Molecular Detection of Aminoglycoside Resistance Genes in Acinetobacter Baumannii Clinical Isolates from Najaf Hospitals, Iraq

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Abstract

Background: Acinetobacter baumannii is an opportunistic infection that quickly acquires resistance to frequently administered antimicrobials in hospitalised patients globally. Aminoglycosides are still the preferred treatment for Acinetobacter infections; however, resistance has increased over the past few years. There are several different routes for aminoglycoside resistance, and they almost always interfere with the activity of aminoglycoside-modifying enzymes.Aim of the study: This study studied most of the genes in A. baumannii that code for aminoglycoside-modifying enzymes in clinical samples from hospitals in Najaf, Iraq.Materials and Methods: Twenty-eight samples of A. baumannii were taken from hospitals in Najaf, Iraq. The antimicrobial resistance patterns of of these isolates to different antimicrobial drugs were tried using the disc diffusion method, and the species were determined utilising the automated VITEK-2 system. The genes encoding aminoglycoside modifying enzymes (ACC (6')-Ib, aacC1, amrA, and aac(3)-IIa) were investigated by the PCR technique.Results: Antimicrobial tests found that all isolates resistant to tetracycline ciprofloxacin, and ceftazidime, whereas colistin (100%) and imipenem (71%) showed the greatest sensitivity. The aminoglycoside resistance rates for gentamicin, amikacin, tobramycin, and neomycin were 93, 86, 82, and 78%, respectively. ACC (6')-Ib gene was the most prevalent aminoglycoside-resistance gene among A. baumannii clinical isolates, as identified in 16 isolates (57%) by PCR, followed by aacC1 in 5 isolates (18%) and amrA in 3 isolates (11%) whereas all isolates were negative for aac(3)-IIa gene. We identified 4 isolates with 2 AME genes, 3 with ACC (6')-Ib and aacC1 and 1 with ACC (6')-Ib and amrA. Only seven (25%) A. baumannii isolates were no amplification for every AME resistance gene.Conclusion: This study showed that clinical isolates of A. baumannii in hospitals in Najaf were often very resistant to aminoglycosides, and most of these isolates had the aac (6')-Ib gene.

Keywords: Acinetobacter baumannii; Aminoglycoside; aminoglycoside-modifying enzymes; ACC (6')-Ib gene

1. Introduction

Acinetobacter baumannii is a Gram-negative bacterium with significant clinical relevance in hospitals. This bacterium causes a variety of nosocomial infections, including as surgical site infections, pneumonia, bacteremia, and urinary tract infections (1,2). A. baumannii is a microbe that is persistent and easily distributed because it is non-susceptible to the majority of antimicrobial agents, desiccation, and disinfectants (3). These species in Acinetobacter genus that naturally resist many β-lactam antibiotics have led to a rise in the resistance profile (1,4). Additionally, unrestricted usage of broad-spectrum antibiotics has changed them into multi-drug resistant microbes, which are now a globally significant problem (4).

Aminoglycosides have been used for a long time to regale infections caused by A. baumannii, and they continue to be an important therapeutic option. But in recent years, these bacteria have become more resistant to aminoglycosides (5,6). Three mechanisms impair the effectiveness of aminoglycosides in the case of nosocomial infections caused by A. baumannii. These include lower absorption or decreased cell permeability, changes at the ribosome binding sites, or the development of aminoglycoside-modifying enzymes (7,8). The main mechanism of A. baumannii aminoglycoside-resistance is provided by aminoglycoside-modifying enzymes (AMEs). The structure of aminoglycosides is destroyed by enzymes like acetyltransferases, phosphotransferases, and adenyltransferases (5). Several studies have shown that a diversity of MDR A.baumannii isolates produces aminoglycoside-modifying enzymes (9,10). A study conducted in Saudi Arabia found a multidrug-resistant A. baumannii strain containing four aminoglycoside-modifying enzymes (11). Another Iranian study indicated that all strains of A.baumannii contain enzymes that modify aminoglycosides, indicating that these enzymes are common in A.baumannii (12). According to our knowledge, Iraq has very little data on the frequency of aminoglycoside-modifying enzyme genes in A. baumannii isolation from clinical samples. The purpose of this study was to identify

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the antimicrobial non-susceptible of A. baumannii strains and to examine the presence of ACC (6')-Ib, aacC1, armA, and aac(3)-IIa aminoglycoside resistance genes from the strains collected from patients referred to main hospitals in Najaf city, Iraq.

2. Materials and Methods

Bacteria identification: This cross-sectional study evaluated some aminoglycoside-modifying enzyme genes in A. baumannii isolates obtained from major hospitals in Najaf province, Iraq. A total of 28 different clinical specimens (burns wounds, urine, blood, and sputum) were collected from patients admitted to AL-Sadar teaching hospital and Al-Hakim teaching hospital in Najaf province, Iraq. These samples were collected during the period between January to June 2022. Before sampling, each patient's verbal agreement was obtained.

The samples were taken to the department of microbiology's lab at Kufa University's faculty of science, where all isolates were streaked on MacConkey, Blood, and Chrom agars and identified using standard biochemical tests and growth capacity at 44°C. In addition to the morphological characteristics of colonies on Chrom agar, the Vitek-2 system was used to confirm the species identification.

Antimicrobial Susceptibility Test

The modified Kirby-Bauer disc diffusion test was performed to assess antibiotic resistance patterns of 28 *A. baumannii* isolates against ten antimicrobial agents, including ceftazidime, ciprofloxacin, tetracycline, colistin, gentamicin, amikacin, tobramycin, neomycin, imipenem, and meropenem (MAST, Merseyside, UK). The results are interpreted following CLSI (2022) recommendations, which classify bacteria as sensitive, intermediate, or resistant to various antimicrobial agents.

Detection of aminoglycoside resistance genes

According to the manufacturer's instructions, fresh subcultures on MacConkey agar plates were used to extract the genomic DNA of A. *baumannii* strains using the ABC DNA Isolation Kit (Applied Biotechnology, Canada). PCR was used to detect the presence of the aminoglycoside resistance genes ACC(6')-Ib, aacC1, armA, and aac(3)-IIa using the specific primers listed in Table 1. PCR products were recognised using 1.5% agarose gel electrophoresis, stained with ethidium bromide (Shimadzu, Japan), and seen under ultraviolet light (Shimadzu, Japan).

Table1: Primers used in this study.

Primer	Primer Sequences	Product Size, bp	Annealing Temperature, °C	Reference
ACC(6')-Ib-F	ATGACTGAGCATGACCTTGC	519	57	13
ACC(6')-Ib-R	TTAGGCATCACTGCGTGTTC			
aacC1-F	ACCTACTCCCAACATCAGCC	630	55	14
aacC1-R	ATATAGATCTCACTACGCGC			
armA-F	TTATTTCTGAAATCCACTAGTAATTA	774	56	13
ArmA-R	CCTAGCGTCCATCCTTTCCTC			
aac(3)-IIa-F	ATGCATACGCGGAAGGC	822	55	15
aac(3)-IIa-R	TGCTGGCACGATCGGAG			

Statistical analysis: The data were interpreted with SPSS version 21.0 (IBM Corp., USA). P-values of <0.05 were regarded as significant. LSD values and mean difference values were compared.

3. Results

Bacteria Isolates and Antimicrobial Susceptibility

Twenty-eight *A. baumannii* isolates were taken from hospitalised patients at Al-Sader medical city and Al-Hakeem general hospital in Najaf, Iraq. The isolates were collected from various sources, including burn wounds 14(50%), urine 9 (32%), blood 3(11%), and sputum 2 (7%), respectively.

The results of the antibiotics susceptibility tests showed that ciprofloxacin (100%), ceftazidime (100%), and tetracycline (100%) had the most elevated level of resistance, while colistin (100%), imipenem (71%), and meropenem (64%) had the most heightened level of susceptibility (Figure 1). Resistance to aminoglycosides was highest for gentamicin (93%), followed by amikacin (86%), tobramycin (82%), and neomycin (78%). Neomycin was the most effective aminoglycoside tested.

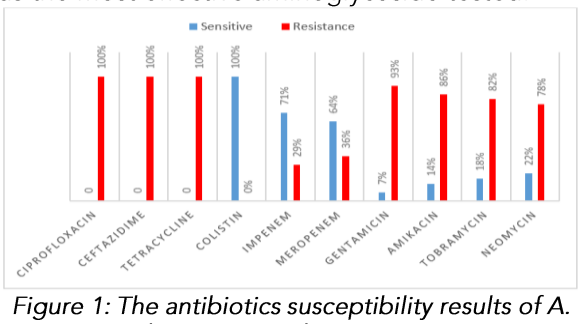

Figure 1: The antibiotics susceptibility results of A. baumannii isolates (n= 28)

Detection of AME Resistance Genes:

In a PCR-based screen for resistance genes in aminoglycoside-resistant isolates (Figure 2,3,4), only seven isolates (25%) isolates did not have any gene of aminoglycoside resistance. Acc(6)-Ib gene was found in 16 of the isolates (57%), aacC1 gene was found in 5 of the isolates (18%), and armA gene was found in 3 of the isolates (11%). We did not find any isolates with aac(3)-IIa. Surprisingly, we found 4 isolates with 2 aminoglycoside resistance genes.

Three had aacC1 and Acc(6)-lb resistance genes, and the other had both Acc(6)-lb and armA resistance genes. Table 2 displays the occurrence of aminoglycoside-resistant genes among A. baumannii isolates.

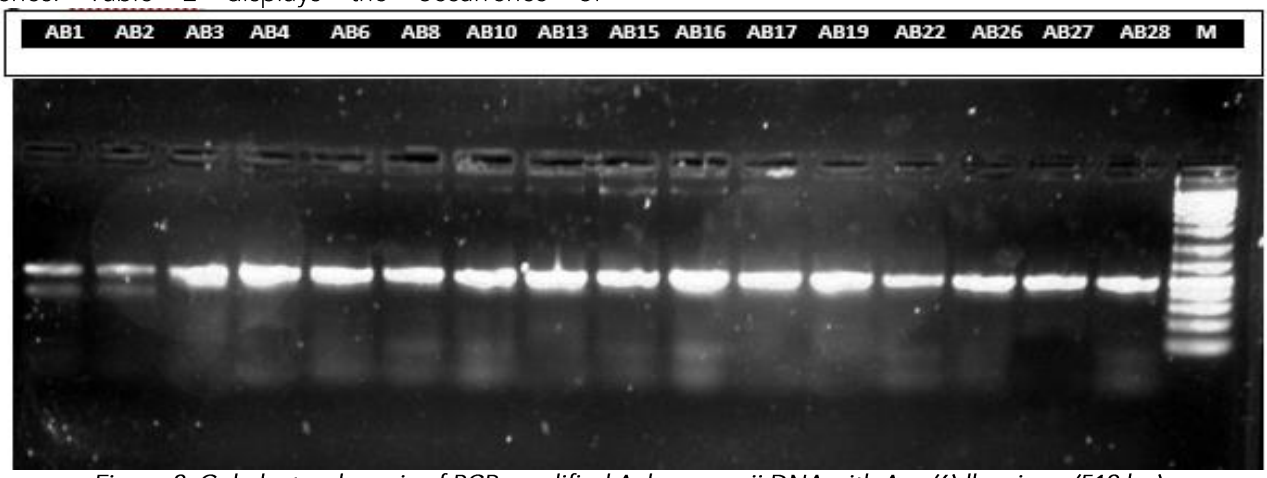

Figure 2: Gel electrophoresis of PCR amplified A. baumannii DNA with Acc (6)-lb primer (519 bp).

80 min at 70 volts was used for electrophoresis. M: DNA molecular marker; AB: *A. baumannii*

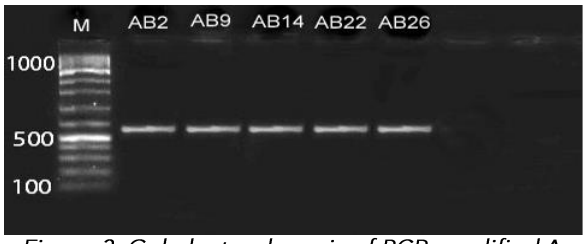

Figure 3: Gel electrophoresis of PCR amplified A. baumannii DNA with aacC1 primer (630 bp).

80 min at 70 volts was used for electrophoresis. M: DNA molecular marker; AB: A. baumannii

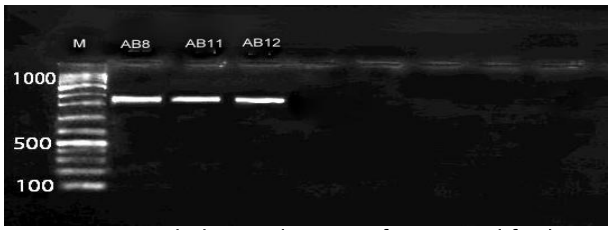

Figure 4: Gel electrophoresis of PCR amplified A. baumannii DNA with armA primer (774 bp).

80 min at 70 volts was used for electrophoresis. M: DNA molecular marker; AB: A. baumannii

Table 2: Occurrence of various aminoglycosides-resistance genes and their combinations and antibiotics resistant profiles isolates (n=28)

Isolate code	Gene encoding	Source	Antibiotics resistance profile
AB1	ACC(6')-lb	Urine	CAZ, CIP,TE, GEN,AMK,TOB,IMI ,MEM
AB2	ACC(6')-lb, aacC1	Wound	CAZ, CIP,TE, GEN, AMK, TOB,NEO, IMI
AB3	ACC(6')-lb	Blood	CAZ, CIP,TE, GEN, AMK, NEO ,MEM
AB4	ACC(6')-lb	Urine	CAZ, CIP,TE, GEN, AMK, TOB, NEO, IMI
AB5	No detect	Wound	CAZ, CIP,TE, GEN, TOB , NEO
AB6	ACC(6')-lb	Wound	CAZ, CIP,TE, TOB, NEO
AB7	No detect	Blood	CAZ, CIP,TE, GEN, AMK, , NEO,MEM
AB8	ACC(6')-lb, armA	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO, IMI
AB9	aacC1	Urine	CAZ, CIP,TE, GEN, AMK, , NEO ,MEM
AB10	ACC(6')-lb	Urine	CAZ, CIP,TE, GEN, AMK, TOB , NEO ,MEM
AB11	armA	Urine	CAZ, CIP,TE, AMK, TOB, NEO
AB12	armA	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO,MEM
AB13	ACC(6')-lb	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO
AB14	aacC1	Urine	CAZ, CIP,TE, GEN, AMK, TOB, NEO
AB15	ACC(6')-lb	Blood	CAZ, CIP,TE, GEN, AMK, TOB , NEO, IMI
AB16	ACC(6')-lb	Wound	CAZ, CIP,TE, GEN, AMK, TOB,
AB17	ACC(6')-lb	Urine	CAZ, CIP,TE, GEN, AMK, ,MEM
AB18	No detect	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO
AB19	ACC(6')-lb	Sputum	CAZ, CIP,TE, GEN, TOB , ,MEM
AB20	No detect	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO
AB21	No detect	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO ,MEM
AB22	ACC(6')-lb, aacC1	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO, IMI
AB23	No detect	Urine	CAZ, CIP,TE, GEN, AMK, TOB,
AB24	No detect	Wound	CAZ, CIP,TE, GEN, AMK
AB25	No detect	Sputum	CAZ, CIP,TE, GEN, TOB, NEO ,MEM
AB26	ACC(6')-lb, aacC1	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO, IMI
AB27	ACC(6')-lb	Wound	CAZ, CIP,TE, GEN, AMK, TOB, NEO
AB28	ACC(6')-lb	Urine	CAZ, CIP,TE, GEN, AMK, TOB, NEO, IMI

AB: *A. baumannii*, AMK: amikacin, CAZ: ceftazidime, CIP: ciprofloxacin, GEN: gentamicin, NEO: neomycin, IMI: imipenem, MEM: meropenem, TE: tetracycline, TOB: tobramycin.

4. Discussion

The progress of multidrug resistance in A. baumannii was pushed by the overuse and misuse of antibiotics, which resulted in the development of different mechanisms of action. Aminoglycoside modifying enzymes have drawn significant attention and emerged as a significant resistance determinant due

to their wide substrate specificity and widespread distribution among various bacterial species (16,17). This study focuses mostly on the aminoglycoside resistance genes present in several clinical strains of A. baumannii isolated from various clinical samples in Najaf Hospitals, Iraq.

According to the results of the current study, A. baumannii was most frequently isolated (50%) from burn wound infection, which is consistent with several earlier surveillance investigations in Najaf (18, 19). A significant prevalence of these isolates was shown in previous research conducted in burn centers in different regions throughout Iraq (20, 21). A. baumannii was the confirmed etiological agent in 9 patients with UTI (32%). This percentage was comparable to research that found 35% of A. baumannii infections in urine in Najaf (19).

The results of the current study revealed the following patterns of drug resistance: high resistance to ceftazidime ciprofloxacin, and tetracycline; moderate resistance to aminoglycosides; low resistance toward carbapenems (imipenem and meropenem); and no resistance to colistin. Alike to other studies conducted in Iraq, antibiotic susceptibility patterns were observed there, with carbapenem and colistin having the lowest rates of resistance (19, 22).

The resistance of A. baumannii to aminoglycosides in Iraq was noted by several researchers (21,23) reported that the resistance rate of A. baumannii strains to gentamicin, tobramycin, and amikacin was 100%, 72.72%, and 45.5%, respectively. Comparing the rates reported by other authors globally, the aminoglycoside resistance rates were greater (24). The fact that this class of antibiotics is used so frequently in hospitals in Iraq may be to reason for the high resistance to them.

The existence of aminoglycoside resistance genes was examined by PCR in 28 isolates that were resistant to the aminoglycoside antibiotic. We found out that the majority of the aminoglycoside-modifying genes in isolates of A. baumannii from Najaf hospitals was Acc (6)-Ib which was detected in 16 isolates (57%) followed by the aacC1 gene which was detected in 5(18%) while armA gene was detected in 3 (11%). These results agree with an Iraqi study in 2014, which found that the Acc (6)-Ib gene is the most common (65.11%), whereas aacC1 and armA were found in 60.46 and 27.9 % of the strains, respectively.

Doi et presented that raised aminoglycoside no susceptibility in A. baumannii strains is correlated to Acc (6)-Ib (25).

An additional study found that 68.5% of A. baumannii isolates contained the Acc (6)-ib aminoglycoside acetyltransferase and that another gene armA was found in 31% of the isolates (26). In contrast to earlier studies, the prevalence armA gene was lower in this study (27).

Worryingly, the co-existence of aminoglycoside genes in A. baumannii isolates also has been found in this study, including aacC1and Acc (6)-Ib in 3 (11%) isolate, and Acc(6)-Ib/armA in 1 (3.5%) isolates. In association with other studies, Gholami et al. (2017) found co-existence of Acc (6)-Ib and armA gene in two isolates of A. baumannii in Iran (28). Nowak et al. (2014) also reported that four aminoglycosides - resistant A. baumannii isolates from Specialized Hospital in Cracow, Poland have aacC1 and Acc (6)-Ib genes (29). Furthermore, Aishwarya (2020) found two isolates of A. baumannii harbored both Acc (6)-Ib and armA genes in India (30).

Among the aminoglycoside modifying enzyme genes, aac(3)-IIa was not detected in the studied A. baumannii isolates. However, more recently, Atasoy reported that nosocomial A. baumannii isolates from Turkey patients more frequently contain the aminoglycoside-modifying enzyme aac(3)-IIa (31). Due to these variations, it seemed that different genotypically dissimilar groupings of A. baumannii strains included the aminoglycoside resistance genes. Consequently, these results may suggest an extensive occurrence and clonal variety of nosocomial A. baumannii strains in different hospitals in Iraq.

5. Conclusion

High levels of non-susceptible aminoglycoside were found in A. baumannii clinical isolates taken from patients in Najaf hospitals. most of the isolates were recovered from the burn center. Acc (6)-Ib gene was predominately detected in isolates resistant to aminoglycosides, and some isolates were found to possess more than one gene encoding the enzyme that modifies aminoglycosides.

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