAAATCGCGCCGAAGGATGT CGCTGCCGACTGGGCAATGGAGCGCCTGCCGGCCCAGTATCAGCCCGTCATACTTGAAGCTAGACAGGC TTATCTTGGACAAGAAGAAGATCGCTTGGCCTCGCGCGCAGATCAGTTGGAAGAATTTGTCCACTACGT GAAAGGCGAGATGACCAAGGTAG

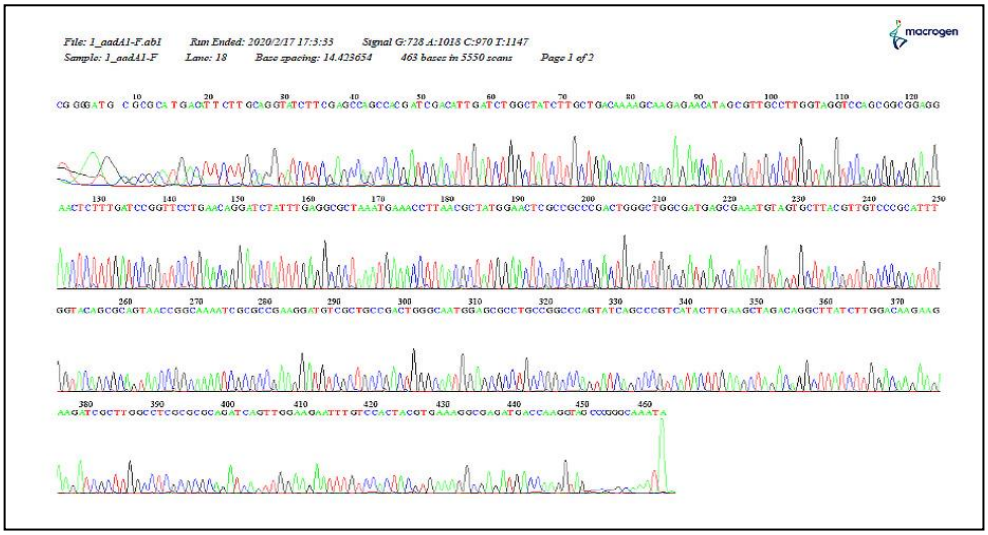

Figure 3. Sequence analysis of the *aadA1* gene of *A. baumannii* isolates no (1)

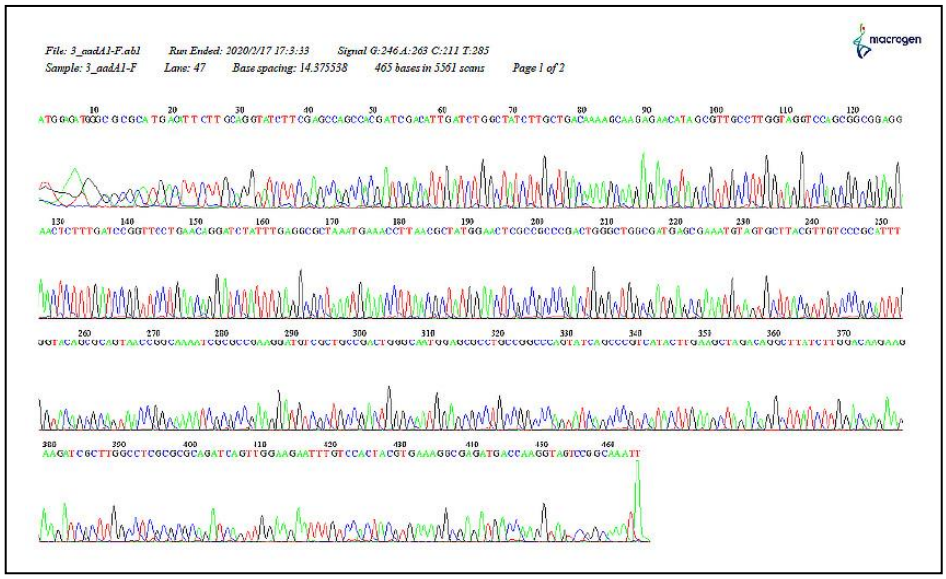

Figure 4. Sequence analysis of the *aadA1* gene of *A. baumannii* isolates no (3)

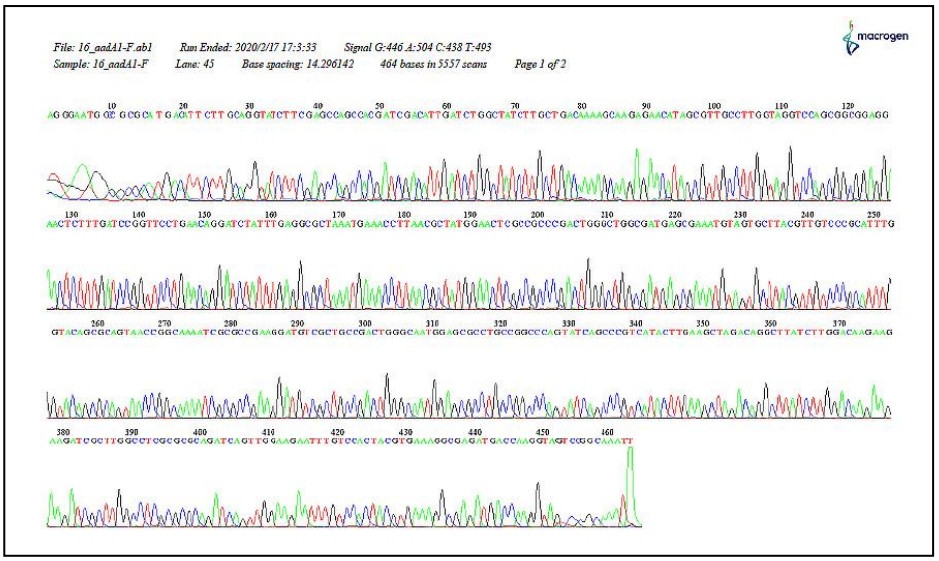

Figure 5. Sequence analysis of the *aadA1* gene of *A. baumannii* isolates no (16)

The 3-N-acetyltransferase IV (*aac 3-IV*) gene is a resistance gene encoding the aminoglycoside, an enzyme resistant to Gentamicin and Tobramycin (38). Regarding the detection of the *aac (3)-IV* gene in *A. baumannii* isolates, the results showed no detection of the *aac (3)-IV* gene in 20 isolates of *A. baumannii*, which is in contrast with a study conducted by Davies and O'Connor (39), who showed that all isolates carried the *aac (3)-IV* gene. The *aac (3)-IV* gene was not detected in any isolates, and this was incompatible with other studies that reported aminoglycoside resistance genes in 95% of the isolates in which the predominance was to *aac (3)-IV* in their population. The *aac (3)-IV* gene was detected only in gram-negative bacterial strains, and the genomic data of the *aac (3)-IV* gene varied among the species of bacteria. Accordingly, *K. pneumoniae* has the highest rates among other species. For *A. baumannii*, the prevalence of the *aac (3)-IV* gene was low, compared to other bacterial species (15).

Authors' Contribution

Study concept and design: E. A. H. and H. Q. H.
Acquisition of data: A. M. A.
Analysis and interpretation of data: R. M. A.
Drafting of the manuscript: H. Q. H.
Critical revision of the manuscript for important intellectual content: H. Q. H.
Statistical analysis: H. Q. H.
Administrative, technical, and material support: H. Q. H.

Conflict of Interest

The authors declare that they have no conflict of interest.

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