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ORIGINAL RESEARCH

Prevalence of Aminoglycoside Resistance and Aminoglycoside Modifying Enzymes in *Acinetobacter baumannii* Among Intensive Care Unit Patients, Ismailia, Egypt

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Background: *Acinetobacter baumannii* is an opportunistic pathogen that rapidly develops antibiotic resistance against commonly prescribed antimicrobial agents in hospitalized patients worldwide. Aminoglycosides are commonly used in the treatment of *A. baumannii* health care-associated infections (HAIs). Aminoglycosides resistance mechanisms are varied and commonly involve production of aminoglycoside-modifying enzymes (AME) and efflux systems.

Aim: This study aimed to provide an insight into the frequency of genes encoding AME in *A. baumannii* strains isolated from different clinical specimens in intensive care units (ICU).

Methodology: A total of 52 multidrug-resistant (MDR) *A. baumannii* strains were isolated from ICU, Suez Canal University Hospitals. Species identification and antibiotics susceptibility testing were done by the automated system VITEK 2. The genes encoding AME were detected by PCR.

Results: Aminoglycosides resistance (amikacin, gentamicin and tobramycin) was observed in 35 isolates (67.3%). We found that aacC1 gene was the predominant AME resistance gene among *A. baumannii* isolates, detected in 14 isolates (40%), aphA6 in 11 isolates (31.4%) and addA1 in 5 isolates (14.2%). We found 5 isolates containing 2 AME genes, 3 of them with aacC1 and aphA6 and the remaining 2 with both aacC1 and aadA1 genes. Nearly, 5 isolates (14.2%) were negative for all AME resistance genes.

Conclusion: Our study indicated that AME encoding genes are predominant in *A. baumannii* strains in our region which stressed on the importance of preventive measures to control spreading of resistance genes.

Keywords: *Acinetobacter baumannii*, aminoglycoside-modifying enzymes; AME, aacC1, aphA6, aadA1, aadB

Introduction

A. baumannii can cause severe health care-associated infections (HAIs) of the skin and soft tissue, wound infections, urinary tract infections, pneumonia and secondary meningitis. The highest mortality rates, however, are seen in ventilator-associated pneumonia (VAP) and bloodstream infections especially in intensive care units (ICUs).[1]

Acinetobacter may develop antibiotic resistance very rapidly.[2,3] A significant element of *A. baumannii*, empowering its endurance and spread inside the health care system, is its capacity to upregulate inherent as well as to gain outside components of

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antimicrobial resistance.[4] They also had the ability to acquire a wide variety of antibiotic resistance genes and rapid development of multidrug-resistant (MDR), extensively drug-resistant (XDR) and even pan drug-resistant (PDR) strains. MDR *A. baumannii* strains are resistant to beta-lactams, aminoglycosides, carbapenems and fluoroquinolones.[5] Decreased membrane permeability due to loss of porins, acquisition of extended-spectrum β - lactamase, and multi-drug efflux systems are mechanisms claimed for *A. baumannii* multidrug resistance.[6]

The aminoglycoside antibiotic class is commonly used in the treatment of HAIs from gram-negative bacilli, including *A. baumannii* strains. The bactericidal activity of aminoglycosides depends on their concentration rather than on the exposure duration to inhibitory concentrations of them. Recently, different resistance mechanisms had been developed against these antimicrobial drugs.[5]

Development of resistance to newer semisynthetic aminoglycosides such as tobramycin, isepamicin, amikacin and sisomicin are being described in many countries worldwide.[7] The resistance mechanisms of *A. baumannii* to aminoglycoside agents are different and typically include aminoglycoside-modifying enzymes (AME) production, which might be classified into aminoglycoside phosphotransferases (APH) (3′)-Via (aphA6), aminoglycoside acetyltransferases (AAC) (3)-Ia (aacC1), besides aminoglycoside nucleotidyltransferases (ANT) (2′′)-Ia (aadB) and ANT(3″) -Ia (aadA1). The genes of those enzymes are carried on plasmids and transposons and are transferred easily among *A. baumannii* population.[8] APH (3′)-VI, AAC (3)-I and ANT(3″)-I synthesis were found to be predominant by many researches on *A. baumannii* isolates, but there are substantial regional differences in their genotypes.[8]

The main mechanism of aminoglycoside resistance is enzymatic alteration of amino-or hydroxyl-groups of aminoglycosides. Aminoglycoside enzymatic modification results in decreased binding to the ribosome of the aminoglycoside molecule. Previous studies suggested several mechanisms of aminoglycoside resistance in *Acinetobacter* spp.[9] Enzymatic inactivation by AAC, ANT, and APH is the most prevalent resistance mechanism.[10]

Although aminoglycosides present nephrotoxicity risks and other side effects, they are considered to be important antimicrobial agents and are used to treat HAIs. The high rates of aminoglycoside resistance could cause a serious issue for combination therapy of aminoglycoside with broad-spectrum β-lactams including cephalosporins and

carbapenems against *A. baumannii* infections. AME and efflux pumps are the most important sources of aminoglycoside resistance among *A. baumannii* isolates where their genes encoding these aminoglycoside resistance mechanisms can be distributed mobile elements.[11]

The overall prevalence of aminoglycoside resistance genes and the mechanisms of resistance among *A. baumannii* clinical isolates in ICU have not been well studied in our country. Therefore, the aim of the present study was to provide an insight into the frequency of gene encoding AME in *A. baumannii* strains isolated from different clinical specimens in ICU.

Materials and Methods
Study Design
This cross-sectional descriptive study was carried out during the period from January 2019 to December 2019. Samples were processed in microbiology laboratory, Faculty of Medicine, Suez Canal University. This study included only patients during ICU stay and developed HAIs. We excluded patients on antibiotic treatment, patients with *A. baumannii* as a colonization not infection. Non-duplicated MDR *A. baumannii* strains were isolated from patients with HAIs in Suez Canal University Hospitals. In case of duplicate isolates (>1 isolates from the same patient) the first isolate was selected for further analysis. Approval was obtained from the ethics committee of Suez Canal University Faculty of Medicine. The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Written, informed consent was obtained from each patient included in this study.

Bacterial Isolation, Identification and Antibiotics Susceptibility
The collected samples (blood, urine, sputum, pus and endotracheal aspirate) were cultured on blood and Mac Conkey (Oxoid, UK) media. Bactec-Alert 3D (Becton Dickinson, Sparks, The United State of America) blood culture incubation system was used for the isolation of bacteria from the blood cultures.

Colonies were identified as *A. baumannii* by colony morphology, Gram stain and different biochemical reactions.[12] A gram stain was performed to determine an organism's gram reaction and morphology prior to selecting which identification card to inoculate Suspected isolates were oxidase negative, catalase positive, indole negative, citrate positive and non-motile. Finally, species identification and antibiotic susceptibility tests were

performed by VITEK 2 Compact 5 (bioMérieux, Inc., Durham, NC, USA). MICs were interpreted as being in susceptible, intermediate, or resistant groups according to the breakpoints recommended by the CLSI standards.[13] Testing was performed according to the manufacturer's instructions. Vitek 2 testing was performed using software version 5.04 and AST-GN71 REF 413,402 cards, according to the manufacturer's instructions.[14,15] *A. baumannii* isolates that were resistant to more than three of the existing antibiotics determined were identified as MDRs. MDR is defined as non-susceptibility to at least one agent in three or more antimicrobial categories. XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two categories).[16] Antibiotics used were ampicillin/sulbactam, cefazoline, ceftriaxone, cefepime, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, tigecycline and trimethoprim/sulphamethoxazole. The isolates were preserved at −80°C in glycerol 15% in brain heart infusion broth (BHIB, Oxoid, Basingstoke, UK) and then subcultured in brain heart infusion broth (BHIB) at 37°C for 24h.

DNA Extraction

DNA was extracted from aminoglycoside resistant strains using Spin ABT Kit (Applied Biotechnology Co. Ltd, Egypt) from fresh subculture on MacConkey agar plates according to manufacturer's instructions. DNA concentration and the purity were assessed by a Nanodrop. A yield of more than 20 μg should be obtained and the A260/A280 ratio should be between 1.7–1.9. DNA was finally eluted in water and preserved at −20°C until use.

Detection of Aminoglycoside Resistance Genes

The genes encoding AME, including phosphotransferases APH (3′)-Via (aphA6), acetyltransferases AAC (3)-Ia (aacC1), nucleotidyl transferases ANT (2″)-Ia (aadB) and ANT (3″) -Ia (aadA1), were detected by PCR in only aminoglycoside resistant isolates. The sequences of primers used in the PCR amplification[5] are demonstrated in Table 1.

PCR reactions were performed in a final volume of 25 μL (24 μL PCR master mix plus 1 μL of template DNA). Reaction mixtures without a DNA template were used as negative control. PCR amplification was carried out in a thermal cycler (Peltier Thermal cycler, MJ Research, USA) with the following conditions: initial denaturation at 95°C for 3 minutes, followed by 35 cycles of 60 seconds, denaturation at 94°C for 60 seconds, then 60 seconds for annealing at primers annealing temperatures (Table 1) and 45 seconds for extension at 72°C with a final extension at 72°C for 5 minutes.

The amplicon size was analyzed by electrophoresis on 1.2% agarose gel in a tris-acetate-EDTA (TAE) buffer at 90 volts alongside a 1 Kb DNA ladder (Applied Biotechnology Co. Ltd, Egypt). Electrophoresis was performed for one hour and finally the gels were stained with ethidium bromide and visualized using the gel documentation system.

Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Science program (SPSS version 22 for windows). Continuous data were summarized by mean, standard deviation and range while qualitative data were summarized by frequencies. In analytical data, the chi-square test was used to detect the difference between qualitative data. Statistical significance was considered at p.value ≤ 0.05.

Table I Primers Used in This Study[5]

Primer	Primer Sequence	Product Size	Annealing Temperature
aacC1 F	ATG GGC ATC ATT CGC ACA TGT AGG	456 bp	52
aacC1 R	TTA GGT GGC GGT ACT TGG GTC		
aadB F	ATG GAC ACA ACG CAG GTC GC	534 bp	55
aadB R	TTA GGC CGC ATA TCG CGA CC		
aadA1 F	ATG AGG GAA GCG GTG ATC G	792 bp	52
aadA1 R	TTA TTT GCC GAC TAC CTT GGT G		
aphA6 F	ATG GAA TTG CCC AAT ATT ATT C	797 bp	55
aphA6 R	TCA ATT CAA TTC ATC AAG TTT TA		

Results

Clinical Specimens and MDR *A. baumannii*

A total of 52 non duplicated MDR *A. baumannii* isolates were recovered from clinical specimens of hospitalized patients with HAIs that had been admitted to the ICUs in Suez Canal University Hospitals, Ismailia, Egypt. The isolates were obtained from invasive and non-invasive sites, including endotracheal aspirates (29/52, 55.8%), urine (11/52, 21.2%), sputum (5/52, 9.6%), blood (5/52, 9.6%) and pus from wound (2/52, 3.8%). The mean ± SD of age was 48 years± 1.334, with a range of 1 to 86 years.

Distribution of MDR *A. baumannii* According to Age

Most MDR *A. baumannii* isolates were observed in the age group, 60–<80 years old (33/52, 63.4%) (p=0.001) followed by 20–<40 years (8/52, 15.4%), 40–<60 (6/52, 11.5%), more than 80 years old (4/52, 7.7%) and only 1 strain was isolated from 0–<20 (1/52, 2%).

Antimicrobial Susceptibility of MDR *A. baumannii* Isolates

The highest resistance was against cefazoline (100%), ampicillin/sulbactam (80.8%) and cefepime (78.8%). On the other hand, all strains were susceptible to tigecycline (100%). Resistance to at least one aminoglycoside (amikacin, gentamicin and/or tobramycin) was observed in 35 isolates (67.3%). All isolates with amikacin and gentamicin resistance were Tobramycin resistant (Table 2).

Table 2 Antimicrobial Resistance Pattern of *A. baumannii* Against Different Antibiotics (N=52)

Antibiotics	Resistance No. (%)	Intermediate No. (%)	Sensitive No. (%)
Ampicillin/sulbactam	42 (80.8%)	0 (0.0%)	10 (19.2%)
Cefazoline	52 (100%)	0 (0.0%)	0 (0.0%)
Ceftriaxone	39 (75%)	2 (3.8%)	11 (21.1%)
Cefepime	41 (78.8%)	0 (0.0%)	11 (21.1%)
Imipenem	36 (69.2%)	0 (0.0%)	16 (30.7%)
Meropenem	36 (69.2%)	0 (0.0%)	16 (30.7%)
Amikacin	5 (9.6%)	0 (0.0%)	47 (90.4%)
Gentamicin	27 (51.9%)	0 (0.0%)	15 (28.8%)
Tobramycin	35 (67.3%)	0 (0.0%)	17 (32.7%)
Ciprofloxacin	39 (75%)	0 (0.0%)	13 (25%)
Tigecycline	0 (0.0%)	0 (0.0%)	52 (100%)
Trimethoprim/ sulphamethoxazole	19 (36.5%)	0 (0.0%)	33 (63.5%)

In relation to age, most aminoglycoside resistant isolates was observed in the age group, 60–<80 years old (27/35, 77.1%), only 6 isolates were equally distributed between the 2 age groups 20–<40 years and 40–<60 (3/35, 8.6% for each) and 2 isolates were more than 80 years old (2/35, 5.7%) (p=0.001).

Genotypic Detection of AME Resistance Genes

Aminoglycoside resistant isolates (35 isolates) were screened for the presence of AME resistance genes by PCR. Nearly, 5 isolates (14.2%) were negative for all AME resistance genes. Interestingly, aacC1 gene was detected in 14 isolates (40%), aphA6 in 11 isolates (31.4%) and addA1 in 5 isolates (%). We did not detect aadB in any isolates. Surprisingly, we found 5 isolates containing 2 AME resistance genes, 3 of them with aacC1 and aphA6 and the remaining 2 were carrying both aacC1 and aadA1 resistance genes (Figures 1–3).

Distribution of AME Resistance Genes According to the Clinical Isolates

Among the MDR *A. baumannii* isolated from endotracheal aspirates (29 isolates), 22 isolates (75.8%) were aminoglycoside resistance phenotypically. One of them (4.5%) was negative by PCR for AME genes, 12 isolates (54.6%) were carrying aaccC1 gene, 8 isolates with aphA6 gene (36.4%) and only one isolate (4.5%) was carrying aadA1. Resistance genes were detected in only 4 urine samples distributed equally to aacC1 and aadA1 genes (2 isolates for each, 33.3%). On the other hand, we detect aphA6 and aaadA1 resistance genes in sputum (2 isolates for each, 50%) while only aphA6 gene was detected in blood samples (1 isolate). No AME resistance genes were detected in pus specimens (Table 3).

Discussion

The present study aims to determine the prevalence of aminoglycosides genes of resistance among different clinical isolates of *A. baumannii* isolated from ICUs in Suez Canal University Hospitals, Ismailia, Egypt. We found that 71% (37/52) of our *A. baumannii* patients aged over 60 years old. In 2010, Sengstock and his colleagues studied MDR *A. baumannii* as an emerging pathogen among elderly in community hospitals and nursing homes. They found that 68% of the patients aged over 60 years.[17] Aging was considered one of the risk factors of MDR *A. baumannii*
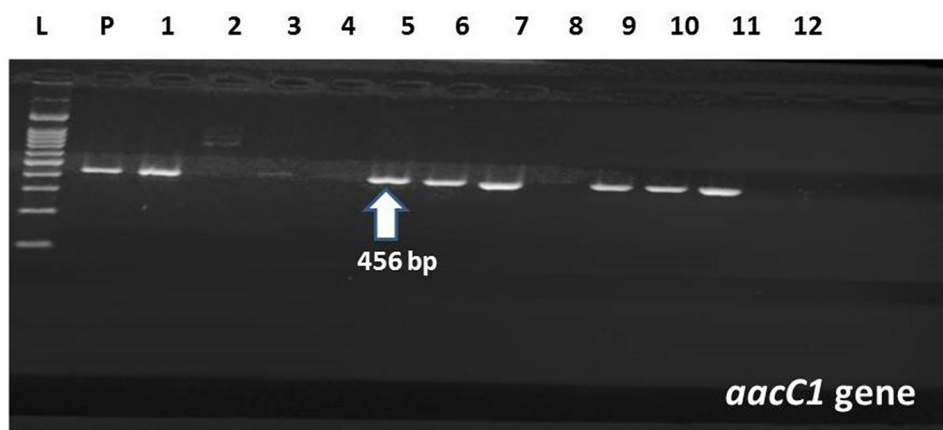
Figure 1 Amplification of *aaaC1* gene in MDR *A. baumannii* by PCR; Lane (L) shows 100-bp molecular size ladder, lane (P) is the positive control, lanes 1,5,6,7,9,10,11 are the positive samples carrying *aaaC1* gene (456 bp). Lanes 2,3,4,8 are negative samples. Lane (12) is the negative control.
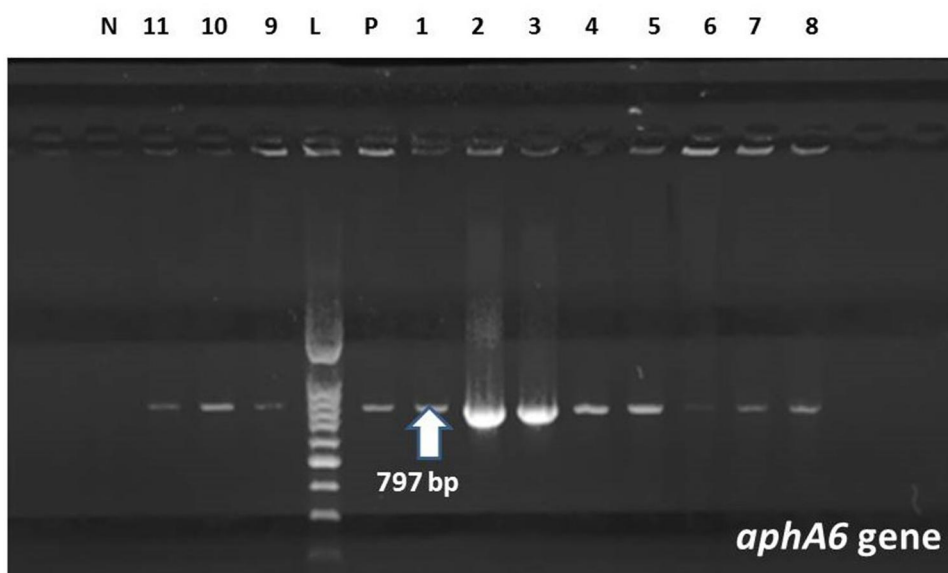

Figure 2 Amplification of *aphA6* gene in MDR *A. baumannii* by PCR; Lane (L) shows 100-bp molecular size ladder, lane (P) is the positive control, lanes 1–11 are the positive samples carrying *aphA6* gene (797 bp). Lane (N) is the negative control.

infection as reported by a multi-centre study.[18] Another research reported aging as a significantly associated factor with mortality in *A. baumannii* infection[19] with worse outcomes than the young.[20,21] This is due to impaired adaptive and innate immune systems in elderly which might influence the prevention and treatment of *A. baumannii* infection.[22–24] So, preventing *A. baumannii* infection in the elderly would be an important public health issue.

In our study, aminoglycosides resistance was observed by MIC in 35 isolates (67.3%) [Amikacin (9.6%), gentamicin (51.9%) and tobramycin (67.3%)]. All isolates with amikacin and gentamicin resistance were Tobramycin resistance. In 2010, Akers et al, reported that the aminoglycoside antibiotics susceptibility were 96.6% to gentamicin and 77.5% to

tobramycin.[25] Previously, a high rate of resistance to aminoglycosides was reported by several investigators in Iran.[5,26–28]

Thirty-five aminoglycoside resistant isolates were investigated for the presence of AME resistance genes by PCR. We found that aacC1 gene was the predominant aminoglycoside-modifying gene among the *A. baumannii* isolates from ICU patients which detected in 14 isolates (40%), aphA6 in 11 isolates (31.4%) and addA1 in 5 isolates (14.2%).These findings were compatible with an Iranian study performed in 2014 reported that aacC1 gene in is the most prevalent one (65.11%), while aphA6, aadA1 was detected in (60.46%), (27.9%) of the isolates respectively.[5]

Nie et al proposed that increased aminoglycoside resistance in A. baumannii is related to acc (6′).[29] Another
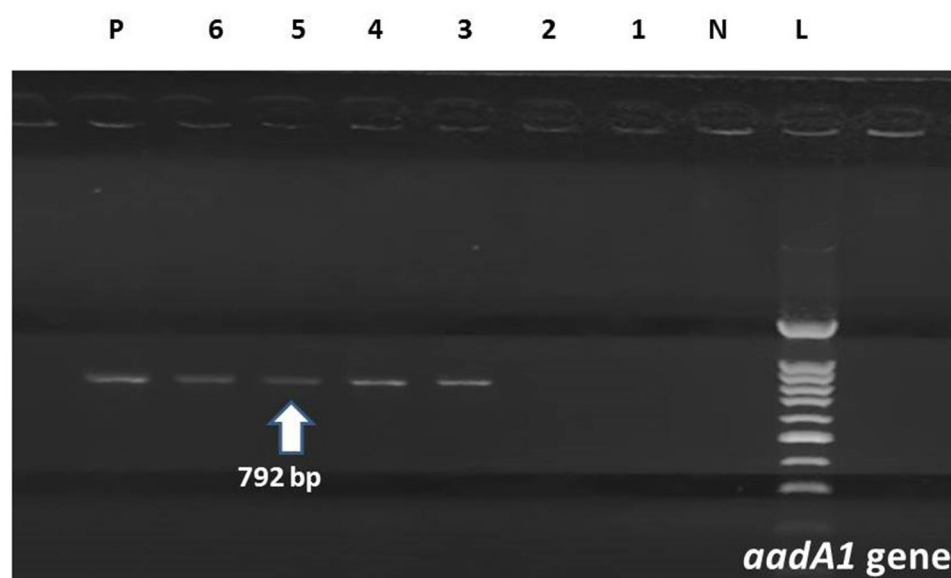
Figure 3 Amplification of *aadA1* gene in MDR *A. baumannii* by PCR; Lane (L) shows 100-bp molecular size ladder. Lane (N) is the negative control. Lanes 3–6 are the positive samples carrying *aadA1* gene (792 bp). Lanes 1.2 are negative samples. Lane (P) is the positive control.

study reported the presence of acetyltransferase genes (aacC1) in 63.3% of *Acinetobacter* isolates and other genes including aphA6, aadA1 and aadB were detected in 65%, 41.7% and 3.3%, of the isolates, respectively.[30] In our study the prevalence of aphA6 and aadA1 genes was less prevalent than reported previously.[31,32] However, Farsiani et al reported a prevalence of aphA1 (75%) among A. baumannii isolates from HAIs in the north-east of Iran while aadA1 gene was not identified in the examined isolates.[33] More recently in 2018, another study reported that aadA1 is the most frequent AMEs amongst nosocomial A. baumannii isolates from Iranian patients.[34]

We did not detect aadB in any isolates. This was incompatible with other studies reported high prevalence of aadB genes in their population.[5,30] In a Korean study, the majority of AME genes detected by PCR were aacC1 (56%), aadB (48%), and aphA6 (71%).[35]

In Czech Republic, investigators assessed the variety of genes encoding AME and their relation to class 1 integrons in *A. baumannii*. They reported aminoglycoside

resistance genes in 95% of isolates where the predominance was to aacC1 gene followed by aadA1, aphA6, and aadB.[36] Previously, a high rate of resistance to aminoglycosides was reported by several investigators in Iran.[5,26,28,31]

Considering these differences, it appeared that the aminoglycoside resistance genes were distributed among various genotypically distinct groups of *A. baumannii* strains. Thus, these data could reflect a widespread occurrence and clonal variation in HAIs *A. baumannii* isolates in various regions.

Limitations of the Study

Our study was a single center, short study in our institution only with small number of isolates. So, it is lack in A. baumannii strain variety. Also, we cannot evaluate of the clonal relatedness of the 52 isolates. The correlation between the detected AMEs and the resistance patterns is unclear. Role of other mechanisms of aminoglycoside resistance was not studied (e.g., efflux pumps and 16S-ribosomal RNA methyltransferases).

Table 3 Distribution of AME Resistance Genes According to the Clinical Isolates

Gene	Endotracheal Aspirate No. (%)	Urine No. (%)	Sputum No. (%)	Blood No. (%)	Pus No. (%)
aacC1 (N=14)	12 (54.6%)	2 (33.3%)	0 (0%)	0 (0%)	0 (0%)
aphA6 (n=11)	8 (36.4%)	0 (0%)	2 (50%)	1 (50%)	0 (0%)
aadA1 (n=5)	1 (4.5%)	2 (33.3%)	2 (50%)	0 (0%)	0 (0%)
No genes (n=5)	1 (4.5%)	2 (33.3%)	0 (0%)	1 (50%)	1 (100%)
Total	22 (100%)	6 (100%)	4 (100%)	2 (100%)	1 (100%)

Conclusion

Our study results showed a significant variety of genes encoding AME in our region. We also found that the genes related to AME are prevalent in the *A. baumannii* strains giving a special concern to the importance of prevention and control measures to prevent their spread.

Data Sharing Statement

All authors claim that all data and materials as well as software application or custom code support our published claims and comply with field standards.

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted * Dove Medical Press Ltd *; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no conflicts of interest for this work.

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