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**Dissemination and Molecular Characterization
of Aminoglycoside Resistant *Acinetobacter
baumannii* Isolates in Babylon Province**

A Dissertation

Submitted to the Council of College of Science, University of
Al-Qadisiyah in Partial Fulfillment of the Degree of Doctorate
of Philosophy in Biology

By

Baidaa Shaheed Tuamaa

B.Sc. Biology (2005)

M.Sc. Biology (2022)

Supervised by

Prof. Dr.Firas Srhan Abd Al-Mayahi

2026 A.D.

1448 A.H.

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Prof. Dr. Firas Srhan Abd Al-Mayahi

University of Al-Qadisiyah

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In view of the available recommendation, I forward this Research for debate by the Examination Committee.

Prof.Dr. Layth Sareea Mutar

Head of the Biology Department

University of Al-Qadisiyah

Date: / / 2026

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Scientific Evaluation: Prof. Dr. Ahlam Kadhem Naeem

Date: / / 2026

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Chairman

Signature:

Prof.Dr.

University of

College of

Date: / /2026

Member

Signature:

Prof.Dr.

University

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Date: / /2026

Member

Signature:

Prof.Dr.

University of

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Member

Signature:

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Signature:

Prof.Dr

University

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Member

Signature:

Prof.Dr.

University of

College of

Date: / /2026

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Qadisiyah

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Prof. Dr. Salwan Ali Abed

Address: Dean of the College / University of Al-Qadisiyah

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Dedication

TO...

Imam Al-Mahdi (the Awaited), the beacon of hope,

My father, my strength and support,

My mother, the source of tenderness,

My brothers, sisters, and My husband,

And to everyone who helped me.

Baidaa

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because this research has been completed through Their blessings

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Summary

Acinetobacter baumannii is an important opportunistic pathogen and one of the main causes of healthcare-associated infections, particularly among critically ill and immunocompromised patients. It has been increasingly isolated from various clinical sources, including burn wounds, blood, sputum, wound swabs, and urine. The emergence of aminoglycoside-resistant *A. baumannii* isolates has become a major concern in clinical settings as it limits therapeutic options and complicates infection management. This study was conducted to determine the occurrence and characteristics of aminoglycoside-resistant *A. baumannii* isolates recovered from different clinical specimens in hospitals of Babylon Province, Iraq.

This cross-sectional study was conducted on a total of 519 clinical specimens randomly collected from hospitalized patients (inpatients) and non-hospitalized patients (outpatients) attending four major hospitals in Babylon Province over a period of ten months during 2025. All *A. baumannii* isolates were identified based on phenotypic characteristics and using conventional biochemical tests and confirmed using the VITEK-2 system.

Antimicrobial susceptibility was evaluated using the disc diffusion method against a panel of aminoglycoside antibiotics and other antibiotics, in addition to determining the minimum inhibitory concentration (MIC) for both colistin and polymyxin B.

A. baumannii was isolated from 75 specimens (14.45%). Aminoglycoside resistance was detected in 40 isolates (53.33%), of which 37 isolates (92.50%) were classified as extensively drug-resistant

(XDR), whereas 3 isolates (7.50%) were recognized as possible pandrug-resistant (PDR). High MAR index values ranging from 0.67 to 1.00 were observed among all aminoglycoside-resistant *A. baumannii* isolates, with all isolates exceeding the critical threshold of 0.2. These findings indicate extensive exposure to antimicrobial agents and a high prevalence of multiple antibiotics resistance among the studied isolates. Molecular confirmation at the species level was achieved using polymerase chain reaction (PCR) targeting the *bla*_{OXA-51-like} and 16S *rRNA* genes, followed by 16S *rRNA* gene sequencing for molecular identification and final confirmation of *A. baumannii* isolates. Aminoglycoside resistance genes were also detected, including seven genes encoding (AMEs), three genes encoding 16S rRNA methylases, and the class 1 integron gene *intI1* using conventional PCR techniques.

Investigation of aminoglycoside resistance genes revealed a high prevalence of aminoglycoside-modifying enzyme (AME) genes. Among aminoglycoside acetyltransferase (AAC) genes, *aac(6')-Ib* was the most prevalent gene (50.00%), followed by *aacC1* (20.00%) and *aac(3)-II* (15.00%). Regarding aminoglycoside phosphotransferase (APH) genes, *aph(3')-VI* (*aphA6*) was detected in 47.50% of isolates. Concerning aminoglycoside nucleotidyltransferase (ANT) genes, *ant(4')-IIb* was the most common gene (45.00%), followed by *aadA1* (7.50%), while all isolates were negative for *aadB*. In addition, 16S rRNA methylase genes were detected, including *rmtB* (30.00%), *rmtD* (15.00%), and *armA* (12.50%). Class 1 integrons were also detected in 22.50% of isolates based on amplification of the *intI1* gene.

Furthermore, sequencing analysis was performed for selected isolates harboring aminoglycoside resistance genes, including those encoding (AMEs) and 16S rRNA methylases. In addition, the genetic

relatedness among 40 aminoglycoside-resistant isolates was determined using Enterobacterial Repetitive Intergenic Consensus-PCR (ERIC-PCR).

In conclusion, the high prevalence of aminoglycoside resistance determinants, together with extensively drug-resistant (XDR) and possible pandrug-resistant (PDR) phenotypes, highlights the serious threat posed by *A. baumannii* in Babylon hospitals. The elevated MAR index values indicate high pressure resulting from antibiotic use. Genetic relatedness analysis using ERIC-PCR demonstrated possible clonal dissemination among the isolates. Therefore, rapid detection and strict implementation of infection control measures are essential to limit further spread. To the best of our knowledge, this study is the first report documenting aminoglycoside resistance determinants in *A. baumannii* isolates in Babylon Province, Iraq.

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List of Abbreviations

Abbreviation	Key
16S rRNA	16S Ribosomal Ribonucleic Acid
<i>aac(3)-II</i>	Aminoglycoside 3-N-Acetyltransferase Type II
<i>aac(6')-Ib</i>	Aminoglycoside 6'-N-Acetyltransferase Type Ib
<i>aacC1</i>	Aminoglycoside 3-N-Acetyltransferase C1
AACs	Aminoglycoside Acetyltransferases
<i>aadA1</i>	Aminoglycoside Adenylyltransferase A1
<i>AadB</i>	Aminoglycoside Adenylyltransferase B
<i>A. baumannii</i>	<i>Acinetobacter baumannii</i>
AbGRI1	<i>Acinetobacter baumannii</i> Genomic Resistance Island 1
ACB	<i>Acinetobacter calcoaceticus</i> – <i>baumannii</i> Complex
AdeABC	AdeABC Efflux Pump System
AMEs	Aminoglycoside-Modifying Enzymes

AMR	Antimicrobial Resistance
<i>ant(4')-IIb</i>	Aminoglycoside Nucleotidyltransferase (4')-IIb
ANTs	Aminoglycoside Nucleotidyltransferases
<i>aph(3')-VI</i> (<i>aphA6</i>)	Aminoglycoside Phosphotransferase (3')-VI
APHs	Aminoglycoside Phosphotransferases
ArmA	Aminoglycoside Resistance Methyltransferase A
AG	Aminoglycoside
BHI	Brain Heart Infusion
<i>Bla</i>	β -Lactamase
BLAST	Basic Local Alignment Search Tool
Bp	Base Pair
CFU	Colony Forming Unit
CLSI	Clinical and Laboratory Standards Institute
°C	Degree Celsius
DNA	Deoxyribonucleic Acid
DW	Distilled Water
ERIC-PCR	Enterobacterial Repetitive Intergenic Consensus- Polymerase Chain Reaction
G1405	Guanine at Position 1405 of 16S Rna
GB	GenBank
Gb	Gigabase
GC	Percentage of guanine and Cytosine Bases
H ₂ O ₂	Hydrogen Peroxide
H ₂ S	Hydrogen Sulfide
HGT	Horizontal Gene Transfer
ICU	Intensive Care Unit
IBM	International Business Machines Corporation
IN	Intermediate

intI1	Class 1 Integron Integrase Gene
ISAb ₁₂₅	Insertion Sequence Ab ₁₂₅
KOH	Potassium Hydroxide
LOS	Lipooligosaccharide
LPS	Lipopolysaccharide
MDR	Multidrug Resistant
MEGA	Molecular Evolutionary Genetics Analysis
Mg	Milligram
MIC	Minimum Inhibitory Concentration
ml	Milliliter
Mm	Millimolar
mRNA	Messenger Ribonucleic Acid
MR-VP	Methyl Red–Voges–Proskauer
NCBI	National Center for Biotechnology Information
ng	Nano gram
Nm	Nano meter
OMPs	Outer Membrane Proteins
ORF	Open Reading Frame
<i>OXA-51</i>	Variant of Oxacillinases
PCR	Polymerase Chain Reaction
PDR	Pandrug Resistant
Ph	Potential of Hydrogen
PICU	Pediatric Intensive Care Unit
PMF	Proton Motive Force
<i>RmtB</i>	16S rRNA Methyltransferase B
<i>RmtD</i>	16S rRNA Methyltransferase D
RNA	Ribose nucleic acid
RND	Resistance–Nodulation–Division
SPSS	Statistical Package for the Social Sciences
T1SS	Type I Secretion System

T2SS	Type II Secretion System
TBE	Tris-Borate-EDTA Buffer
TE	Tris-EDTA Buffer
TSI	Triple Sugar Iron Agar
U	Units
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
VITEK 2	VITEK 2 Compact System
WHO	World Health Organization
XDR	Extensively Drug Resistant
Mg	Microgram
ml	Microliter

CHAPTER ONE

INTRODUCTION

1. Introduction

Acinetobacter baumannii is a Gram-negative opportunistic pathogen that has emerged as one of the most important causes of hospital-acquired infections worldwide. (Nguyen & Joshi, 2021). It is commonly associated with a wide range of clinical infections, including burn wound infections, bloodstream infections, ventilator-associated pneumonia, urinary tract infections, and wound infections (Stanley *et al.*, 2023). Among these, burn wound infections represent the highest proportion of cases in many hospital settings and are associated with increased morbidity and mortality, particularly in critically ill patients. The persistence of *A. baumannii* in hospital environments and its ability to survive for prolonged periods on inanimate surfaces contribute significantly to its transmission and epidemiological success. (Javadi, 2024).

Moreover, this organism has a remarkable capacity to acquire antimicrobial resistance determinants through mobile genetic elements such as plasmids, transposons, and integrons, facilitating rapid adaptation to antimicrobial pressure (Noel *et al.*, 2022). Antimicrobial resistance (AMR) represents a major global health threat and contributes substantially to the global burden of infectious diseases, particularly in healthcare settings (WHO, 2023). The World Health Organization (WHO) recognizes AMR as one of the most serious challenges facing modern medicine due to its impact on treatment failure, increased mortality, prolonged hospitalization, and limited therapeutic options, especially in infections caused by multidrug-resistant Gram-negative bacteria (Dubey *et al.*, 2025). The treatment of *A. baumannii* infections has become increasingly difficult due to the global emergence of (MDR), (XDR), and (PDR) strains (Rafailidis *et al.*, 2024).

Worldwide, a high proportion of isolates exhibited XDR and PDR phenotypes, reflecting a severe limitation in effective therapeutic options and representing a major public health concern in hospital environments. Aminoglycoside antibiotics, including gentamicin, amikacin, and tobramycin, are widely used for the treatment of severe Gram-negative bacterial infections (Reina *et al.*, 2022; Thy *et al.*, 2023). These antibiotics exert their bactericidal effect by binding irreversibly to the 30S ribosomal subunit, thereby inhibiting protein synthesis in a concentration-dependent manner. However, resistance to aminoglycosides in *A. baumannii* has become increasingly prevalent (Nicholson *et al.*, 2020).

The main mechanisms of aminoglycoside resistance include the production of (AMEs), such as ACC (*aacC1*, *aac(3)-II*, *aac(6')-Ib*), APH (*aph(3)-VIa*), and ANT (*aadA1*, *aadB*, *ant(4'')-IIb*), which chemically modify and inactivate the aminoglycoside antibiotic (Saleh *et al.*, 2023; Scoffone *et al.*, 2025). Another important mechanism is high-level resistance mediated by 16S rRNA methylase, including *armA*, *rmtB*, and *rmtD*, which prevent aminoglycoside binding to the ribosomal target site. In addition, alterations in membrane permeability and other intrinsic bacterial mechanisms may contribute to reduced intracellular accumulation of aminoglycosides (Yang & Hu, 2022).

These resistance determinants are frequently located on mobile genetic elements such as plasmids, transposons, and integrons, facilitating horizontal gene transfer and rapid dissemination among clinical isolates. Class 1 integrons (*intI1*) play a key role in the accumulation and spread of antimicrobial resistance genes in hospital environments (Nemec *et al.*, 2004; Lin *et al.*, 2010; Firoozeh *et al.*, 2023). The intrinsic *bla*_{OXA-51}-like gene is commonly present in *A. baumannii* and used as a molecular marker for species confirmation (Al-Dahlaki, 2020). In addition, the 16S rRNA

gene was also used for molecular identification and confirmation of all isolates (Ghaima *et al.*, 2015).

In Iraq, particularly in Babylon Governorate, limited data are available regarding the molecular characterization of aminoglycoside-resistant *A. baumannii* isolated from different clinical sources, including burn wounds, blood, sputum, wound swabs, and urine. The lack of comprehensive surveillance systems and the widespread misuse of antibiotics have contributed to the increasing burden of antimicrobial resistance in hospital environments. Therefore, understanding the molecular mechanisms underlying aminoglycoside resistance and the epidemiology of resistant *A. baumannii* isolates is essential for improving infection control strategies and guiding appropriate therapeutic approaches.

1.1. Aim of the Study

The present study aims to investigate the occurrence and molecular characterization of aminoglycoside-resistant *A. baumannii* isolates recovered from different clinical specimens in hospitals of Babil Governorate, Iraq.

This study aim to achieve the following objectives:

1. To isolate, identify, and confirm *A. baumannii* from different clinical specimens.
2. To determine the antibiotic susceptibility profiles of the isolates and classify them into (MDR), (XDR), and (PDR) phenotypes.
3. To detect the 16S rRNA and *bla*_{OXA-51-like} genes and sequence the 16S rRNA gene for molecular identification and confirmation of *A. baumannii* isolates.
4. To investigate aminoglycoside resistance genes, including aminoglycoside-modifying enzymes (AMEs) and 16srRNA

methylase genes and *intI1* among aminoglycoside- resistant isolates using PCR technique.

5. To perform sequencing of the aminoglycoside resistance genes in selected resistant isolates to confirm molecular results.
6. To assess the genetic relatedness and clonal diversity of aminoglycoside-resistant isolates using ERIC-PCR typing.

CHAPTER TWO

LITERATURES REVIEW

2. Literature Review

2.1. Historical Overview and General Characteristics of *A. baumannii*

Acinetobacter. baumannii is a relatively recent addition to the list of major hospital-acquired pathogens, although members of the genus *Acinetobacter* were first described in the early 20th century. Early reports of similar organisms date back to 1911; however, formal taxonomic classification of the genus was not established until later (Yehya *et al.*, 2025). In 1954, Brisou and Prévot proposed the genus *Acinetobacter*, emphasizing its non- motile nature, which is reflected in its name derived from the Greek word *akinetos* meaning "non-motile Subsequent systematic microbiological studies in the 1960s led to the recognition of multiple species within the genus."including the identification of *A. baumannii* as a distinct clinically important species (Muleshkova *et al.*, 2025).

Although initially considered an environmental organism with limited clinical relevance, *A. baumannii* has progressively emerged as a significant opportunistic pathogen, particularly in hospital environments. The clinical importance of *A. baumannii* increased markedly during the 1970s and 1980s, when it began to be frequently isolated from intensive care unit (ICU) patients (McNeilly, 2023). Its global significance further escalated in the early 2000s during military conflicts, when MDR strains were isolated from injured soldiers returning from Iraq, leading to the informal designation "Iraqibacter" (Guide, 2010). *A. baumannii* appears as short Gram-negative coccobacilli. In some clinical specimens, it may resemble Gram-positive cocci due to variable decolorization during Gram staining (Rogers, 2021). The cells typically measure approximately 1.0-1.5 um and may occur singly, in pairs, or short chains (Sufa *et al.*, 2023; Dave, 2025).

A. baumannii is an obligate aerobic organism. It is catalase-positive and oxidase-negative, a key diagnostic feature that distinguishes it from other non-fermenting Gram-negative bacilli (Ghimire, 2020). It does not ferment glucose and is indole- and urease-negative. The genomic GC content ranges from 39% to 47% (Muleshkova *et al.*, 2025). On culture media, it grows readily on blood agar and MacConkey agar, forming smooth, non-pigmented colonies. On MacConkey agar, colonies remain colorless due to its non- lactose fermenting nature (Mohamed, 2016). A distinctive phenotypic characteristic is its ability to grow at temperatures up to 44°C, which differentiates it from other *Acinetobacter* species (Nurjadi & Boutin, 2022).

One of the most clinically significant features of *A. baumannii* is its remarkable environmental resilience. It can survive for prolonged periods on dry surfaces such as hospital beds, medical equipment, and door handles, contributing to nosocomial transmission. This persistence is supported by its genetic adaptability and ability to acquire antimicrobial resistance determinants (Abiola, 2019). The organism has demonstrated a strong capacity to acquire resistance to multiple antibiotic classes, including carbapenems, cephalosporins, and aminoglycosides (Kyriakidis *et al.*, 2021). Major resistance mechanisms include efflux pumps, β -lactamase production, and alterations in outer membrane proteins (Dolma & WM, 2022). *A. baumannii* is now recognized as one of the most important causes of healthcare-associated infections worldwide, particularly in ICUs. It is frequently associated with ventilator- associated pneumonia, bloodstream infections, wound infections, and urinary tract infections (Chandra *et al.*, 2022). Due to its XDR, the WHO has classified it as a "critical priority pathogen" for antibiotic research and development (da Silva *et al.*, 2024).

2.2. Taxonomy of *A. baumannii*

Acinetobacter. baumannii belongs to the genus *Acinetobacter*, which was first formally proposed by Brisou and Prévot in 1954. Early classification within this genus was primarily based on phenotypic characteristics; however, subsequent taxonomic studies led to the recognition of multiple distinct species with clinical and environmental significance, including *A. baumannii*, *A. calcoaceticus*, *A. Iwoffli*, *A. haemolyticus*, *A. Johnsonii*, and *A. junii* (Mateo-Estrada *et al.*, 2019). Currently, the genus *Acinetobacter* comprises more than 50 validly recognized species (Qin *et al.*, 2021). Among these, several closely related species form the *Acinetobacter calcoaceticus-baumannii* (ACB) complex, which includes *A.baumannii*, *A. pittii*, *A. nosocomialis*, *A. seifertii*, and *A. dijkshoorniae* (Carvalho, 2021; Pidsadny, 2025). These species are phenotypically similar and often indistinguishable using routine laboratory methods, which complicates accurate species-level identification in clinical diagnostics. The taxonomic classification of *A. baumannii* is summarized as follows (Van Assche, 2019):

Domain: Bacteria

Phylum: Proteobacteria

Class: Gamma proteobacteria

Order: Pseudomonadales

Family: Moraxellaceae

Genus: *Acinetobacter*

Species: *A. baumannii*

2.3. Identification of *A. baumannii*

Accurate laboratory identification is essential for the proper diagnosis, epidemiological surveillance, and antimicrobial management of infections caused by *A.baumannii*. Initial isolation is performed using routine microbiological culture techniques on standard media, followed by phenotypic characterization and confirmatory molecular assays (Munson & Carroll, 2019; Mc Andrew, 2023). The organism grows well on blood agar and tryptic soy agar, producing small, smooth, convex, gray-white colonies after 18-24 hours of incubation at 37°C. It is a strictly aerobic, non-fermentative Gram-negative coccobacillus that is catalase-positive and oxidase-negative, which assists in preliminary differentiation from other non- fermenting Gram-negative bacilli (Munson & Carroll, 2019).

Selective and chromogenic media have been developed to facilitate rapid detection of MDR strains by inhibiting competing flora and allowing presumptive identification based on enzymatic reactions and colony coloration; however, these methods cannot reliably distinguish *A. baumannii* from other members of the *Acinetobacter calcoaceticus-baumannii* (ACB) complex, and therefore require confirmatory testing (Santos, 2016; Saad, 2022). Phenotypic identification is further supported by its non- motility, inability to ferment carbohydrates, and absence of urease and indole production, although these biochemical characteristics are insufficient for definitive species identification. Molecular diagnostic methods provide the highest level of accuracy, particularly through detection of the intrinsic *bla*_{OXA-51-like} gene, which encodes a class D carbapenemase and serves as a reliable species-specific genetic marker for *A. baumannii*. PCR-based detection of this gene enables rapid and precise identification directly from clinical isolates (McAndrew, 2023; Ayams, 2023). Currently, molecular techniques such as enterobacterial repetitive

intergenic consensus polymerase chain reaction (ERIC-PCR) and sequencing are considered important tools in epidemiological investigations and molecular characterization of *A. baumannii* isolates. ERIC-PCR is widely used to determine the genetic relatedness and genetic diversity among bacterial isolates, whereas sequencing is used for accurate detection and confirmation of antimicrobial resistance genes. These techniques contribute to a better understanding of the transmission dynamics and dissemination of resistant strains in healthcare settings (Aljindan *et al.*, 2018; Anwer *et al.*, 2026).

2.4. Epidemiology of *A. baumannii*

The global burden of healthcare-associated infections caused by MDR Gram-negative bacteria has risen substantially, with *A. baumannii* now recognized as a leading opportunistic pathogen in ICUs, particularly among critically ill and immunocompromised patients (Teerawattanapong *et al.*, 2018). *A. baumannii* is strongly associated with hospital environments, where it is frequently isolated from diverse clinical specimens, reflecting its ecological adaptability and capacity for both colonization and invasive disease (Abban *et al.*, 2023; Ceparano, 2025). It is implicated in ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound and burn infections, and, less commonly, meningitis, with invasive infections particularly bloodstream and respiratory tract infections being associated with severe outcomes such as sepsis, organ failure, and increased mortality (Dolma & WM, 2022; Cavallo *et al.*, 2023; Ahuatzin-Flores *et al.*, 2024).

The epidemiological impact is most pronounced in high-risk populations, including intensive care patients, elderly individuals, neonates, burn victims, and patients exposed to prolonged hospitalization or invasive devices such as mechanical ventilation, urinary catheters, and central venous

lines (García-Patiño *et al.*, 2017; Haque *et al.*, 2018; Yao *et al.*, 2023). These conditions facilitate both colonization and subsequent infection, particularly in settings where skin or mucosal barriers are compromised. The global dissemination of *A. baumannii* is primarily driven by the emergence of MDR clones, extensive antimicrobial pressure, and healthcare-associated transmission, factors that have led to its designation by the World Health Organization as a critical-priority pathogen requiring urgent therapeutic innovation (Rangel & De-Simone, 2024).

Although community-acquired cases remain uncommon, the organism exhibits significant geographical variability influenced by infection control practices and antimicrobial stewardship policies (Agyepong *et al.*, 2023; Asmare *et al.*, 2025). *A. baumannii* represents a major nosocomial threat due to its ecological resilience, propensity for severe invasive disease in vulnerable hosts, and strong association with multidrug resistance, collectively complicating global prevention and treatment strategies (Zhang *et al.*, 2021).

2.5. Pathogenicity of *A. baumannii*

The clinical success of *A. baumannii* as a nosocomial pathogen is largely attributed to its remarkable ability to survive under stressful environmental conditions and efficiently adapt within the host, particularly in critically ill and immunocompromised patients (Sehree *et al.*, 2021; Rangel & De-Simone, 2024). A key feature contributing to its pathogenic potential is its ability to persist under adverse environmental conditions, including desiccation and exposure to commonly used hospital disinfectants, which allows prolonged survival on inanimate surfaces and facilitates nosocomial transmission (Javadi, 2024).

Another important virulence-associated mechanism is biofilm formation, where the organism develops structured microbial communities on biological tissues and medical devices such as catheters, ventilator tubing, and implants. These biofilms are embedded in an extracellular matrix composed of polysaccharides, proteins, and extracellular DNA, which enhances bacterial protection and persistence while reducing antimicrobial penetration and host immune clearance (Gedefie *et al.*, 2021; Roy *et al.*, 2022). This contributes to chronic and difficult-to-treat infections. The pathogenic success of *A. baumannii* is further supported by multiple factors that enhance survival under antimicrobial pressure, including the production of β -lactamases (particularly OXA-type enzymes), overexpression of efflux pump system and modifications in outer membrane proteins that reduce antibiotic uptake (Abdi *et al.*, 2020; Kyriakidis *et al.*, 2021). Infections caused by *A. baumannii*, including ventilator-associated pneumonia, bloodstream infections, wound infections, and meningitis, are associated with high morbidity and mortality rates, particularly in intensive care units where patients are highly vulnerable and treatment options are limited (Ju *et al.*, 2018), the combination of environmental persistence, biofilm formation, and adaptive resistance mechanisms makes *A. baumannii* a highly successful and clinically significant pathogen (Roy *et al.*, 2022).

2.6. Association of *A. baumannii* with Multiple Infections

The clinical significance of *A. baumannii* is largely attributed to its broad involvement in healthcare-associated infections, driven by its remarkable ability to colonize diverse anatomical sites and persist within hospital environments (Abban *et al.*, 2023). This adaptability enables the organism to be frequently isolated from a wide range of clinical specimens, reflecting its capacity to establish both localized and systemic infections in

susceptible patients. Clinical isolates of *A. baumannii* generally demonstrate enhanced pathogenic potential compared to environmental strains. This increased virulence is linked to the acquisition of specialized determinants that support host interaction and survival, including outer membrane proteins involved in adhesion, iron acquisition systems essential for growth under iron-limited conditions, and secretion systems that facilitate host cell interaction and tissue colonization (Havenga *et al.*, 2022; EL-Hakeem *et al.*, 2023).

A key factor contributing to its success as a hospital-adapted pathogen is its high genetic plasticity. Horizontal gene transfer plays a central role in the acquisition of both virulence traits and antimicrobial resistance determinants, promoting the emergence and persistence of successful MDR clones in healthcare settings (Sykes, Deo, & Kumar, 2020). At the molecular level, structural components such as the polysaccharide capsule, lipooligosaccharide (LOS), and outer membrane proteins enhance bacterial adhesion, immune evasion, and survival within the host. In particular, LOS contributes to membrane stability and reduces susceptibility to antimicrobial agents, thereby improving persistence under hostile conditions (Ayoub & Halat, 2020; Powers, 2020; Simpson *et al.*, 2021).

Secreted virulence factors, including phospholipase D, proteases, and outer membrane vesicles, play a significant role in host tissue damage and the induction of inflammatory responses during infection. Furthermore, the ability of *A. baumannii* to form biofilms on indwelling medical devices such as catheters and ventilator equipment significantly enhances its persistence and contributes to the development of chronic and difficult-to-treat infections (Ma & McClean, 2021; Shadan *et al.*, 2023). The combined effects of host adaptability, multifactorial virulence

mechanisms, and biofilm-mediated persistence underpin the ability of *A. baumannii* to cause a wide spectrum of healthcare-associated infections with significant clinical impact (Lucidi *et al.*, 2024).

2.7. Virulence factors of *A. baumannii*

Several virulence-associated determinants contribute to the persistence, adaptability, and pathogenic potential of *A. baumannii*. These traits are further enhanced by environmental resilience and tolerance to disinfectants, which collectively support bacterial survival on hospital surfaces and facilitate infection in vulnerable patients (Zeighami *et al.*, 2019; Morris *et al.*, 2019). A central component of its virulence is the polysaccharide capsule, which provides protection against host immune responses, particularly phagocytosis, and enhances bacterial survival during infection. In addition, biofilm-forming capacity represents a major determinant of persistence, allowing the organism to adhere to abiotic surfaces and medical devices while increasing tolerance to antibiotics through the formation of a protective extracellular matrix. This biofilm-associated lifestyle is strongly linked to chronic and device-related infections (Lucidi *et al.*, 2024).

Iron acquisition systems are also essential for survival in host environments, where free iron is limited. These systems enable efficient sequestration of iron from host proteins, supporting bacterial growth and metabolic activity during infection. Outer membrane proteins contribute further to virulence by mediating adhesion to host tissues and facilitating initial stages of colonization (Zhang *et al.*, 2025). Secretion systems play an additional role in pathogenicity by exporting effector molecules that interact with host cells. These systems promote bacterial adaptation and contribute to tissue damage and modulation of host responses, thereby supporting infection progression (Li *et al.*, 2023). These virulence factors

interact to enhance bacterial adaptation, persistence, and survival within host-associated environments, enabling successful establishment of infection across multiple anatomical sites and contributing to the clinical significance of this pathogen (Lucidi *et al.*, 2024).

2.7.1. Outer Membrane Proteins (Porins)

The permeability of the outer membrane in Gram-negative bacteria plays a critical role in determining the intracellular accumulation and overall efficacy of antimicrobial agents, including aminoglycosides (Xu *et al.*, 2026). OMPs form β -barrel channel structures embedded in the outer membrane, which are involved in regulating permeability to small molecules, including aminoglycosides. Among these proteins, OmpA is a major structural component that maintains membrane integrity and influences membrane permeability. Alterations in OmpA expression or structure may reduce membrane permeability, thereby limiting aminoglycoside entry into the bacterial cell and directly contributing to reduced antimicrobial efficacy (Nie *et al.*, 2020; Tan & Cao, 2023). Since aminoglycosides require active intracellular accumulation to bind to the 30S ribosomal subunit and inhibit protein synthesis, any reduction in outer membrane permeability represents a critical mechanism influencing bacterial susceptibility. Therefore, changes in OMPs can directly affect aminoglycoside uptake and play a key role in the development of resistance in *A. baumannii* (Kyriakidis *et al.*, 2021).

2.7.2. Efflux Pump System

Active transport systems that extrude toxic compounds constitute an important bacterial defense strategy against antimicrobial stress. *A. baumannii* possesses efficient efflux pump systems that play an important role in lowering intracellular antibiotic concentrations, thereby reducing drug effectiveness and contributing to resistance (Sharma *et al.*, 2023).

Efflux pumps in Gram-negative bacteria are typically composed of a tripartite structure spanning the inner membrane, periplasmic space, and outer membrane, forming a continuous channel that enables direct export of toxic compounds (Abdi *et al.*, 2020).

Among these systems, the Resistance-Nodulation-Division (RND) family is the most clinically important in *A. baumannii*. In particular, the AdeABC efflux system is strongly associated with multidrug resistance and reduced antibiotic susceptibility. This system contributes to decreased intracellular accumulation of several antibiotics, including aminoglycosides, thereby limiting their therapeutic effectiveness (Kyriakidis *et al.*, 2021). Efflux pump systems therefore represent a key resistance mechanism that reduces intracellular drug concentration and contributes to therapeutic failure and persistence of infection in healthcare-associated environments (Russo & Serapide, 2025).

2.7.3. Biofilm Formation and Quorum Sensing

Surface-associated bacterial communities provide a highly protective environment that enhances survival under antimicrobial pressure. *A.baumannii* is characterized by its strong ability to form biofilms, which play an important role in bacterial persistence and reduced susceptibility to antibiotics, including aminoglycosides. Biofilms consist of bacterial communities embedded in a protective extracellular matrix that limits antibiotic penetration and reduces drug effectiveness (López- Martin *et al.*, 2021). Biofilm formation proceeds through sequential stages including initial surface attachment, development of bacterial microcolonies, and maturation into a structured multicellular community. This structured organization contributes to increased resistance by restricting antimicrobial access and promoting bacterial survival under antimicrobial pressure (Gedefie *et al.*, 2021).

Key surface components such as OmpA contribute to bacterial adhesion and early biofilm development, enhancing the ability of *A. baumannii* to colonize surfaces and medical devices (Pathoor *et al.*, 2024). In addition, extracellular polysaccharides play a central role in stabilizing the biofilm structure and strengthening bacterial aggregation, thereby increasing tolerance to antibiotics (Flannery, 2019). Cell-to-cell communication through quorum sensing further regulates biofilm development. In *A. baumannii*, signaling molecules such as acyl-homoserine lactones (AHLs) control the expression of genes involved in adhesion and biofilm maintenance, promoting bacterial persistence under stressful conditions (Saipriya *et al.*, 2020). Biofilm formation represents an important non-genetic mechanism that reduces aminoglycoside susceptibility by limiting antibiotic penetration and enhancing bacterial survival (Mohamed *et al.*, 2023).

2.7.4. Protein Secretion System

Protein export systems are essential for bacterial adaptation and interaction with the host environment, contributing significantly to pathogenicity and persistence. Protein secretion systems in *A. baumannii* play an important role in bacterial survival, adaptation, and persistence under antimicrobial pressure by facilitating the export of proteins involved in host interaction and colonization (Li *et al.*, 2023). Among the main systems, the Type I secretion system (T1SS) enables direct translocation of effector proteins across the bacterial membranes, while the Type II secretion system (T2SS) is responsible for exporting enzymes that contribute to bacterial adaptation and interaction with the surrounding environment (Li *et al.*, 2023; Lucidi *et al.*, 2024).

The Type V secretion system (T5SS), represented by adhesin proteins such as Ata, mediates bacterial attachment to host surfaces and contributes to

colonization and biofilm formation, which are important factors in bacterial persistence (Pereira & Hartwig, 2025). protein secretion systems support the ability of *A. baumannii* to colonize and persist in host environments, thereby enhancing its survival under antimicrobial pressure, including exposure to aminoglycosides (Li *et al.*, 2023).

2.8. Antibiotic Resistance and Treatment Options

The therapeutic challenge posed by *A. baumannii* is mainly driven by its increasing resistance to multiple antibiotic classes, leading to the global emergence of (MDR) and (XDR) strains associated with healthcare-associated infections. These resistant phenotypes are frequently linked to outbreaks in intensive care settings, where treatment options are severely limited (Vázquez-López *et al.*, 2020). Resistance extends to most commonly used antimicrobial agents, including β -lactams, carbapenems, fluoroquinolones, tetracyclines, chloramphenicol, and aminoglycosides among these, aminoglycoside resistance is particularly clinically significant due to its impact on combination therapy used in severe infections (Kyriakidis *et al.*, 2021). The spread of resistance is largely mediated by horizontal gene transfer through plasmids, transposons, and integrons, enabling rapid dissemination of resistance determinants among bacterial populations (Noel *et al.*, 2022). This process is strongly amplified by inappropriate antibiotic use, which creates selective pressure favoring resistant strains (Roy *et al.*, 2022).

Multiple molecular mechanisms contribute to resistance, including antibiotic-modifying enzymes, reduced membrane permeability, efflux pump overexpression, and mutations in antibiotic target sites (Vrancianu *et al.*, 2020). Environmental persistence on medical surfaces and equipment further facilitates transmission within clinical environments (Ding *et al.*, 2026). Biofilm formation enhances this resistance profile by

limiting antibiotic penetration and promoting genetic exchange between bacterial cells, which supports persistent and recurrent infections (Roy *et al.*, 2022).

2.9. Mobile Genetic Elements in *A. baumannii*

Genetic adaptability in bacteria is largely driven by mobile DNA elements that facilitate the acquisition and dissemination of antimicrobial resistance determinants. These elements significantly enhance genome plasticity and contribute to bacterial adaptation under antimicrobial selective pressure (Wu *et al.*, 2023; Scoffone *et al.*, 2025). The spread of resistance genes in *A. baumannii* occurs mainly through horizontal gene transfer mechanisms, particularly transformation and conjugation, which facilitate the exchange of genetic material between bacterial cells and promote rapid adaptation to antibiotic exposure (Godeux *et al.*, 2022). This process is further supported by mobile genetic platforms such as plasmids, transposons, and integrons, which act as key vehicles for the accumulation and dissemination of resistance genes, including those involved in aminoglycoside resistance (Naderi *et al.*, 2023). The combined activity of these genetic mechanisms increases genome flexibility and accelerates the evolution of resistance traits, allowing *A. baumannii* to persist under strong antimicrobial pressure. This genetic adaptability plays a major role in the survival of resistant strains and contributes to the difficulty of controlling their spread in clinical environments (Weinberg *et al.*, 2020).

2.9.1. Plasmids

Plasmids are extrachromosomal DNA elements that play a key role in antimicrobial resistance dissemination by carrying genes that enhance bacterial survival under antibiotic pressure (Weber *et al.*, 2016). Their ability to replicate independently and transfer between

bacteria makes them major drivers of resistance evolution. Conjugative transfer represents the main mechanism for plasmid spread, enabling direct gene exchange between bacterial cells and facilitating rapid dissemination of MDR (Ababneh *et al.*, 2023). Many plasmids in *A. baumannii* carry genes encoding resistance to multiple antibiotic classes, including aminoglycosides, which remain important in severe Gram-negative infections (Vrancianu *et al.*, 2020). These resistance genes often coexist with other determinants, forming complex MDR regions, while some additional resistance factors may also be chromosomally encoded (Chapartegui-González *et al.*, 2021). Plasmid diversity contributes to variability in resistance patterns among clinical isolates and plays a major role in the global spread of MDR strains (Salgado-Camargo *et al.*, 2020). The interaction between plasmid-borne and chromosomal resistance determinants has been central to the emergence of highly resistant clinical lineages (Nasr, 2020; Chakravarty, 2020).

2.9.2. Transposable Elements

Genome plasticity in *A. baumannii* is strongly influenced by mobile genetic elements, including transposable sequences capable of relocating within or between genomic regions, thereby promoting genetic adaptation and facilitating the dissemination of antimicrobial resistance determinants under selective antibiotic pressure (Noel *et al.*, 2022). Insertion sequences are the most abundant form in *A. baumannii* and, although they encode only transposase enzymes, they can strongly influence gene expression when located near resistance genes. In some cases, they enhance transcription by providing promoter sequences or form composite transposons when flanking resistance genes (Xie *et al.*, 2025). ISAba125 has been associated with

aminoglycoside resistance genes such as *aphA6*, increasing expression and facilitating horizontal transfer of resistance determinants (Naderi *et al.*, 2023). Other genes such as *aadB* and *aacC1* are also frequently linked to mobile genetic platforms contributing to aminoglycoside resistance (Rizk & Abou El-Khier, 2019). These elements collectively enhance genetic flexibility and accelerate the evolution of resistant strains under antibiotic selective pressure (Popa *et al.*, 2018).

2.9.3. Gene Cassettes and Integrons

The accumulation of antimicrobial resistance genes is frequently mediated by specialized genetic platforms that enable the capture and expression of multiple resistance determinants within a single genetic framework. Among these elements, integrons are particularly important due to their ability to acquire gene cassettes through site-specific recombination leading to the assembly of diverse resistance genes within a common genetic structure (Xu *et al.*, 2020). Class 1 integrons are the most clinically relevant and are widely associated with MDR clinical isolates. These integrons function as genetic platforms that capture and express gene cassettes encoding resistance to multiple antibiotic classes, including aminoglycosides. Common gene cassettes include *aacA4*, *aadA1*, *aacC1*, *aphA1b*, and *armA*, which contribute to resistance against gentamicin, amikacin, and tobramycin through enzymatic modification or target alteration (Huang *et al.*, 2015; Xu *et al.*, 2020). These gene cassettes are frequently embedded within mobile genetic elements such as plasmids and transposons, which facilitates their horizontal transfer between bacterial strains. This organization significantly enhances the spread of aminoglycoside resistance genes across clinical isolates and

contributes to the persistence of MDR populations in healthcare-associated settings (Anderson *et al.*, 2020). In addition to standalone integrons, these gene cassette arrays are often incorporated into larger genomic resistance islands, such as AbGRI1-AbGRI5. These regions serve as reservoirs of multiple antibiotic resistance determinants and may contain several aminoglycoside resistance genes, including *strA*, *strB*, *aadB*, *aadA2*, *aacC1*, *aphA1b*, and *armA*. The coexistence of these genes within the same genetic structure enables bacteria to resist antibiotics through different enzymatic mechanisms such as acetylation, phosphorylation, adenylation, and ribosomal modification (Blackwell *et al.*, 2017; Chan *et al.*, 2020; Hua *et al.*, 2021). The accumulation of gene cassettes within integron systems significantly increases the adaptability of *A. baumannii*, allowing rapid response to antimicrobial pressure and contributing to the persistence and dissemination of resistant strains. Continuous molecular surveillance of these genetic structures is therefore essential for understanding the evolution of aminoglycoside resistance and tracking its spread among clinical isolates (Ilyas *et al.*, 2024).

2.10. Aminoglycoside Antibiotics

Aminoglycosides are a class of bactericidal antibiotics that exhibit strong activity primarily against aerobic Gram-negative bacteria, including clinically significant pathogens such as *A.baumannii*. Commonly used agents in this group include amikacin, gentamicin, tobramycin, neomycin and netilmicin, which are frequently applied in the treatment of severe and life-threatening infections caused by MDR organisms (Kishk *et al.*, 2021). The history of aminoglycosides began in 1944 with the discovery of streptomycin, the first aminoglycoside antibiotic, isolated from *Streptomyces*

griseus by Selman Waksman and his colleagues. Subsequently, additional aminoglycosides were identified from various *Streptomyces* species, including neomycin in 1949 and kanamycin in 1957. In the 1960s, gentamicin was isolated from *Micromonospora purpurea*, followed by the discovery of other naturally occurring aminoglycosides such as tobramycin. During the 1970s, semi-synthetic derivatives including amikacin, dibekacin, isepamicin, and netilmicin were developed to overcome emerging resistance mechanisms associated with earlier aminoglycosides (Mingeot-Lecleroq *et al.*, 1999; Mitscher, 2002). Because most aminoglycosides were originally isolated from either *Streptomyces* or *Micromonospora* species, their nomenclature is largely based on their microbial source (Paul, 2009).

Structurally, aminoglycosides are composed of amino-modified sugars linked to an aminocyclitol core, as shown as Figure (2-1) which allows them to interact electrostatically with negatively charged bacterial cell components. Their chemical structure is directly related to their ability to penetrate bacterial cells and bind to ribosomal targets (Crotteau *et al.*, 2023). The antibacterial activity of aminoglycosides is based on their ability to bind to the 30S ribosomal subunit, specifically targeting the 16S rRNA within the decoding site. This binding disrupts the fidelity of protein synthesis by interfering with codon-anticodon recognition, leading to misreading of mRNA, incorporation of incorrect amino acids, and production of abnormal or nonfunctional proteins (Morgan *et al.*, 2020). The accumulation of these defective proteins ultimately results in disruption of cellular processes and bacterial cell death (Gedefie *et al.*, 2021).

A key characteristic of aminoglycosides is their concentration-dependent bactericidal activity, meaning that higher drug concentrations result in faster and more effective bacterial killing. This property also explains their clinical use in once- daily dosing regimens in certain infections to maximize efficacy while minimizing toxicity (Pagkalis *et al.*, 2011). The uptake of aminoglycosides into bacterial cells is oxygen-dependent, which limits their activity to aerobic organisms. Initial entry occurs through interaction with the bacterial outer membrane, followed by energy-dependent transport into the cytoplasm. This process is essential for achieving sufficient intracellular concentrations required for ribosomal binding and antibacterial activity (Powers, 2020; Lang *et al.*, 2023).

Aminoglycosides are used in the management of severe infections, including ventilator-associated pneumonia, bloodstream infections, urinary tract infections, and complicated wound infections. In many cases, they are administered as part of combination therapy to enhance antibacterial efficacy and reduce the risk of resistance development (Kyriakidis *et al.*, 2021). Despite their effectiveness, the clinical utility of aminoglycosides is limited by their narrow therapeutic index and potential toxicity, particularly nephrotoxicity and ototoxicity. These adverse effects restrict their prolonged use and require careful monitoring of serum drug levels during treatment (Thy *et al.*, 2023).

Due to the increasing prevalence of resistant organisms, aminoglycosides remain important therapeutic agents in combination regimens, especially when treating infections caused by MDR Gram-negative bacteria, where few alternative options are available (Karakonstantis *et al.*, 2020)

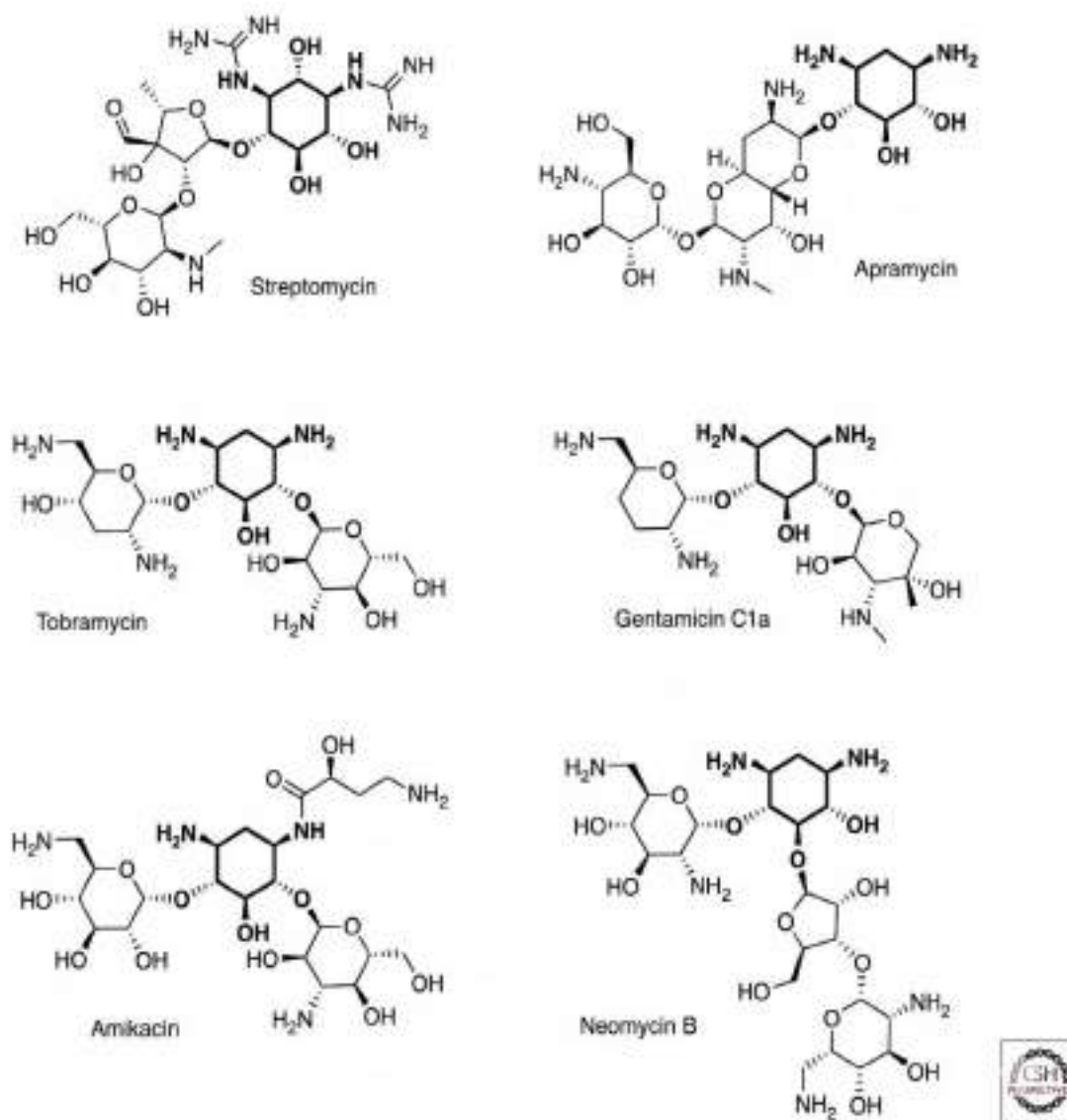


Figure (2-1): Structures of representative aminoglycosides, including the atypical aminoglycosides streptomycin and apramycin, 4,6-substituted AGs tobramycin, gentamcin, and amikacin, and the 4,5-substituted AG neomycin. The deoxystreptamine or streptidine rings are in bold (Krause *et al.*, 2016)

2.11. Mechanism of Action and Clinical Use in *A. baumannii*

Aminoglycoside antibiotics exert their antibacterial activity through a multistep process that begins with interaction at the bacterial outer surface and culminates in inhibition of protein synthesis. The initial stage involves electrostatic binding between the positively charged aminoglycoside molecules and negatively charged components of the outer membrane, particularly (LPS) as shown as

Figure (2-2) . This interaction destabilizes the outer membrane and facilitates initial penetration of the drug (Lang *et al.*, 2023). Following this step, aminoglycosides may enter the periplasmic space through porin channels or membrane disruptions. The subsequent uptake into the cytoplasm occurs in an energy- dependent manner. This process is divided into two distinct phases. The first phase (Energy-dependent phase I) is a slow transport process driven by the proton motive force (PMF), which allows limited intracellular accumulation of the antibiotic. During this stage, transport systems associated with carbohydrate uptake may also contribute to aminoglycoside entry, although the exact relationship between these systems and PMF-dependent transport remains not fully clarified (Powers, 2020).

Once a threshold intracellular concentration is reached, energy-dependent phase II is initiated. This phase is characterized by accelerated uptake of aminoglycosides, which is strongly associated with membrane damage caused by mistranslated proteins. The accumulation of defective proteins in the cytoplasmic membrane increases permeability, further enhancing antibiotic entry and creating a self-amplifying cycle that ultimately leads to rapid bacterial cell death (Shi *et al.* , 2024).

Inside the bacterial cell, aminoglycosides bind irreversibly to the 30S ribosomal subunit, specifically targeting the 16S rRNA at the decoding (A) site. This interaction interferes with codon- anticodon recognition during translation, resulting in misreading of mRNA, incorporation of incorrect amino acids, and production of nonfunctional or toxic proteins (AL-Dahlaki *et al.*, 2020 Morgan *et al.*, 2020). The accumulation of abnormal proteins disrupts essential cellular processes and contributes to membrane instability and

metabolic collapse (Morris *et al.*, 2019). In addition to ribosomal targeting, the production of aberrant proteins triggers a cascade of cellular stress responses, including membrane dysfunction and disruption of normal ion gradients. These effects collectively contribute to irreversible bacterial injury and cell death under aerobic conditions, where active electron transport is required for drug uptake (Monem *et al.*, 2020). The clinical efficacy of aminoglycosides against *A. baumannii* is significantly influenced by the presence of resistance mechanisms that interfere with each step of this pathway. Aminoglycoside-modifying enzymes (AMES) chemically inactivate the drug before ribosomal binding, while 16S rRNA methyltransferases such as *armA* alter the target site, preventing antibiotic interaction with the ribosome (Ayoub & Hammoudi, 2020; Martinez-Trejo *et al.*, 2022).

Due to its mechanism-dependent activity, aminoglycosides remain clinically important in the treatment of severe infections caused by *A. baumannii*, including ventilator-associated pneumonia, bloodstream infections, and wound infections. However, their use is often restricted to combination therapy with other antimicrobial agents such as carbapenems, polymyxins, or tigecycline, to improve therapeutic efficacy and reduce the risk of resistance development (Karakonstantis *et al.*, 2020; Thy *et al.*, 2023).

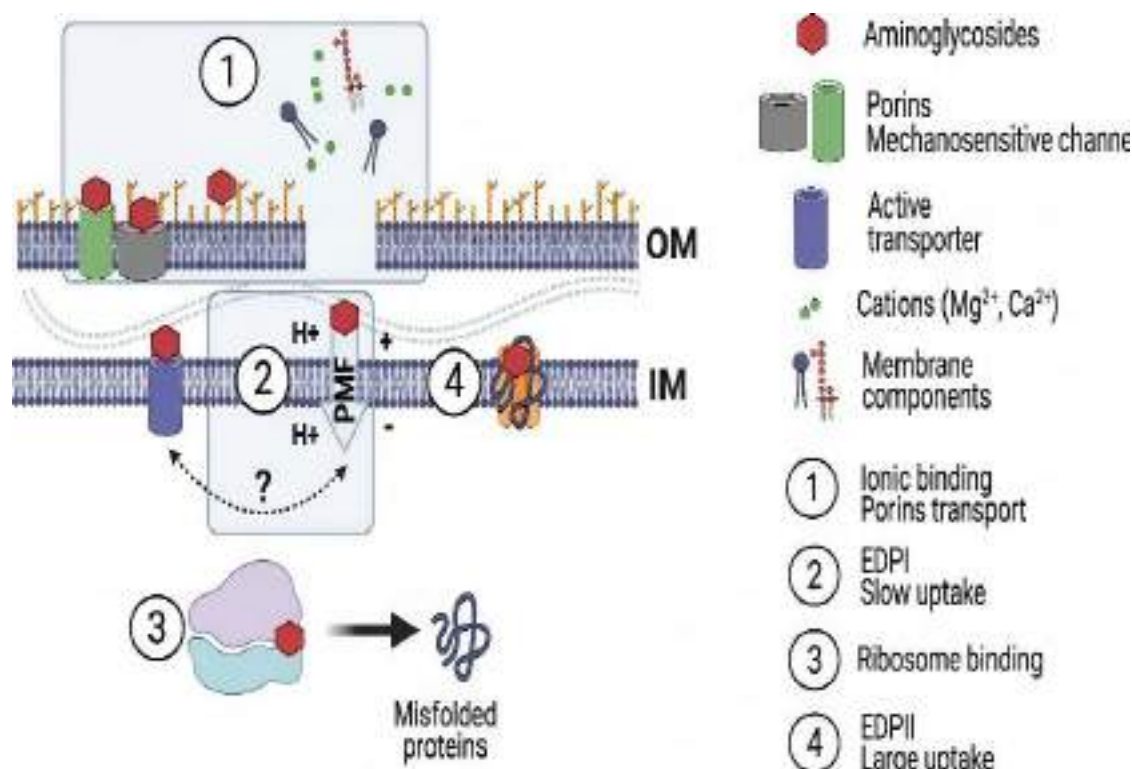


Figure (2-2): Model of aminoglycosides (AG) entry in Gram-negative bacteria.

(1) Electrostatic binding. The interaction between AG and the outer membrane induces its destabilization. Entry through porins is possible. (2) Energy-dependent phase I. Slow uptake, proton motive force dependent. Active transporters (notably carbohydrates transporters) are also able to carry AG. The link between active transporters and the role of the PMF in AG entry remains to be fully elucidated. (3) Ribosome binding. AGs induce protein mistranslations. (4) Energy-dependent phase II. Misfolded protein induces membrane permeability and large uptake, preceding cells death. “Active transporter” corresponds to carbohydrate transporters and others. (Lang *et al.*, 2023).

2.12. Aminoglycoside Resistance

The effectiveness of aminoglycoside therapy has been increasingly compromised by the emergence of multiple bacterial resistance mechanisms. These mechanisms act at different stages of antibiotic activity and collectively reduce treatment efficacy under clinical selective pressure. One of the earliest barriers to aminoglycoside activity is the reduction of intracellular drug concentration (Ayswarya *et al.*, 2026). This occurs through decreased

outer membrane permeability and alterations in uptake processes, which limit antibiotic entry into the bacterial cell. In parallel, efflux pump systems such as AdeABC actively export aminoglycosides out of the cell, preventing their accumulation to bactericidal levels (Kyriakidis *et al.*, 2021; Shi *et al.*, 2024).

Aminoglycoside- modifying enzymes (AMEs), including acetyltransferases, phosphotransferases, and nucleotidyltransferases, chemically modify the antibiotic molecule, thereby preventing its effective binding to the ribosomal target site (Khurshid *et al.*, 2020). These enzymes are often associated with mobile genetic elements, which facilitates their horizontal transfer among bacterial populations and enhances their spread in clinical settings (Martinez-Trejo *et al.*, 2022). In addition to enzymatic modification, target-site alterations contribute significantly to resistance. Mutations in the 16s rRNA or ribosomal proteins of the 30S subunit reduce aminoglycoside binding affinity, leading to decreased susceptibility in certain strains (Nicholson *et al.*, 2020). A more potent mechanism involves 16S rRNA methylation mediated by enzymes such as ArmA, which modifies the aminoglycoside binding site and results in high-level resistance to almost all clinically important aminoglycosides, including amikacin, gentamicin, and tobramycin (Jouybari *et al.*, 2021).

These mechanisms may occur simultaneously within the same bacterial isolate, producing a cumulative resistance phenotype that severely limits treatment options. The coexistence of reduced drug uptake, active efflux, enzymatic inactivation, and target modification explains the high level of aminoglycoside resistance observed in

clinical isolates of *A. baumannii* and highlights its strong adaptive capacity under antimicrobial pressure (Kashani *et al.*, 2021).

2.12.1. Aminoglycoside-Modifying Enzymes (AMEs)

Aminoglycoside-modifying enzymes represent one of the major mechanisms by which *A. baumannii* develops resistance to aminoglycoside antibiotics. These enzymes are classified into three main functional groups as shown as Figure (2-3) according to the type of chemical modification they mediate on aminoglycoside molecules (Khurshid *et al.*, 2020) N-acetyltransferases (AACs), which acetylate amino groups using acetyl-CoA; O-nucleotidyltransferases (ANTs), which adenylate hydroxyl groups using ATP; and O-phosphotransferases (APHs), which phosphorylate hydroxyl groups using ATP. These enzymatic modifications occur at the amino (-NH₂) or hydroxyl (-OH) groups located on the 2-deoxystreptamine nucleus or on the attached sugar moieties of aminoglycosides (Salimizand *et al.*, 2018; Khurshid *et al.*, 2020). Such chemical alterations significantly reduce the ability of aminoglycosides to bind to their target site on the 30S ribosomal subunit, thereby impairing their antibacterial activity (Lang *et al.*, 2023).

Consequently, reduced susceptibility and high-level resistance to aminoglycosides may develop. In *A. baumannii*, genes encoding AMES are frequently associated with mobile genetic elements such as plasmids, integrons, and transposons, which facilitate horizontal gene transfer and contribute to the rapid dissemination of resistance determinants among bacterial populations (Al-Jabri, 2018).

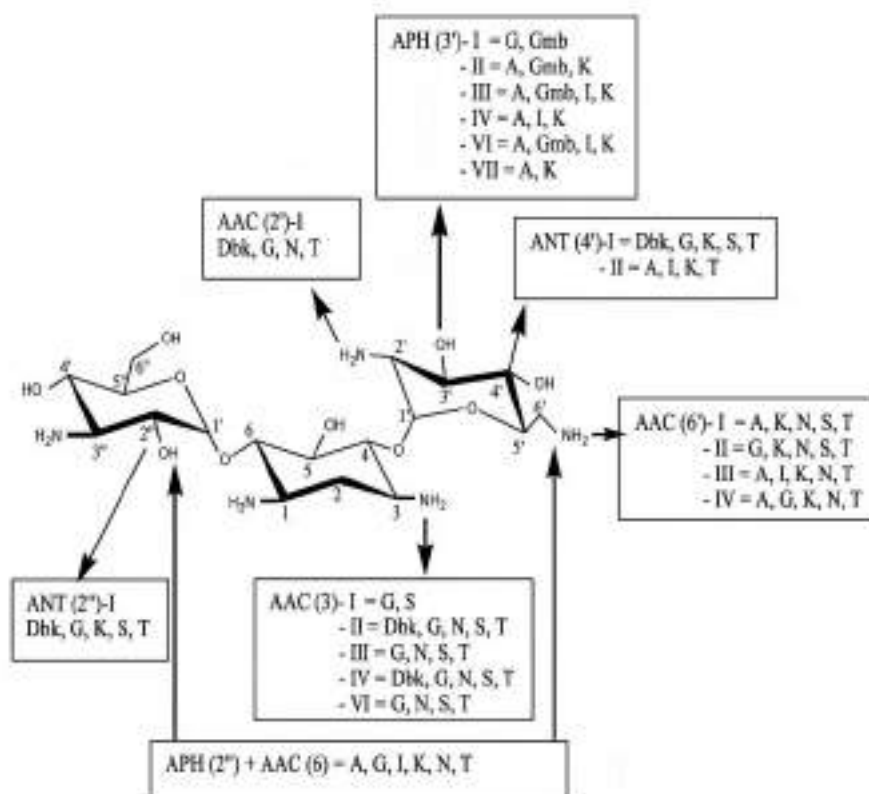


Figure (2-3): Aminoglycoside modifying enzymes and their substrates. A, amikacin; Dbk, dibekacin; G, gentamicin; Gmb, gentamicin B; I, isepamicin; K,kanamycin A; N, netilmicin; S, sisomicin; T, tobramycin (Hermann, 2007).

Two principal nomenclature systems are commonly used for the classification and naming of AMES. The first system utilizes a three-letter abbreviation indicating the enzymatic activity (AAC, ANT, or APH), followed by the site of modification in parentheses, a Roman numeral representing the subclass, and a lowercase letter identifying the specific enzyme variant, such as AAC(6')-Ib (Hasib, 2018). Although a hyphen has traditionally been used to separate the class designation from the subclass, recent literature frequently omits it. The second nomenclature system is gene-based and employs the prefixes *aac*, *aad*, or *aph*, followed by a capital letter corresponding to the site of modification on the aminoglycoside molecule. For example, *aacA*, *aacB*, and *aacC* encode acetyltransferases acting on different positions of the antibiotic molecule (Ramirez & Tolmasky, 2010).

2.12.1.1. Aminoglycoside N-Acetyltransferases (AACs)

Chemical acetylation of aminoglycosides represents an important enzymatic mechanism that reduces the antibacterial activity of these agents in *A. baumannii*. These enzymes mediate resistance through the acetylation of amino groups (-NH₂) on aminoglycoside molecules using acetyl-CoA as the acetyl donor, resulting in structural modification of the antibiotic and reduced affinity for its ribosomal target (Rizk & Abou El-Khier, 2019). The acetylation process typically occurs at specific positions on the aminoglycoside structure, most commonly at the 6'-amino group of the 2-deoxystreptamine nucleus or its sugar moieties. This modification interferes with the ability of the antibiotic to bind to the 30S ribosomal subunit, thereby inhibiting its antibacterial activity and leading to reduced susceptibility or complete resistance (Stogios *et al.*, 2017). Among AAC enzymes, the AAC(6')-type, particularly the gene *aac(6')-Ib*, is the most prevalent and clinically significant in *A. baumannii*. This gene is strongly associated with resistance to several clinically important aminoglycosides, including amikacin, gentamicin, kanamycin, and tobramycin. Its wide distribution among clinical isolates highlights its major role in treatment failure in hospital-associated infections (Harmer *et al.*, 2023).

Other AAC enzymes, such as *aac(3)-II* and *aacC1*, have also been identified in *A. baumannii* isolates. These enzymes mainly confer resistance to gentamicin and tobramycin by modifying different amino positions on the aminoglycoside molecule, further expanding the resistance spectrum of this bacterial species (Martinez-Trejo *et al.*, 2022). Genes encoding AAC enzymes are frequently located on mobile genetic elements such as plasmids, transposons, and integrons, this genetic localization facilitates horizontal gene transfer between bacterial strains and contributes to the rapid spread of aminoglycoside resistance within healthcare

environments. In many cases, these genes are also found within MDR regions, where they coexist with other resistance determinants, enhancing the overall resistance phenotype of clinical isolates (Kishk *et al.*, 2021; Rashvand *et al.*, 2021). The widespread occurrence of AAC enzymes, particularly AAC(6)-Ib, represents a key mechanism underlying aminoglycoside resistance in *A. baumannii* and significantly limits the effectiveness of aminoglycoside-based therapies in severe infections (Tamma *et al.*, 2024).

2.12.1.2. Aminoglycoside O-Nucleotidyltransferases (ANTs)

Bacterial enzymatic adenylation of aminoglycosides contributes significantly to the inactivation of these antimicrobial agents in *A. baumannii*. These enzymes mediate resistance through adenylation of hydroxyl (-OH) groups on aminoglycoside molecules by transferring an adenosine monophosphate (AMP) group from ATP, resulting in structural modification that prevents effective binding to the bacterial ribosome (Zhang *et al.*, 2017). The enzymatic modification occurs at specific hydroxyl positions on the aminoglycoside structure, leading to reduced affinity for the 30S ribosomal subunit and subsequent inhibition of protein synthesis. This modification significantly decreases the antibacterial activity of aminoglycosides and contributes to the development of resistant phenotypes in clinical isolates (Kyriakidis *et al.*, 2021). Among the ANT enzymes identified in *A. baumannii*, the *aadB* [*ant*(2'')-Ia] gene is one of the most clinically relevant. It confers resistance to several aminoglycosides, including gentamicin, tobramycin, and kanamycin, through modification at the 2'' hydroxyl position. This gene is frequently located on plasmids and is often associated with mobile genetic elements, which facilitates its horizontal transfer among bacterial populations (Naderi *et al.*, 2023). Another important gene is *aadA1* [*ANT*(3')-Ia] which

encodes. an enzyme that catalyzes adenylation at the 3' hydroxyl position. This gene mainly confers resistance to streptomycin and spectinomycin and is commonly found within class 1 integrons and plasmids, enhancing its dissemination among multidrug-resistant strains (Rizk & Abou El-Khier, 2019). In addition, the *ant (4')-IIb* gene has been reported in some clinical isolates of *A. baumannii*. This enzyme is associated with resistance to aminoglycosides such as tobramycin and amikacin. although its distribution is less widespread compared to *aadB* and *aadA1*. Its presence reflects the genetic diversity of ANT- mediated resistance mechanisms in this species (Al-Kadmy *et al.*, 2015). Genes encoding ANT enzymes are commonly associated with mobile genetic platforms such as plasmids and integrons, which enhance their ability to spread between bacterial strains. This genetic mobility contributes significantly to the dissemination of aminoglycoside resistance in hospital environments and supports the persistence of resistant clones in clinical settings (Sezmis *et al.*, 2023). ANT enzymes such as *aadB*, *aadA1*, and *ant (4')-IIb* play a significant role in aminoglycoside resistance in *A. baumannii* by enzymatically inactivating the antibiotic and facilitating its spread through horizontal gene transfer mechanisms (Saleh *et al.*, 2023).

2.12.1.3. Aminoglycoside O-Phosphotransferases (APHs)

phosphorylation-mediated inactivation represents another major mechanism contributing to aminoglycoside resistance in Gram- negative bacteria, with particular relevance in *A. baumannii*. These enzymes mediate resistance through ATP- dependent phosphorylation of hydroxyl (-OH) groups on aminoglycoside molecules, leading to structural modification (Kyriakidis *et al.*, 2021). that reduces or eliminates antibiotic activity. This enzymatic alteration prevents effective interaction of aminoglycosides with their target site on the bacterial 30S ribosomal

subunit, thereby contributing to reduced susceptibility and therapeutic failure (Vrancianu *et al.*, 2020). The phosphorylation process occurs at specific hydroxyl positions on the aminoglycoside structure, which alters the chemical configuration of the drug and prevents its effective binding to the 30S ribosomal subunit. As a result, inhibition of protein synthesis is blocked, leading to reduced susceptibility or complete resistance to aminoglycosides (Kakoullis *et al.*, 2021).

Among the APH enzymes identified in *A. baumannii*, the *aph(3')-VIa* gene (*aphA6*) is considered the most clinically important determinant. This enzyme mediates phosphorylation at the 3'-hydroxyl position and confers high-level resistance to several aminoglycosides, particularly amikacin, kanamycin, and neomycin. Due to its strong impact on resistance, *aphA6* is widely recognized as a key contributor to therapeutic failure in clinical infections (Tahbaz *et al.*, 2019; Kyriakidis *et al.*, 2021). The *aphA6* gene is frequently associated with mobile genetic elements such as plasmids and transposons, which facilitate its horizontal transfer between bacterial strains. This mobility enhances its dissemination among clinical isolates and contributes to the rapid spread of aminoglycoside resistance in healthcare-associated environments. In many cases, *aphA6* is located within multidrug resistance regions that contain multiple resistance determinants, further increasing its clinical significance (Khongfak *et al.*, 2022). Although other APH genes such as *aph(3')-Ia* and *aph(3')-Ib* have been reported in *A. baumannii*, their contribution to resistance is generally less prominent compared to *aphA6*. However, their presence indicates the genetic diversity of phosphorylation-mediated resistance mechanisms and their potential role in expanding the aminoglycoside resistance profile of this species (Jouybari *et al.*, 2021). APH enzymes, particularly APH(3')-VIa, represent an important mechanism of aminoglycoside resistance in *A.*

baumannii acting through enzymatic inactivation of the drug and facilitating its spread via mobile genetic elements in clinical environments (Amyes & Young, 2020).

2.12.2. 16S rRNA Methylation

High-level aminoglycoside resistance is frequently mediated by methylation of the 16S rRNA target site, resulting in complete inhibition of aminoglycoside binding and subsequent loss of antibacterial activity (Khurshid *et al.*, 2020). These enzymes act by methylating specific nucleotides within the 16S rRNA of the 30S ribosomal subunit, particularly at the A-site decoding region. This modification alters the structure of the ribosomal binding site, preventing aminoglycosides from interacting with their target and resulting in high-level resistance across nearly all clinically important agents in this class (Joubert, 2020). Among the identified methyltransferase genes, *armA* is the most widespread and clinically significant in *A. baumannii*. The ArmA enzyme methylates the G1405 position of 16S rRNA and confers high-level resistance to almost all aminoglycosides, including amikacin, gentamicin, and tobramycin. The *armA* gene is frequently located on plasmids and is often associated with mobile genetic elements such as transposons, which facilitates its horizontal transfer and rapid dissemination among clinical isolates (De Silva & Kumar, 2019; Jouybari *et al.*, 2021).

Another important gene is *rmtB*, which is also predominantly plasmid-mediated and contributes to high-level aminoglycoside resistance. The RmtB enzyme targets the same ribosomal region and confers resistance to multiple aminoglycosides, including amikacin and gentamicin. Its association with mobile genetic elements enhances its spread among bacterial populations and contributes to the emergence of multidrug-resistant strains (Tada *et al.*, 2013). In addition, the *rmtD* gene

has been reported in some clinical isolates of *A. baumannii*. Although less frequently detected compared to *armA* and *rmtB*, it also encodes a 16S rRNA methyltransferase that targets the G1405 site and contributes to high-level resistance to aminoglycosides. Its presence indicates the ongoing emergence of diverse methylation-mediated resistance mechanisms within this species (Shi *et al.*, 2024). The coexistence of genes such as *armA*, *rmtB*, and *rmtD* significantly enhances the level of aminoglycoside resistance in clinical isolates. Their frequent association with plasmids and mobile genetic elements facilitates rapid horizontal gene transfer, contributing to the dissemination and persistence of MDR *A. baumannii* strains in healthcare environments. (dos Santos Rodrigues, 2023). 16S rRNA methylation represents one of the most potent mechanisms of aminoglycoside resistance, as it directly modifies the drug target and results in complete loss of antimicrobial activity, making it a major challenge in the treatment of infections caused by *A. baumannii* (Vrancianu *et al.*, 2020).

CHAPTER THREE

MATERIALS AND METHODS

3. Materials and Methods

3.1. Materials

3.1.1. Laboratory Equipments

Table (3-1): Equipment

Equipment	Company	Origin
Autoclave	Marubeni	Japan
Bench Centrifuge	Hettich	Germany
Deep Freezer	GFL	Germany
Electric Oven	Memmert	Germany
Electrophoresis Unit	FSF-SPBT	UK
Gel Documentation System	E-Graph	Japan
Hot Plate	Labtech	Korea
Incubator	Memmert	Germany
Laminar Flow Cabinet	Marubeni	Japan
Magnetic Stirrer	Scilogex	Korea
Microscope	Olympus	Japan
Mini Spin Centrifuge	Eppendorf	Germany
Nanodrop	Avans	China
PCR Cabinet	Aura PCR	Italy
PCR Vortex	Exispin	European
pH Meter	Radiometer	Denemark
Refrigerator	Gree	China
Sensitive Balance	Kern	Germany
Thermocycler Apparatus	Bioneer	Korea
VITEK-2 System	BioMerieux	France
Vortex	Vortex mixer	Taiwan
Water Bath	Memmert	Germany

3.1.2. Laboratory Instruments

Table (3-2): Instruments

Instruments	Company	Origin
Dry Cotton Swabs	Difco	UK
Ependroff Tubes	Sterellin Ltd	UK
Forceps	China	China
Glass Flasks (100-1000) ml	Jlassco	India
Micropipette Set (1-1000) ml	Eppendorf	Germany
Millipore Filters (0.22) mm	Difco	UK
PCR Tubes	Eppendorf	Germany
Petri Dishes	Himedia	India
Slides with Covers	Sliprand	China
Standard Loop (0.01) ml	Himedia	India
Swabs with Media	Difco	UK
Test Tubes	Pyrex	UK
Tips (10, 100, 1000) ml	Difco	UK
Wooden Sticks	Superem	China

3.1.3. Chemicals

Table (3-3): The Chemicals that Have Been Used in the Study.

Chemicals	Company	Origin
Agar-agar	Himedia	India
Agarose	Promega	USA
Bromophenol Blue	Promega	USA
EDTA	AppliChem	Germany
Ethanol (96%)	BDH	UK
Ethidium Bromide	Sigma	USA
Glycerol (C ₃ H ₈ O ₃)	Fluke	Germany
Gram Stain Kit	Himedia	India
Hydrochloric Acid (HCl)	BDH	UK
Hydrogen Peroxide (H ₂ O ₂)	Teba	Iraq
Isoamyl Alcohol	BDH	UK
Kovac's Reagent	Himedia	India
McFarland (0.5) Standard	BioMerieux	France
Methyl Red	BDH	UK
Normal Saline	Alhikma	Iraq
Nuclease Free Water	Ambion	USA
Peptone	Himedia	India

Potassium Hydroxide (KOH)	BDH	UK
Sodium Chloride (NaCl)	BDH	UK
Sodium Hydroxide (NaOH)	Fisher	Germany
Sucrose	Difco	UK
Sulphuric Acid	BDH	UK
Tetramethyl-P-Phenylene Diamine Dihydrochloride	BDH	UK
Tris-Borate-EDTA Buffer	Promega	USA
Tris-EDTA Buffer	Promega	USA
Trypton	Difco	USA
Urea Solution	Mast Diagnostic	UK
Yeast Extract	Bioworld	USA
α -Naphthol (C ₁₀ H ₈ O)	BDH	UK

3.1.4. Culture Media

Table (3-4): The Culture Media that Have Been Used in the Study.

Medium	Company	Origin
Blood Agar Base	Himedia	India
Brain Heart Infusion Broth	Himedia	India
MacConkey Agar	Oxoid	UK
MR-VP Broth	Oxoid	UK
Muller-Hinton Agar	Oxoid	UK
Nutrient Broth and Agar	Himedia	India
Pepton Water	BDH	UK
Simmon-Citrate Agar	Himedia	India
Triple Sugar Iron Agar	Biolife	Italy
Tryptic Soy Broth	Biolife	Italy
Urea Base Agar	Difco	USA

3.1.5. Antibiotics Used in the Study.

A total of 24 antimicrobial agents belonging to 9 different classes were included in this study. These antibiotics were selected for antimicrobial testing against *A. baumannii* isolates. Each agent was evaluated using the disk diffusion method, while selected antibiotics were additionally assessed by minimum inhibitory concentration (MIC) determination, as indicated in Table (3-5).

Table (3-5): Antibiotics Used in the Study against *A. baumannii* Isolates. (Bioanalyse,Turkey, bioMérieux).

Class of Antibiotics	Antibiotic Subclass	Antibiotic Name	Symbol	Content (µg)
Aminoglycosides		Gentamicin	CN	10 µg
		Tobramycin	TOB	10 µg
		Amikacin	AK	30 µg
		Netilmicin	NET	30 µg
		Kanamycin	K	30 µg
Carbapenem		Imipenem	IPM	10 µg
		Meropenem	MEM	10 µg
		Doripenem	DOR	10 µg
Quinolones	Fluoroquinolone	Ciprofloxacin	CIP	5 µg
		Levofloxacin	LEV	5 µg
Penicillin/β-Lactamase Inhibitor		Piperacillin-Tazobactam	TPZ	100/10 µg
		Ampicillin-Sulbactam	AMS	10/10 µg
		Ticarcillin-Clavulanic Acid	TCC	75/10 µg
Cephams	Cephalosporin III	Cefotaxime	CTX	30 µg
		Ceftriaxone	CTR	30 µg
		Ceftazidime	CAZ	30 µg
	Cephalosporin IV	Cefepime	FEP	30 µg
Folate Pathway Inhibitor		Trimethoprim-Sulfamethoxazole	TMP	1.25/23.75 µg
Penicillin	Ureidopenicillin (Antipseudomonal)	Piperacillin	PI	100 µg
Lipopeptide		Polymyxin B	PB	N/A*
		Colistin	CT	N/A*
Tetracycline		Doxycycline	DO	30 µg
		Minocycline	MIN	30 µg
		Tetracycline	TE	30 µg

Colistin and polymyxin B were tested by MIC (broth microdilution) only (0.25–128 µg/mL) according to CLSI guidelines; disk diffusion was not applicable. (*) indicates MIC-only testing.

3.1.6. Kits

Table (3-6): The Kits that Have Been Used in the Study

Kit	Components	Company (Country)
DNA Ladder	13 dsDNA fragments (100, 200, 300, 400, 500, 600, 700, 800, 900, 1000 bp)	Bioneer (Korea)
GoTaq® Green Master Mix	2X Green Taq Reaction buffer (pH 8.5), 400 µM dNTPs (dATP, dGTP, dCTP, dTTP), 3mM MgCl ₂	Promega (USA)
Presto™ Mini gDNA Bacteria Kit	GT Buffer, Proteinase K, GB Buffer, Wash (W1 & W2) Buffers, Elution Buffer, Collection tubes, GD Columns	Geneaid (Taiwan/USA)
VITEK® 2 System Cards 21341	VITEK glass test tubes, Densichek meter, VITEK ID cards	BioMérieux (France)

3.1.7. Primers

3.1.8.1. Primers for Detection of Aminoglycosides Resistance Genes (ALPHA-Canada)

Table (3-7) Primers Oligonucleotide Sequences Used in This Study

Groups	Name Gene		Primer sequence (5'-3')	PCR product (bp)	References
Genotypic Detection	16Sr RNA		F:TGCGTAGGCGGCTTATTAAGTCGG R:ATCCGRAATGCTGCAAGTAAG	546	(Fukatsu <i>et al.</i> , 2023)
	<i>bla</i> _{OXA-51}		F- TAATGCTTTGATCGGCCTTG R- TCGATTGCACTTCATCTTGG	353 bp	(Roberts <i>et al.</i> , 2001)
Aminoglycoside-Modifying Enzymes (AMEs)	(AACs)	<i>aacC1</i>	F:ATGGGCATCATTCGCACATGTAGG R:TTAGGTGGCGGTACTTGGGTC	465	(Shafigh <i>et al.</i> , 2018)
		<i>aac(3)-II</i>	F:TGAAACGCTGACGGAGCCTC R:GTCGAACAGGTAGCACTGAG	370	(Rashvand <i>etal.</i> , 2021)
		<i>aac(6')-Ib</i>	F:TTGCGATGCTCTATGAGTGGCTA R:CTCGAATGCCTGGCGTGTTT	482	(Khorsi <i>etal.</i> , 2015)
	(APHs)	<i>aph(3')-VI</i> (<i>aphA6</i>)	F:ATGGAATTGCCCAATATTATTC R:TCAATTCAATTCATCAAGTTTTA	780	(Tawfick <i>et al.</i> , 2020)
	(ANTs)	<i>aadA1</i>	F:ATGAG GGAAGCGGTGATCG R:TTATTTGCCGACTACCTTGGTG	792	(Aliakbarzade <i>etal.</i> , 2014)
		<i>aadB</i>	F:ATGGACACAACGCAGGTCGC R;TTAGGCCGCATATCGCGACC	534	(Aliakbarzade <i>et al.</i> , 2014)

		<i>ant(4')-IIb</i>	F:TATCTCGGCGGGCGGTCGAGT R:CACGCGGGGAAACGCGAGAA	364	(Rashvand <i>et al.</i> , 2021)
16S rRNA methylases		<i>armA</i>	F: ATTCTGCCTATCCTAATTGG R: ACCTATACTTTATCGTCGT	315	(Zahra <i>et al.</i> , 2021)
		<i>rmtD</i>	F: CGGCACGCGATTGGGAAGC R: CGGAAACGATGCGACGAT	401	(Wang <i>et al.</i> , 2016)
		<i>rmtB</i>	F:GCT TTC TGC GGG CGA TGT AA R:ATG CAA TGC CGC GCT CGT AT	173	(Nie <i>et al.</i> , 2014)
Class 1 integrons		<i>Int1</i>	F:CAGTTGACATAAGCCTGTTC R:CCCGAGGCATAGACTGTA	160	(Koeleman <i>et al.</i> , 2001)
ERIC Seq.		<i>Eric</i>	ERIC-1(TGTAAGCTCCTGGGGATTAC) ERIC-2 (AAGTAAGTGACTGGGGTGAGCG)	Variable	Coudron <i>et al.</i> ,2000

Abbreviations:

AMEs:Aminoglycoside-Modifying

AACs : Aminoglycoside N-acetyltransferase

ANTs: Aminoglycoside O-nucleotidyltransferase

APHs: Aminoglycoside O-phosphotransferase

3.2.Methods

3.2.1. Study Design

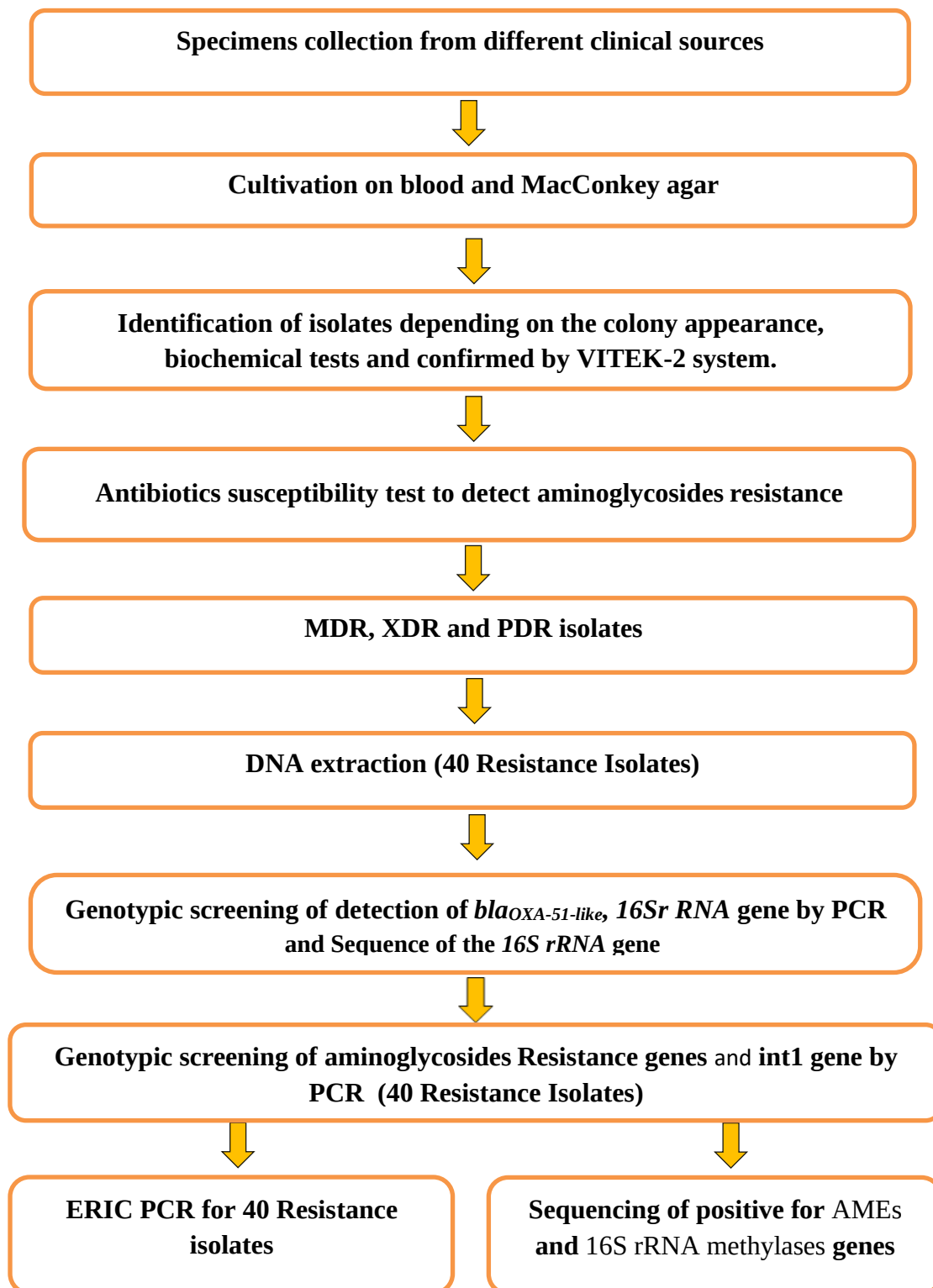


Figure (3-1): Design of the Study

3.2.2. Specimens Collection

This cross sectional descriptive study was conducted at four major teaching hospitals in Babylon Governorate, Iraq, namely Al-Sadia (AS) Teaching Hospital, Al- Hilla Teaching Hospital, Marian Teaching Hospital, and Babil Teaching Hospital, over a period of ten months, from January to October 2025. Ethical approval was obtained from the Ethics Committee of the College of Science at University of Al-Qadisiyah (Approval No. 135), and the study was conducted in accordance with ethical standards, ensuring patient confidentiality and informed consent. A total of 519 clinical specimens were collected randomly from patients attending or admitted to the above-mentioned hospitals who were clinically suspected by physicians of having bacterial infections. The specimens were obtained from the microbiology laboratories of the hospitals and included urine, burn swabs, blood, sputum, and wound swabs.

All specimens were labeled properly and transported immediately to the Microbiology Laboratory at the Al-Sadia (AS) Teaching Hospital, under appropriate conditions for further microbiological investigation. For burn and wound swabs, sterile swabs were used to collect specimens from infected sites under aseptic conditions and were immediately placed in transport media for delivery to the laboratory. Sputum samples were collected in sterile wide-mouthed containers after instructing patients to produce deep cough specimens, preferably in the early morning following mouth rinsing to reduce contamination. Clean-catch midstream urine samples were collected in sterile, screw-capped containers from patients suspected of urinary tract infections and transported immediately to the laboratory. Blood specimens were collected

aseptically after skin disinfection with 70% ethanol followed by iodine solution; approximately 10 mL was collected from adults and 1-2 mL from pediatric patients, then inoculated into brain heart infusion broth and incubated at 37°C for 24 hours up to 7 days, after which positive cultures were subcultured onto solid media for further analysis.

3.2.3. Cultivation of Specimen

All clinical specimens, including burn swabs, blood, sputum, wound swabs, and urine samples, were cultured aseptically on blood agar and MacConkey agar plates, then incubated aerobically at 37°C for 24 hours. Urine specimens were inoculated using the standard calibrated loop method. For blood specimens, any broth showing turbidity was subcultured onto solid media using sterile techniques, while clear broths were further incubated and considered negative if no visible growth appeared after 7 days of incubation. After incubation, the culture plates were carefully examined for bacterial growth. The isolates demonstrated good growth on all media. On MacConkey agar, the colonies appeared as pale, non-lactose fermenting colonies, which is a characteristic feature of *A. baumannii*. On blood agar, the colonies were typically smooth, opaque, grayish-white, and non-hemolytic.

Following overnight incubation, the plates were re-examined to confirm colony purity. Bacterial isolates were initially identified based on colony morphology and microscopic characteristics. Gram staining revealed Gram-negative coccobacilli, consistent with *A. baumannii* (Parasuraman *et al.*, 2024). Further identification was carried out using conventional biochemical tests. The oxidase test was performed using fresh colonies and showed no color change within 10 seconds, indicating a negative reaction. The catalase test was

conducted by adding 3% hydrogen peroxide to the colonies, producing immediate effervescence, which confirmed a positive reaction (Lal *et al.*, 2019).

For final confirmation, all isolates were identified using the VITEK-2 Compact system according to the manufacturer's instructions. The results showed a high level of agreement in identifying the isolates as *A. baumannii*

3.2.4. Culture Media Preparation

3.2.4. 1. Ready-Prepared Culture Media

The culture media used in the present study included blood agar and MacConkey agar. All media were prepared according to the manufacturer's instructions. Sterilization was performed using an autoclave at 121°C and 15 lbs/in² for 15 minutes.

After sterilization, the media were allowed to cool under sterile conditions and then poured into sterile Petri dishes. The prepared plates were left to solidify and were incubated at 37°C for 24 hours to ensure sterility and absence of contamination. After incubation, the culture media were inspected and those showing no signs of contamination were stored at 4°C until use. For blood agar preparation, sterile defibrinated blood was added aseptically to the sterilized and cooled base medium (approximately 45- 50°C) to obtain a final concentration of 5%. The medium was gently mixed to ensure even distribution of blood before being poured into sterile Petri dishes. All prepared culture media were properly labeled and stored under refrigerated conditions at 4°C until required for bacterial cultivation.

3.2.4.2. Maintenance Medium

The maintenance medium consisted of brain heart infusion (BHI) broth as a basal medium supplemented with 20% glycerol. The medium was prepared according to standard procedures and sterilized by autoclaving at 121°C under a pressure of 15 lbs/in² for 15 minutes. After sterilization, the medium was allowed to cool to approximately 56°C, then dispensed aseptically into sterile cryovials in aliquots of 5 ml each and stored at 4°C until use. For long-term preservation, pure isolates of *A. baumannii* were inoculated into the prepared BHI-glycerol medium and stored at -70°C for several months to maintain viability (Chapartegui-González *et al.*, 2018). For short-term maintenance, a pure bacterial colony was streaked onto nutrient agar slants and incubated aerobically at 37°C for 18-24 hours. The slants were then stored at 4°C in a refrigerator for further use. For revival of preserved isolates, frozen stock cultures were subcultured onto fresh blood agar plates and incubated aerobically at 37°C for 24 hours to ensure viability and purity of the bacterial growth (Nielsen *et al.*, 2015).

3.2.4.3. Motility Medium

The motility medium was prepared by dissolving 0.5 g of agar-agar in 100 ml of brain heart infusion (BHI) broth. The mixture was dispensed into test tubes and autoclaved at 121°C for 15 minutes (MacFaddin, 2000). *A. baumannii* showed no motility in the prepared medium.

3.2.5. Biochemical Test

The present study employed several conventional biochemical tests to identify *A. baumannii*, following the guidelines set by MacFaddin (2000). All tests are presented in Table (3-8).

3.2.5.1. Catalase Test

To conduct the catalase test, a sterile inoculating loop or wooden applicator stick was used to pick the center of a colony from an overnight

culture plate of *A. baumannii*. The specimen was placed on a clean glass slide, and a drop of 3% (v/v) hydrogen peroxide (H₂O₂) was added to the smear. The rapid production of bubbles, indicating the release of oxygen, signifies a positive result; all tested isolates showed immediate bubbling, indicating a positive catalase reaction.

3.2.5.2. Citrate Utilization Test

Fresh colonies of *A. baumannii* were inoculated onto Simmons' citrate agar slant using a sterile straight wire loop and incubated at 37°C for 48-72 hours. A positive result was indicated by a color change of the medium from green to blue along with visible bacterial growth on the slant surface indicating a positive citrate utilization reaction.

3.2.5.3. Indole Test

For the indole test, the conventional tube method was utilized. Fresh colonies of *A. baumannii* were inoculated into a tube of peptone water and incubated for 18-48 hours at 37°C. After incubation, a few drops of Kovac's reagent (composed of 5 g of para- dimethyl amino benzaldehyde in 57 ml of isoamyl alcohol, with 25 ml of concentrated hydrochloric acid added gradually) were added to the broth culture and mixed gently. The formation of a pink to red ring at the surface of the medium indicated a positive result; however, no color change or ring formation was observed, indicating a negative indole reaction.

3.2.5.4. Methyl Red Test

Colonies from an overnight culture of *A. baumannii* were inoculated into a Methyl Red/Voges-Proskauer (MR-VP) broth tube and incubated at 37°C for 24 hours. The methyl red reagent was prepared by dissolving 0.1 g of methyl red in 300 ml of 96% ethanol and then diluting it to 500 ml with distilled water. Five drops of the reagent were added to the tube and the result was read immediately. A positive test was indicated

by the development of a red color; however, no red coloration was observed, indicating a negative Methyl Red reaction.

3.2.5.5. Voges-Proskauer Test

Methyl Red/Voges-Proskauer broth was inoculated with an overnight culture of *A. baumannii* and incubated at 37°C for 48 hours. Two drops of 40% potassium hydroxide (KOH) solution (40 g of KOH in 100 ml of distilled water) followed by six drops of 5% a-naphthol solution (5 g of a-naphthol in 100 ml of absolute ethanol) were added to the tube. The tube was then gently shaken and allowed to stand for 15 minutes. A positive test was indicated by the development of a pink to red color; however, no color change was observed, indicating a negative Voges-Proskauer reaction.

3.2.5.6. Urease Test

Colonies of *A. baumannii* from an overnight culture were streaked onto the surface of a urea agar slant. The tube was incubated at 37°C and was examined after 24 hours and daily for up to 6 days. A positive test was indicated by the development of a purple to pink color due to alkalization of the medium however, no color change was observed, indicating a negative urease reaction.

3.2.5.7. Oxidase Test

The oxidase reagent was prepared by dissolving 0.1 g of tetramethyl-p- phenylenediamine dihydrochloride in 10 ml of distilled water. The solution was stored in a dark bottle. For the test, a strip of filter paper was soaked with a few drops of the freshly prepared 1% oxidase reagent. A colony from an overnight culture of *A. baumannii* was picked using a sterile wooden stick and applied to the filter paper. The

development of a deep purple color within 10 seconds indicates a positive result; however, no color change was observed, indicating a negative oxidase reaction.

3.2.5.8. Triple Sugar Iron Test (TSI)

Using a straight inoculation needle, a colony isolated from an overnight growth culture plate of *A. baumannii* was picked and stabbed into the center of the medium all the way to the bottom of the tube. The needle was then used to streak the surface of the agar slant in a zigzag pattern. The slant was then incubated at 37°C for 24 hours. The expected results are as follows:

- An acid-acid reaction (yellow slant and yellow butt) indicates fermentation of dextrose, lactose, and/or sucrose.
- An alkaline-acid reaction (red slant and yellow butt) indicates fermentation of dextrose only.
- An alkaline-alkaline reaction (red slant and red butt) indicates the absence of carbohydrate fermentation.

Presence of blackening in the medium indicates the presence of hydrogen sulfide (H₂S), while bubbles or cracks in the agar signify gas production. In the present study, *A. baumannii* isolates showed an alkaline- alkaline reaction without blackening or gas production, indicating a non- fermentative profile.

3.2.5.9. Motility Test

An overnight growth colony of *A. baumannii* was inoculated into the motility medium by stabbing the center of the medium to a depth of 2 cm with a straight inoculation needle. The inoculated medium was then incubated at 37°C for 24 to 48 hours. The formation of a diffuse zone of growth spreading from the line of inoculation

indicates a positive motility test; however, no spreading growth was observed, indicating a negative motility reaction.

Table (3-8): The Biochemical Tests for Identification of *A. baumannii* Isolates.

Test	Reaction
Catalase	Positive
Simmons Citrate	
Indole	Negative
Methyl Red	
Voges–Proskauer	
Urease	
Oxidase	
Motility	
TSI	Alkaline/Alkaline (K/K), no gas production, no H ₂ S

3.2.5.10. Ability to grow at 44°C

This test was used as a phenotypic screening method to assess the ability of *A. baumannii* isolates to grow at 44°C, which is commonly associated with members of the *A. baumannii* complex. Nutrient agar was inoculated with all isolates and incubated at 44°C for 24 hours, after which bacterial growth was examined (Dijkshoorn, 2012).

3.2.5.11. Confirmatory Diagnosis by VITEK-2 System

The VITEK-2 system is one of the fastest and most accurate automated techniques for identifying bacteria. It utilizes a biochemical profile produced by plastic reagent cards, which contain microliter amounts of various biochemical test media in 30 wells to identify organisms. The system measures color changes in the card caused by the metabolic activity of the microbes using a photometer. An inoculum from

cultivated specimens is automatically deposited into the card, while color changes due to microbial activity are measured periodically. Data is processed, stored, and printed using a computerized database, and the system offers a variety of cards for identifying both Gram-negative and Gram-positive bacteria (Maina and Kagotho, 2014). *A. baumannii* was confirmed using the VITEK-2 system as a Gram-negative non-fermenting bacterium, following the manufacturer's instructions. The procedure is outlined below:

1. In a sterile manufacturer's tube, a single isolated pure colony of *A. baumannii* was suspended in 3 ml of physiological saline.
2. The bacterial suspension was compared to a standard turbidity solution using a turbidity measuring device to adjust the concentration. The final turbidity within the tube should be between 0.5 and 0.63.
3. Following Gram staining, the tubes were placed into the VITEK-2 cassette, which contains a specific strip for Gram-negative bacteria.
4. A rack holding the tubes and cassette was then introduced into the system. Initially, it was positioned in the filler area, where the machine automatically filled the cassette with the bacterial suspension. Once this process was completed, the device signaled the end of the filling.
5. The system cut the cassettes and sent data as a digital signal to the second field reader. Each sample's data was provided by a tube rack that moved away from the instrument and connected to the VITEK-2 system.
6. The cassettes were incubated at 37°C for 6-18 hours, after which the results for bacterial identification were read as *A. baumannii*

3.2.6. Antibiotics Susceptibility Test

The antibiotic susceptibility testing of *A. baumannii* isolates was performed using the Kirby-Bauer disk diffusion method on

Mueller- Hinton agar (Bauer *et al.*, 1966), according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2025). A total of twenty-four antibiotics belonging to nine different classes were tested Table (3-5). All antibiotics were evaluated using the disk diffusion method except colistin and polymyxin B. For disk diffusion testing, isolates were subcultured on a non-selective agar medium and incubated for 18-24 hours. Three to five colonies were suspended in tryptic soy broth and adjusted to a 0.5 McFarland standard, and then used to inoculate Mueller-Hinton agar plates. Antibiotic disk were applied and the plates were incubated at 37°C for 16-18 hours.

For colistin and polymyxin B, the (MIC) was determined using the broth microdilution method according to CLSI guidelines. The inoculum was adjusted to achieve a final concentration of 5×10^5 CFU/mL, and serial two-fold dilutions of antibiotics were prepared in cation-adjusted Mueller-Hinton broth. MIC tubes were incubated at 37°C for 18-20 hours. Interpretation of results was performed according to CLSI breakpoints. Inhibition zone diameters were measured in millimeters for disk diffusion antibiotics, while MIC values were recorded for colistin and polymyxin B. The Multiple Antibiotic Resistance (MAR) index was calculated for each isolate by dividing the number of antibiotics to which the isolate showed resistance by the total number of antibiotics tested (Krumperman, 1983; Duruik *et al.*, 2022).

$$\text{MAR index} = \frac{\text{Number of antibiotics against which isolate showed resistance}}{\text{Total number of antibiotics tested}}$$

A MAR index value greater than 0.2 indicates high-risk exposure to antibiotics, while lower values suggest limited exposure (Barosa, 2020).

3.2.7. Screening Test for Aminoglycosides Resistance

Based on CLSI (2025) recommendations, the disk diffusion test was performed to determine aminoglycoside resistance among all *A. baumannii* isolates using gentamicin (10 µg), tobramycin (10 µg), kanamycin (30 µg), netilmicin (30 µg), and amikacin (30 µg). Susceptibility testing was carried out as described above (Section 3.2.6). The zone diameters for gentamicin, tobramycin, and netilmicin were interpreted as susceptible (≥ 15 mm), intermediate (13–14 mm), or resistant (≤ 12 mm), whereas amikacin was interpreted as susceptible (≥ 17 mm), intermediate (15–16 mm), or resistant (≤ 14 mm), according to CLSI (2025). Kanamycin (30 µg) was tested using the disk diffusion method, and the inhibition zone diameters were interpreted as susceptible (≥ 18 mm), intermediate (14–17 mm), or resistant (≤ 13 mm), following the interpretive criteria applied to *A. baumannii* by Kim *et al.* (2010), based on the NCCLS (2007) criteria. For colistin and polymyxin B, minimum inhibitory concentrations (MICs) were determined using the broth microdilution method according to CLSI (2025) guidelines. Serial two-fold dilutions ranging from 0.25 to 128 µg/mL were used. Isolates were interpreted as susceptible at MIC ≤ 2 µg/mL and resistant at MIC ≥ 4 µg/mL.

3.2.8. Solutions Used in Molecular Screening

According to Bartlett and Stirling (1998), the solutions for molecular screening were prepared as follows:

3.2.8.1. Tris-Borate-EDTA (TBE) Buffer

The working buffer (1X) was prepared by diluting the 10X stock solution. Specifically, 100 ml of TBE buffer (10X) was added to 900 ml of DW. This solution was stored at room temperature until it was used for agarose gel electrophoresis.

3.2.8.2. Tris-EDTA (TE) Buffer

The TE buffer was prepared at a concentration of 1X by diluting the stock TE solution with DW. This solution was stored at 4°C until it was needed for use with primers.

3.2.8.3. Loading Dye Solution

The loading dye was prepared by dissolving 0.25 g of bromophenol blue and 4 g of sucrose in 10 ml of DW. The total volume was then adjusted to 100 ml, and the solution was stored at 4°C until it was used.

3.2.8.4. Ethidium Bromide Solution

This solution was prepared by dissolving 0.05 g of ethidium bromide in 10 ml of DW to achieve a final concentration of 5 mg/ml. It was stored in a dark reagent bottle to protect it from light.

3.2.9. DNA Extraction

In this study, DNA was extracted from Aminoglycosides resistant *A. baumannii* isolates following the instructions provided by a commercial extraction kit. The procedure is outlined below:

1. Up to 1×10^9 bacterial cells of overnight growth were transferred to a 1.5 ml microcentrifuge tube and centrifuged at 14000 rpm for 1 minute. The supernatant was then discarded.
2. Using a pipette, 180 μ l of GT buffer was added to the cell pellet.
3. Next, 20 μ l of Proteinase K was added, and the mixture was incubated in a water bath at 60°C for at least 10 minutes, inverting the tube every 3 minutes.
4. Then, 200 μ l of GB buffer was added to the sample, which was mixed by vortexing for 10 seconds. The sample was incubated at 70°C for at least 10 minutes, inverting the tube every 3 minutes during the incubation.

5. After that, 200 μ l of absolute ethanol was added to the sample lysate, and the mixture was shaken vigorously.
6. The resulting mixture was transferred to the GD column and centrifuged for two minutes in a collecting tube. The collection tube was discarded and replaced.
7. Four hundred μ l of W1 buffer was added to the GD column and centrifuged for 30 seconds. The collection tube was then replaced, and the flow-through was discarded.
8. Six hundred μ l of wash buffer (W2) was added to the GD column and centrifuged for 30 seconds. The collection tube was replaced, and the column was centrifuged for an additional 3 minutes after each substitution.
9. Finally, 100 μ l of pre-heated elution buffer at 70°C was added to the center of the column matrix. The column was allowed to stand for at least 3 minutes, and then centrifuged for 30 seconds to elute the purified DNA, and stored at -20°C.

3.2.10. DNA Concentration Estimation

The extracted genomic DNA is analyzed using a NanoDrop spectrophotometer to measure DNA concentration (ng/ μ l) and assess DNA purity by reading the absorbance at 260/280 and 260/230 nm wavelengths. The procedure is as follows:

1. To prepare the device, add 1 μ l of sterile DW to the lens, ensuring that it does not touch the lens.
2. Next, add 1 μ l of the DNA sample to the lens and read the absorbance at 260/280 and 260/230 nm.

An optical density of 1 corresponds to approximately 50 ng/ μ l of double-stranded DNA. The acceptable ratio for DNA purity is between 1.8 ± 0.2 .

3.2.11. Primers Preparation

The oligonucleotide primers used in this study are listed in Table 3-8, including both forward and reverse primers. Following the supplier's recommendations, these primers were supplied in lyophilized form and were dissolved in nuclease-free water to achieve a final concentration of 100 picomoles/ul. To prepare a working solution at a concentration of 10 pmol/ul, 10 ul of this stock solution was resuspended in 90 µl of nuclease-free water. All primer stock solutions were stored in Eppendorf tubes and frozen at -20°C until needed.

3.2.12. Preparation of Polymerase Chain Reaction (PCR) Mixture

Preparation of the PCR mixture was performed with a final volume of 25 ul, following the Go Tag Green manufacturer's instructions Table (3-9).

Table (3-9): Total Volume of PCR Reaction Mixture. Reagent Volume per PCR reaction

Reagent	Volume per PCR reaction
Go tag PCR green master mix	12.5 µl
Forward primer	2 µl
Reverse primer	2 µl
DNA template	4 µl
Nuclease free water	4.5 µl
Total volume	25 µl

3.2.13. PCR Thermocycling Conditions

The PCR thermocycling conditions were optimized based on their reference procedures. The specific program for each primer set is outlined in Table (3-10). The presence or absence of the PCR product was verified PCR were combined in a PCR tube and mixed under sterile conditions while placed on an ice bag, in all 40 Aminoglycosides resistant *A. baumannii* isolates using PCR.

Table (3-10): Programs of PCR thermocycling conditions for all genes in This Study

Gene	Initial denaturatin	Denaturation	Annealing	Extension	Final extension	Cycles
<i>16Sr RNA</i>	94°C/5min	94°C/30 sec	58 / 30 sec	72°C/30sec	72°C/5min	35
<i>bla_{OXA-51}</i>	94°C/5min	94°C / 1 min	55°C/1min	72°C/1min	72°C/10min	30
<i>aacC1</i>	95/15 min	94/30 sec	58/30 sec	72/30 sec	72/10 min	30
<i>aac(3)-II</i>	95/15 min	95/1 min	55/1min	72/5 min	72/10 min	30
<i>aac(6')-Ib</i>	95/1 min	94/45 Sec	55/45 Sec	72/54Sec	72/5 min	34
<i>aph(3')-VI (aphA6)</i>	94/6min	94/50 sec	56/50min	72/80 sec	72/8 min	35
<i>aadA1</i>	94/10min	94/60sec	57/30 sec	72/30 sec	72/10 min	35
<i>aadB</i>	94/10min	94/60sec	55/30 sec	72/30 sec	72/10 min	35
<i>ant (4')-IIb</i>	95/15min	95/1min	55/30 sec	72/5min	72/10min	30
<i>rrmA</i>	94/45sec	94/45sec	53/30 sec	72/1 min	72/10 min	35
<i>rmtB</i>	95/15min	95/1min	60/1 min	72/5 min	72/10min	30
<i>rmtD</i>	94/4min	94/1min	50/1 min	72/90 sec	72/5min	35
<i>Int1</i>	94/5 min	94/30 sec	55/30 sec	72/1 min	72/7 min	35
<i>ERIC</i>	94°C/5min	95°C/1min	52°C/1min	72°C/5min	72°C/10min	35

3.2.14. Agarose Gel Preparation

The gel electrophoresis procedures were performed according to the method outlined by Bartlett and Stirling (1998). An agarose gel was prepared using 100 ml of 1X TBE buffer by adding 1.5 g of agarose powder. The solution was boiled until clear, then cooled to 50°C, after which 0.5 ug/ml of ethidium bromide was added to the agarose solution. The agarose was poured into a pre-prepared gel tray and allowed to cool and solidify. The comb was then gently removed from the tray, creating wells for loading the DNA sample. The wells of the agarose gel were loaded with 5 microliters of the amplified PCR product, alongside a 5 µl DNA ladder for size comparison. The gel tray was placed in the electrophoresis chamber, and enough 1X TBE buffer was added to cover the gel layer. The electrical current was applied at 70 volts for 1.5 to 2 hours. Finally, visualization of the PCR products was achieved using the

E-graph documentation system. Positive results were determined when the DNA band sizes from the samples corresponded to the target size of the DNA product, as indicated by the ladder.

3.2.15. Molecular Confirmation of *A. baumannii* Using 16S rRNA & *bla*_{OXA-51-like} genes

Molecular identification of aminoglycoside-resistant *A. baumannii* isolates was performed by detecting the 16s rRNA gene and the intrinsic *bla*_{OXA-51-like} gene, for the purpose of confirming the bacterial identity at the molecular level. A total of forty clinical isolates resistant to aminoglycosides were included in this study. (PCR) was carried out using specific primers targeting the two genes, as shown in Table (3-7). The reaction mixture was prepared according to the manufacturer's instructions of GoTaq Green Master Mix, with a final reaction volume of 25 µl. Amplification was performed using a T3000 thermocycler under optimized cycling conditions for each primer set, as presented in Table (3-10). All PCR reactions were prepared under sterile conditions on ice to prevent contamination. The PCR products were analyzed by agarose gel electrophoresis, and the presence of specific DNA bands corresponding to the expected sizes of 16s rRNA and *bla*_{OXA-51-like} genes was considered as confirmation of the identity of *A. baumannii* isolates.

3.2.16. Screening of Genes Encoding AMEs and 16S rRNA Methylase

Clinical isolates of *A. baumannii* obtained from different sources were tested for the presence of aminoglycoside resistance genes, including aminoglycoside-modifying enzymes (AMEs) and 16S rRNA methylase genes. A total of ten genes were investigated, including seven AMEs genes and three 16S rRNA methylase genes. The AMEs genes included aminoglycoside N-acetyltransferases (*aacC1*, *aac(3)-II* and *aac(6')-Ib*),

aminoglycoside O-phosphotransferase *aph(3')-VI* (*aphA6*), and aminoglycoside nucleotidyltransferases (*aadA1*, *aadB*, and *ant(4')-IIb*). In addition, three 16S rRNA methylase genes (*armA*, *rmtD*, and *rmtB*) were also screened. The amplification reactions were carried out using monoplex PCR selected genes. The PCR protocols were performed according to the manufacturer's instructions of GoTag Green Master Mix, with a total reaction volume of 25 ul for monoplex PCR (Table 3-8). Amplification was performed using a T3000 thermocycler. The PCR cycling conditions for each primer set were optimized based on previously published protocols and are summarized in Table (3-10).

3.2.17. Integrons Examination

Class 1 integrons were detected in *A. baumannii* isolates by monoplex PCR using primers specific for the *intI1* gene. The reaction was carried out by monoplex PCR. Amplifications were performed in 25 ul volumes according to GoTag Green manufacturer's protocol (Table 3-8). The PCR was performed using a T3000 thermocycler under the conditions listed in Table (3-10). A 100-bp DNA ladder was used as a molecular size marker.

3.2.18. Sequencing of 16S rRNA and Aminoglycoside Resistance Genes

The PCR products of the 16S rRNA gene and aminoglycoside resistance genes including AMEs (*aac(3)-II*, *aac(6')-Ib*, *aph(3')-VI* (*aphA6*), *aadA1*, *ant(4')-IIb*) and 16S rRNA methylase genes (*armA* and *rmtD*) were sent to Macrogen Company in Seoul, Korea, for sequencing. Chromatograms from the ABI sequence files were thoroughly examined to ensure that the annotations and variations were not a result of PCR or sequencing errors, using Finch TV software for analysis. The virtual locations and additional details of the obtained PCR fragments were determined by comparing the observed nucleic acid sequences of the

local samples with those retrieved from the reference database using Basic Local Alignment Search Tool (BLAST) software from National Center for Biotechnology Information (NCBI). Phylogenetic tree was constructed and the evolutionary distance calculated according to the method of Jukes and Cantor (1969), and the topology was inferred using the neighbor-joining method (Saitou and Nei 1987), based on bootstrap analysis of 1000 trees (using MEGA X software).

3.2.19. Clonal analysis by ERIC-PCR.

To investigate the genetic diversity and the clonal relationship of *A. baumannii* isolates, specific ERIC-PCR primers were used to identify the number of genetic patterns of isolates indicated by Coudron *et al.*, (2000), as shown in Table (3-8). The amplification reaction volume has been 25µl, it was composed of 5ul of template DNA, 1µl of each of the primers, 12.5ul green master mix, then the volume was completed with 25ul of nuclease-free water. While the cycling conditions were as shown in in a Table (3-10). Furthermore, the amplified products were separated by electrophoresis and stained with ethidium bromide on a 1% agarose gel. UPGMA was used to cluster the ERIC patterns, and the coefficient of Dice similarity has been utilized to compare them (Sallman *et al.*, 2018).

2.2.20 Statistical analysis

Data analysis was conducted using SPSS version 24 (IBM Statistical Package for the Social Sciences, Chicago, IL, USA). (IBM Corp., 2020). Data were analyzed using the chi-square (χ^2) test and Fisher's exact test, where applicable, to identify significant differences in resistance or prevalence. A p-value of less than or equal to 0.05 ($p \leq 0.05$) was considered statistically significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

4. Result

4.1. Study Population and Bacterial Isolates

A total of 519 clinical specimens were collected from four hospitals in Babylon Province during the study period. The highest proportion was obtained from Imam Al-Sadiq Teaching Hospital with 260 specimens (50.10%), followed by Al-Hilla Teaching Hospital with 115 specimens (22.16%), Marjan Teaching Hospital with 100 specimens (19.27%), and Babylon Teaching Hospital with 44 specimens (8.48%). According to the distribution of clinical sources, burn specimens represented the highest proportion with 141 specimens (27.17%), followed by blood with 137 specimens (26.40%), sputum with 122 specimens (23.51%), wound swabs with 79 specimens (15.22%), and urine with 40 specimens (7.71%), as shown in Table (4-1). The bacterial isolates were identified using conventional microbiological methods and confirmed by the Vitek-2 automated system, in addition to molecular confirmation. The highest prevalence of *A. baumannii* was detected in burn specimens, followed by blood and sputum specimens, while lower prevalence was recorded in wound and urine specimens. The predominance of *A. baumannii* in burn specimens may be attributed to the loss of skin barrier in burn patients, prolonged hospitalization, and increased exposure to hospital environments, which facilitate opportunistic and nosocomial infections.

These findings are partially consistent with previous studies. Yousif Alnakshabandie *et al.* (2024) reported respiratory specimens as the main source of *A. baumannii* in Duhok, followed by wound and blood samples. Similarly Almasaudi, (2018) reported respiratory tract predominance in Saudi Arabia, while Peleg *et al.* (2008) highlighted the association of *A. baumannii* with ventilator-associated pneumonia, bloodstream, and wound infections among critically ill patients.

In contrast, Vahdani *et al.* (2011) reported respiratory specimens as the primary source followed by urine and wound Specimens, while Iranian studies documented a high prevalence of MDR *A. baumannii* in intensive care units (Ghamari *et al.*, 2025). The variation between studies may be attributed to differences in hospital settings, patient populations, infection control practices, and geographical distribution.

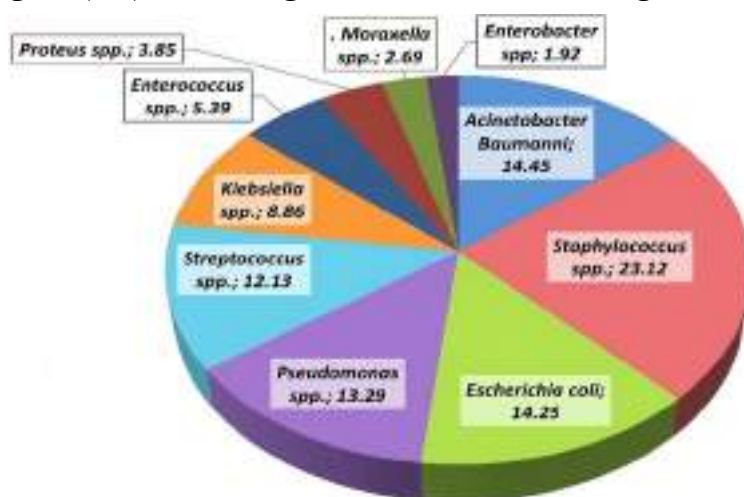
Table(4-1). Distribution of clinical specimens according to source in Babylon hospitals

Specimen source	No. (%) of Clinical specimens
Burn	141 (27.17)
Blood	137 (26.40)
Sputum	122 (23.51)
Wound	79 (15.22)
Urine	40 (7.71)
Total	519 (100.00)

During the study period, a total of 519 clinical specimens were analyzed. The most frequently isolated organisms were *Staphylococcus* spp. (120 isolates, 23.12%), followed by *A. baumannii* (75 isolates, 14.45%), *Escherichia coli* (74 isolates, 14.25%), *Pseudomonas* spp. (69 isolates, 13.29%), and *Streptococcus* spp. (63 isolates, 12.13%). Other bacterial isolates included *Klebsiella* spp. (46 isolates, 8.86%), *Enterococcus* spp. (28 isolates, 5.39%), and *Proteus* spp. (20 isolates, 3.85%), whereas lower frequencies were observed for *Moraxella* spp. (14 isolates, 2.69%) and *Enterobacter* spp. (10 isolates, 1.92%).as shown in Figure (4-1). Statistical analysis. The relatively high prevalence of *A. baumannii* among the recovered isolates highlights its epidemiological importance as one of the demonstrated a significant difference in the distribution of bacterial isolates ($P < 0.001$). major nosocomial pathogens in hospital environments. This organism is characterized by its ability to survive under adverse environmental conditions and to acquire resistance to multiple antimicrobial agents, which contributes to

its persistence and dissemination in healthcare settings. These findings are consistent with several previous local and regional studies. Habash and Jabir (2025) and Obaid, (2025) reported frequent isolation of MDR. *A. baumannii* from hospitalized patients and hospital environments in Iraq. Similarly, studies conducted in Saudi Arabia and Lebanon demonstrated a considerable prevalence of this pathogen among clinical isolates, particularly in healthcare-associated infections (Itani *et al.*, 2023; Alharbi *et al.*, 2025). In contrast, some investigations reported lower prevalence rates of *A. baumannii* compared with other Gram-negative pathogens. Ghsein *et al.* (2026) documented a lower prevalence in a university hospital in Beirut, Lebanon, while Alali *et al.* (2021) found that *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were more prevalent than *A. baumannii* in a tertiary care hospital in Eastern India. Such variations may be attributed to differences in geographical distribution, infection control strategies, antimicrobial usage patterns, specimen types, and patient populations. (Lee *et al.*, 2017).

Figure (4-1): Percentage distribution of microorganisms isolated



from different clinical specimens (N = 519).

4.2. Identification and Prevalence of *A. baumannii* Isolates

A total of 75 (14.45%) *A. baumannii* isolates were identified from 519 clinical specimens collected from four teaching hospitals in Babylon Province. The distribution of isolates varied among hospitals, with 20 isolates

(17.39%) from Al-Hilla Teaching Hospital, 29 (11.15%) from Imam Al-Sadiq Teaching Hospital, 16 (16.00%) from Marjan Teaching Hospital, and 10 (22.73%) from Babylon Teaching Hospital. Regarding specimen sources, the highest number of isolates was recovered from burn specimens (22 isolates, 15.60%), followed by blood (21 isolates, 15.32%), sputum (16 isolates, 13.11%), wound swabs (13 isolates, 16.45%), and urine specimens (3 isolates, 7.50%), as illustrated in Table (4-2). Significant variation in the distribution of *A. baumannii* was observed among specimen types across hospitals, while the strength of association differed according to hospital settings. The results demonstrate of the present study indicate that *A. baumannii* is more frequently associated with burn and bloodstream infections, reflecting its role as an opportunistic pathogen in hospitalized patients. This may be attributed to its ability to survive in hospital environments and persist in intensive care and burn units. These findings are consistent with several previous studies. At the local level in Iraq, research conducted in Al-Najaf Province reported that *A. baumannii* constituted a major proportion of *Acinetobacter* isolates, highlighting its clinical importance in Iraqi healthcare settings (Zghair *et al.*, 2025). Similarly, a study from Saudi Arabia demonstrated that *A. baumannii* represented approximately 11% of clinical bacterial isolates, reflecting its considerable frequency in healthcare facilities across the Middle East (Alharbi *et al.*, 2025). In contrast, lower prevalence rates have been reported in other regions. A multicenter study in the United States found that *Acinetobacter* species accounted for only a small proportion (approximately 1.5%) of nosocomial bloodstream infections (Wisplinghoff *et al.*, 2000). Additionally, reports from military medical facilities in the United States documented outbreaks of *A. baumannii* infections among wounded patients, suggesting variability in occurrence depending on healthcare setting (Scott *et al.*, 2004).

Table (4-2): The Prevalence of *A. baumannii* Isolates Obtained from Various Clinical Specimens in Babylon Hospitals (N=519)

Hospital	Total Samples	Specimen source						X ² or exact Fisher	P. value
		Burn (No.=141)	Blood (No.=137)	Sputum (No.=122)	Wound (No.=79)	Urine (No.=40)	Total		
Al-Hilla Teaching	115	1(0.70)	6(4.37)	0(0)	13(16.45)	0(0)	20(17.39)	32.04	<0.0001**
Imam Al-Sadiq Teaching	260	16(11.34)	1(0.72)	9(7.37)	0(0)	3(7.5)	29(11.15)	22.69	<0.0001**
Marjan Teaching	100	5(3.54)	5(3.64)	6(4.91)	0(0)	0(0)	16(16)	4.97	0.250*
Babylon Teaching	44	0(0)	9(6.56)	1(0.81)	0(0)	0(0)	10(22.72)	14.67	0.001**
Total	519	22(15.6)	21(15.32)	16(13.11)	13(16.45)	3(7.5)	75(14.45)	2.23	0.697*
X ² or exact Fisher		30.46	6.48	13.95	30.09	5.22	5.72		
P. value		<0.001**	0.09*	0.001**	<0.0001**	0.059*	0.126*		

* No significant difference at P<0.05; **Highly significant difference at P<0.01

The demographic distribution of *A. baumannii* isolates among the studied patients is summarized in Table (4-3). The highest number of *A. baumannii* isolates was observed among patients aged 41–60 years (26 cases, 16.15%), followed by patients older than 60 years (20 cases, 15.27%), whereas the lowest number of isolates was recorded among patients younger than one year (4 cases). Although variations were observed in the distribution of isolates among different age groups, these differences were not statistically significant. This pattern may be associated with increased exposure of middle-aged and older patients to hospital environments and a higher prevalence of underlying medical conditions, which may contribute to susceptibility to opportunistic infections. These findings are consistent with García-Garmendia *et al.* (2001), who reported no strong age-dependent distribution of *A. baumannii* infections.

Analysis of the distribution of *A. baumannii* isolates according to gender revealed a slightly higher number of cases among female patients (40 cases, 13.84%) compared with male patients (35 cases, 15.22%). However, the difference between genders was not statistically significant, indicating a similar distribution of *A. baumannii* infections among both sexes. Comparable trends have been reported by Maragakis and Perl (2008) and Almasaudi (2018), indicating no significant association between gender and *A. baumannii* infection.

The distribution of *A. baumannii* isolates among the studied hospitals showed the highest number of isolates in Imam Al-Sadiq Teaching Hospital (29 cases), followed by Al-Hilla

Teaching Hospital (20 cases), Marjan Teaching Hospital (16 cases), and Babylon Teaching Hospital (10 cases). However, the differences among hospitals were not statistically significant. These variations may reflect differences in patient admission rates, hospital workload, and infection control practices rather than true epidemiological differences. Similar findings were reported by Dent *et al.* (2010) and Durante-Mangoni and Zarrilli (2011), emphasizing the widespread presence of *A. baumannii* across healthcare facilities without a consistent hospital-specific pattern.

In this study, the highest number of *A. baumannii* isolates was detected among inpatients (53 cases) compared with outpatients (22 cases). This distribution may be attributed to prolonged hospital stays, increased exposure to invasive procedures, and a higher risk of nosocomial transmission among hospitalized patients. Although the proportion of isolates was higher among outpatients, the difference was not statistically significant. These observations are in agreement with previous studies highlighting the strong association between *A. baumannii* and healthcare settings, particularly among inpatients (Doi *et al.*, 2015).

Table (4-3): Demographic Characteristics of Patients with *A. baumannii* Infections (N=519)

Patient Profile	Status	No. of Specimens (%)	No. of <i>A. baumannii</i> (%)
Age Group (Years)	<1	17 (3.28)	4 (23.53)
	1–20	91 (17.53)	10 (10.99)
	21–40	119 (22.93)	15 (12.61)
	41–60	161 (31.02)	26 (16.15)
	>60	131 (25.24)	20 (15.27)
Total		519 (100.00)	75 (14.45%)
$\chi^2 = 2.79$, df = 4, P > 0.05 No Significant			
Sex	Female	289 (55.68)	40 (13.84)
	Male	230 (44.32)	35 (15.22)
Total		519 (100.00)	75 (14.45%)
Chi-Square	$\chi^2 = 0.20$, df = 1, P > 0.05 No Significant		
Hospital	Al-Hilla Teaching Hospital	115 (22.16)	20 (17.39)
	Imam Al-Sadiq Teaching Hospital	260 (50.10)	29 (11.15)
	Marjan Teaching Hospital	100 (19.27)	16 (16.00)
	Babylon Teaching Hospital	44 (8.48)	10 (22.73)
Total		519 (100.00)	75 (14.45)
Chi-Square	$\chi^2 = 5.72$, df = 3, P > 0.05 No Significant		
Hospitalization	Inpatient	398 (76.69)	53 (13.32)
	Outpatient	121 (23.31)	22 (18.18)
Total		519 (100.00)	75 (14.45)
Chi-Square	$\chi^2 = 1.78$, df = 1, P > 0.05 No Significant		

4.3. Antibiotic Susceptibility of *A. baumannii* Isolates

The findings of the present study indicate high levels of resistance among *A. baumannii* isolates to several antibiotics belonging to the aminoglycoside group. The susceptibility of most antibiotics used in this study, including aminoglycosides (gentamicin, amikacin, netilmicin,

kanamycin, and tobramycin), was analyzed using the standard Kirby-Bauer disc diffusion method according to the CLSI (2025) guidelines. The highest resistance rate was observed for kanamycin (73.33%), followed by tobramycin (69.33%), amikacin (56.00%), and netilmicin (49.33), whereas resistance was comparatively lower for gentamicin (42.67%) as shown in Table(4-4). These findings reflect *A. baumannii* remarkable ability to adapt rapidly to hospital environments and acquire multiple resistance traits due to continuous antimicrobial exposure in healthcare settings, particularly ICUs, which explains the high resistance rates observed. In addition, the frequent use of aminoglycosides in the treatment of severe bacterial infections increases selective pressure on bacterial populations, thereby promoting the survival and dissemination of resistant strains within healthcare facilities. Globally, *A. baumannii* is known for its capacity to persist for prolonged periods on medical equipment and environmental surfaces, facilitating the transmission of resistant strains between patients and contributing to the increased prevalence of antimicrobial resistance in hospital environments (Howard *et al.*, 2012). The results demonstrate of the current study are consistent with several international studies reporting high resistance rates of *A. baumannii* to aminoglycoside antibiotics. For instance, Lee *et al.* (2017) reported that this pathogen is among the most significant nosocomial organisms capable of developing multidrug resistance, including resistance to aminoglycosides, mainly due to the accumulation of resistance mechanisms and the widespread dissemination of MDR strains in hospital settings. Similarly, Kyriakidis *et al.* (2021) highlighted that resistance to aminoglycoside antibiotics has become increasingly common in healthcare systems worldwide due to mechanisms such as aminoglycoside modifying enzymes and efflux pumps that reduce drug susceptibility in *A. baumannii* clinical isolates, limiting available

therapeutic options. Comparable findings have also been reported in regional studies conducted in Iran, where an investigation in Tabriz demonstrated high resistance rates among clinical isolates of *A. baumannii* with approximately 86% resistance to gentamicin, 81% to amikacin, and 63% to tobramycin (Aliakbarzadeh *et al.*, 2014). Furthermore, another study in Tehran, Iran, reported high resistance rates among clinical isolates of *A. baumannii* to aminoglycosides, confirming the widespread problem of aminoglycoside resistance (Mirnejad *et al.*, 2018).

In contrast, some studies reported lower resistance levels to aminoglycosides. For example, a study conducted on 52 *A. baumannii* isolates from ICU patients at Suez Canal University Hospitals in Ismailia, Egypt reported resistance to gentamicin of 51.9%, tobramycin 67.3%, and amikacin 9.6% using automated antimicrobial susceptibility methods (Kishk *et al.*, 2021). Additionally, a systematic review and meta-analysis of *A. baumannii* clinical isolates in Ethiopia reported lower pooled resistance for amikacin (20.3%) compared with gentamicin (57.1%) and tobramycin (47.0%), illustrating geographic differences in aminoglycoside resistance (Asmare *et al.*, 2025). The results demonstrate a high level of resistance among *A. baumannii* isolates to carbapenems. Resistance was highest for imipenem (81.33%) and doripenem (72.00%), whereas meropenem showed lower resistance (54.67%). This may be attributed to extensive use of carbapenems in treating severe infections in hospital settings, particularly ICUs, increasing selective pressure and dissemination of resistant strains (Kramer *et al.*, 2006). The findings of the current study are in agreement with previous studies in Iraq reporting high resistance rates to carbapenems (Hussein *et al.*, 2024; Yousif Alnakshabandi *et*

al., 2024; Abdullah *et al.*, 2024). Resistance to fluoroquinolones was very high, with 92% for levofloxacin and 80% for ciprofloxacin. This likely reflects frequent clinical use of these antibiotics, exerting strong selective pressure. Similar observations have been reported internationally (Aboelenin *et al.*, 2024; Nazari *et al.*, 2025). *A. baumannii* isolates exhibited high resistance to β -lactams and cephalosporins, with elevated rates for piperacillin-tazobactam, ceftazidime, cefepime, ceftriaxone, and trimethoprim-sulfamethoxazole, reflecting widespread MDR strains. Regional studies in Iraq corroborate these findings (AL-Dahlaki, 2020; Habash & Flayyih & Abid, 2022; Abdullah, 2022; Yousif Alnakshabandie *et al.*, 2024; Obaid, 2025; Jabir, 2025). Consistent with these global observations, the results of the present study conducted in Babylon hospitals indicate variable susceptibility of *A. baumannii* isolates to polymyxin and tetracycline antibiotics. Polymyxin B and colistin susceptibility was assessed using the broth microdilution method to determine the (MIC), as these antibiotics cannot be reliably tested by the disk diffusion method. Resistance to polymyxin agents was relatively high, with Polymyxin B showing (56.00%) resistance and colistin (73.33%), highlighting the limitations of these last-line therapies in hospital practice. This trend likely reflects the increased clinical use of these antibiotics for severe infections, which may gradually reduce the efficacy of these critical drugs, as reported in a study conducted in Pakistan (Ahsan *et al.*, 2026). In contrast, tetracycline antibiotics exhibited variable activity. Minocycline had the lowest resistance rate at (14.67%), followed by doxycycline at (20.00%), while tetracycline showed considerably higher resistance of (64.00%). These findings suggest that minocycline remains more effective against MDR *A. baumannii* infection isolates, consistent with

a previous study conducted in the United States of America (Lashinsky *et al.*, 2017), which reported retained activity of minocycline against resistant strains.

Table (4-4): Antibiotic Susceptibility of *A. baumannii* Isolates (N = 75)

Class of Antibiotics	Disc of Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)
Aminoglycosides	Gentamicin	32 (42.67)	3 (4.00)	40 (53.33)
	Tobramycin	52 (69.33)	6 (8.00)	17 (22.67)
	Amikacin	42 (56.00)	15 (20.00)	18 (24.00)
	Netilmicin	37 (49.33)	22 (29.33)	16 (21.33)
	Kanamycin	55 (73.33)	2 (2.67)	18 (24.00)
Carbapenem	Imipenem	61 (81.33)	2 (2.67)	12 (16.00)
	Meropenem	41 (54.67)	0 (0.00)	34 (45.33)
	Doripenem	54 (72.00)	4 (5.33)	17 (22.67)
Fluoroquinolone	Ciprofloxacin	60 (80.00)	6 (8.00)	9 (12.00)
	Levofloxacin	69 (92.00)	4 (5.33)	2 (2.67)
Penicillin/β-Lactamase Inhibitor	Piperacillin-Tazobactam	60 (80.00)	8 (10.67)	7 (9.33)
	Ampicillin-Sulbactam	63 (84.00)	6 (8.00)	6 (8.00)
	Ticarcillin-Clavulanic Acid	68 (90.67)	3 (4.00)	4 (5.33)
Cephalosporin	Cefotaxime	47 (62.67)	6 (8.00)	22 (29.33)
	Ceftriaxone	75 (100.00)	0 (0.00)	0 (0.00)
	Ceftazidime	68 (90.67)	3 (4.00)	4 (5.33)
	Cefepime	71 (94.67)	2 (2.67)	2 (2.67)
Folate Pathway Inhibitor	Trimethoprim-Sulfamethoxazole	59 (78.67)	8 (10.67)	8 (10.67)
Penicillin	Piperacillin	49 (65.33)	3 (4.00)	23 (30.67)
Lipopeptide	Polymyxin B	42 (56.00)	11 (14.67)	22 (29.33)
	Colistin	55 (73.33)	1 (1.33)	19 (25.33)
Tetracycline	Doxycycline	15 (20.00)	5 (6.67)	55 (73.33)
	Minocycline	11 (14.67)	8 (10.67)	56 (74.67)
	Tetracycline	48 (64.00)	8 (10.67)	19 (25.33)

4.4. Clinical Data and Resistance Patterns of Aminoglycoside Resistant *A. baumannii* Isolates

In this study, aminoglycoside non-susceptibility was defined as either resistance or intermediate susceptibility to four or more of the tested aminoglycoside antibiotics, including gentamicin, tobramycin, amikacin, netilmicin, and kanamycin. Among the 75 *A. baumannii* isolates, 40 (53.33%) were classified as non-susceptible to aminoglycosides. Resistance rates varied among the tested antibiotics, with the highest resistance observed for tobramycin (97.5%), followed by amikacin and kanamycin (95.0% each), netilmicin (85.0%), and gentamicin (72.5%). High resistance rates were also recorded against several other antimicrobial agents, including imipenem (95.0%), meropenem (65.0%), doripenem (90.0%), ciprofloxacin (97.5%), levofloxacin (100.0%), piperacillin–tazobactam (92.5%), ampicillin–sulbactam (97.5%), ticarcillin–clavulanic acid (100.0%), cefotaxime (85.0%), ceftriaxone (100.0%), ceftazidime (97.5%), cefepime (100.0%), trimethoprim–sulfamethoxazole (90.0%), piperacillin (87.5%), polymyxin B (85.0%, MIC-based), colistin (87.5%, MIC-based), and tetracycline (87.5%), whereas lower resistance rates were observed for doxycycline (35.0%) and minocycline (27.5%), as shown in Figure (4-2). The high level of aminoglycoside and MDR observed in this study reflects the extensive dissemination of resistant *A. baumannii* strains in hospital environments, which may be attributed to the intensive and often inappropriate use of broad-spectrum antibiotics that exerts strong selective pressure, together with the organism's remarkable ability to acquire and accumulate multiple resistance determinants, including genes carried on mobile genetic elements, allowing its persistence and survival in healthcare environments, while the relatively lower resistance to doxycycline and minocycline suggests that tetracycline derivatives may still retain partial

activity due to lower usage and reduced selective pressure compared to other antibiotic classes. These findings are consistent with previous reports at different a study from hospitals in Egypt reported high aminoglycoside resistance and identified several resistance genes including *aadB*, *apha6*, *aadA1*, and *aacC1*, confirming the genetic basis and dissemination of resistance in Egypt (Rizk & Abou El Khier, 2019).

While regionally, studies from Amman, Jordan reported 87.9%, resistance to gentamicin and 76.7%, to tobramycin among clinical isolates, indicating widespread MDR strains in hospital settings (Batarseh *et al.*, 2016); globally, high resistance rates were also reported from Tabriz, Iran (86% gentamicin, 81% amikacin, 63% tobramycin) and Qazvin, Iran (98.4% gentamicin, 97.9% tobramycin), highlighting the broad dissemination of aminoglycoside resistance mechanisms worldwide (Aliakbarzade *et al.*, 2014; Rashvand *et al.*, 2021), whereas lower resistance levels have been documented in some regions such as Ismailia, Egypt (9.6% amikacin, 51.9% gentamicin, 67.3% tobramycin) and Ethiopia in a meta-analysis (20.3% amikacin, 57.1% gentamicin, 47.0% tobramycin), reflecting geographic variability in resistance patterns (Kishk *et al.*, 2021; Asmare *et al.*, 2025), and overall, *A. baumannii* continues to represent a major global healthcare challenge due to the widespread emergence and persistence of multidrug- resistant strains in hospital environments (Naderi *et al.*, 2023), with the high resistance rates in the present study likely driven by excessive and sometimes inappropriate antibiotic use and the organism's strong capacity for horizontal gene transfer and long-term survival under hospital conditions (Jeon *et al.*, 2023).

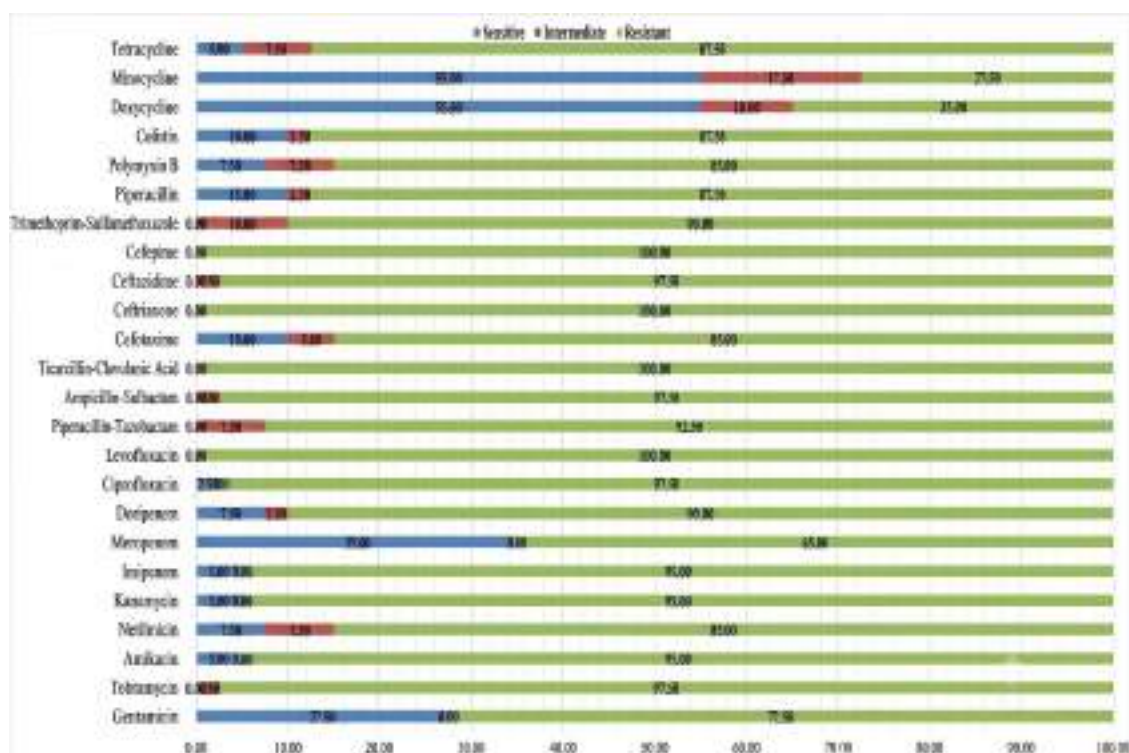


Figure (4-2): Antimicrobial susceptibility results of aminoglycosides resistant *A. baumannii* isolates (N = 40).

As shown in Table (4-5), among a total of 519 clinical specimens analyzed in the present study, 75 (14.45%) yielded *A. baumannii*. *A. baumannii* isolates were recovered from burn specimens (22 isolates, 15.60%), blood (21 isolates, 15.33%), sputum (16 isolates, 13.11%), wound samples (13 isolates, 16.46%), and urine (3 isolates, 7.50%). Regarding aminoglycoside susceptibility, 40 (53.33%) of the *A. baumannii* isolates were non-susceptible. The highest proportion of non-susceptible isolates was detected in urine samples (2 isolates, 66.67%), followed by wound (8 isolates, 61.54%), sputum (9 isolates, 56.25%), burn (12 isolates, 54.55%), and blood samples (9 isolates, 42.86%). Statistical analysis using the Chi-square test revealed no significant association between the type of clinical specimen and aminoglycoside non-susceptibility among *A. baumannii* isolates, indicating that resistance was distributed across different specimen types without a statistically significant pattern ($P > 0.05$).

The findings of the present study demonstrated that non-susceptible *A. baumannii* isolates were distributed across multiple clinical specimens, including wound, respiratory, blood, and urine specimens. This pattern reflects the opportunistic nature of *A. baumannii* and its ability to colonize and infect diverse anatomical sites. The relatively higher proportions of non-susceptible isolates observed in burn and wound specimens may be attributed to the high risk of hospital-acquired infections in these sites and frequent exposure to antimicrobial agents. Comparable observations have been reported in several epidemiological studies from different geographical regions. For example, a study conducted in Lebanon revealed that the majority of the isolates were recovered from respiratory specimens, followed by blood, wound swabs, and urine specimens, confirming a broad clinical distribution within hospital settings (Rafei *et al.*, 2015). Similarly, a long-term epidemiological study conducted in China analyzed 295 clinical isolates collected over 12 years and reported that most isolates originated from respiratory specimens (sputum), with smaller proportions from wound, urine, and other clinical samples, supporting the organism's wide distribution across specimen types (Jiang *et al.*, 2021). Furthermore, clinical and microbiological evidence indicates that nosocomial bacteremia due to *A. baumannii* is frequently associated with respiratory, wound, and urinary tract infections, reinforcing its significant role as a nosocomial pathogen (Cisneros & Rodriguez-Bafio, 2002; Dijkshoorn *et al.*, 2007). Collectively, these findings support the notion that *A. baumannii* is not restricted to a single specimen type but rather exhibits a broad clinical distribution, particularly among hospitalized and critically ill patients.

With regard to aminoglycoside resistance, the present findings demonstrate a high proportion of non-susceptible isolates (53.33%), which is consistent with the global trend of increasing aminoglycoside resistance in *A. baumannii*. Clinical isolates from burn wound infections in Iran have shown high resistance rates to commonly used aminoglycosides, including gentamicin, amikacin, and tobramycin, reflecting the emergence of MDR strains in hospital settings (Tahbaz, Azimi, & Lari, 2019). In contrast, the findings of the present study differ from some reports in other regions. For instance, a study conducted in Tehran, Iran by Ghamari *et al.* (2025) demonstrated that isolates were predominantly recovered from respiratory tract specimens (66.6%), followed by blood samples (18.6%), while wound specimens were reported at a much lower frequency, indicating a distribution pattern that differs from the present findings. Likewise, another study conducted in Tehran, Iran by Vahdani *et al.* (2011) reported that the respiratory tract was the most common source of isolation (39%), followed by urine and blood specimens, whereas wound isolates represented a smaller proportion.

Table (4-5): Distribution of Aminoglycoside -Resistant *A. baumannii* Isolates from Different Clinical Specimens (N = 40)

Type of Specimen	No. of Specimens	No. of <i>A. baumannii</i> Isolates (%)	No. of Aminoglycosides Non-Susceptible <i>A. baumannii</i> (%)
Burn	141 (27.17)	22 (15.60)	12 (54.55)
Blood	137 (26.40)	21 (15.33)	9 (42.86)
Sputum	122 (23.51)	16 (13.11)	9 (56.25)
Wound	79 (15.22)	13 (16.46)	8 (61.54)
Urine	40 (7.71)	3 (7.50)	2 (66.67)
Total	519 (100.00)	75 (14.45)	40 (53.33)
Chi-Square	$X^2 = 1.56, df = 4, p > 0.05$		p-value=0.82

The demographic characteristics of patients from whom the bacteria isolates were obtained are presented in the table above. Analysis of age distribution showed that the highest proportion of resistant isolates was observed in patients aged 41-60 years, with 16 cases (61.54%), followed by those over 60 years with 11 cases (55.00%). Patients aged 21-40 years accounted for 8 cases (53.33%), while 4 cases (40.00%) were reported in the 1-20 year age group. Only one case (25.00%) was detected in patients younger than one year as shown in Table (4-6). Statistical analysis indicated that the differences in resistance across age groups were not significant ($P > 0.05$). Regarding Sex, 21 resistant isolates (52.50%) were collected from female patients and 19 (54.29%) from male patients, with no significant association between sex and resistance ($P > 0.05$). When considering hospital distribution, 10 cases (50.00%) were identified at Al-Hilla Teaching Hospital, 15 cases (51.72%) at Imam Al- Sadiq Teaching Hospital, 8 cases (50.00%) at Marjan Teaching Hospital, and 7 cases (70.00%) at Babylon Teaching Hospital. No statistically significant difference in resistance prevalence was observed between the four hospitals ($P > 0.05$). Finally, analyzing patient hospitalization status showed that 29 resistant isolates (54.72%) came from inpatients, while 11 isolates (50.00%) were from outpatients. This difference was not statistically significant ($P > 0.05$). The findings of the present study demonstrate a noticeable variation in the distribution of aminoglycosides - resistant the bacteria isolates across different age groups. The highest proportion of resistant isolates was observed among patients aged 41-60 years (61.54%), followed by those older than 60 years (55%). Lower proportions were recorded in younger age groups, including 21-40 years (53.33%) and 1-20 years (40%), with the lowest prevalence detected among infants under one year of age. This pattern may be explained by the increased susceptibility of middle-aged and elderly patients to hospital-acquired infections, which can be attributed to factors such as the presence of chronic diseases, weakened

immune responses, prolonged hospital stays, and frequent exposure to antimicrobial therapy and invasive medical procedures. Nevertheless, statistical analysis indicated no significant differences among the age groups ($P>0.05$), suggesting that *A. baumannii* infections can occur across all age categories and are not strictly confined to a particular age group.

The findings of the present study align with established epidemiological evidence indicating that infections are predominantly associated with hospitalized and critically ill patients, particularly in intensive care units (ICUs). *A. baumannii* is recognized globally as an opportunistic nosocomial pathogen capable of causing a wide range of infections, including pneumonia, bloodstream infections, wound infections, and urinary tract infections in patients with compromised health status and prolonged hospital stays (Alibrahim *et al.*, 2025; Almoghrabi *et al.*, 2024). This pathogen's persistence in hospital environments and its ability to rapidly develop resistance to multiple antibiotics contribute to its clinical significance among adult and older patients with underlying medical conditions or extensive exposure to invasive medical procedures (Alibrahim *et al.*, 2025). Such epidemiological trends support the observation in the current study that middle-aged and elderly patients exhibit a higher occurrence of antibiotic-resistant bacteria isolates, although infection can occur across all age groups due to the bacterium's opportunistic nature and adaptability in healthcare settings. However, the results of the present study differ from some reports that documented notable occurrences of *A. baumannii* infections among pediatric populations in intensive care settings. For example, a retrospective investigation conducted in the pediatric intensive care unit (PICU) of Shanghai Children's Hospital, Shanghai, China found that MDR and XDR *A. baumannii* infections were a significant clinical concern among hospitalized children, with isolates recovered from a variety of clinical

specimens including blood, respiratory tract, and other body fluids (Shi *et al.*, 2020). Similarly, molecular characterization of MDR the bacteria strains from PICU patients at the First Affiliated Hospital of Sun Yat-sen University in Guangdong, China showed the presence of resistant isolates associated with serious infections in children (Chen *et al.*, 2018). Additionally, retrospective analysis from the PICU of Shengjing Hospital, Shenyang, China demonstrated high rates of *A. baumannii* drug resistance among isolates from pediatric patients (Xu, 2021). These findings contrast with the current study, where lower proportions of resistant isolates were observed among younger age groups. Such discrepancies may be attributed to differences in study design, sample size, clinical specimen types, and variations in patient characteristics, infection control practices, and antimicrobial usage patterns across hospitals and geographic regions. Although *A. baumannii* infections are often associated with adults and elderly patients in many studies, these pediatric investigations highlight that this pathogen can also affect children, especially in PICU settings where multiple risk factors for nosocomial infection exist.

The results of the present study showed that the distribution of aminoglycoside - resistant isolates was similar between males and females. Resistant isolates were detected in (52.50%) of female patients and (54.29%) of male patients, with no significant association between Sex and resistance ($P > 0.05$). This similarity may be attributed to the fact *that* infections are mainly associated with hospital- related factors such as prolonged hospitalization, exposure to antimicrobial therapy, and the use of invasive medical devices, which affect both sexes in a comparable manner. The findings of the current study indicate that gender is not consistently a major determinant in the epidemiology of infections, as hospital-associated factors such as prolonged hospitalization, use of invasive devices, and prior antimicrobial exposure play a more significant role in the spread and

resistance of this pathogen. However, several studies have reported a predominance of infections among male patients, highlighting potential local variations. For instance, a five-year retrospective study in Beirut, Lebanon found that 67% of isolates were recovered from males (Ghssein *et al.*, 2026). Similarly, a nine-year review in Salerno, Italy showed that most positive cases occurred in male patients (Serretiello *et al.* 2025), and a multicenter analysis in Hebei Province, China reported that nearly two thirds of isolates were from male patients, with males also showing higher resistance rates (Liu *et al.*, 2025). These observations suggest that while sex may influence infection distribution in some settings, local hospital populations, patient characteristics, and infection control practices are likely the main drivers of *A. baumannii* epidemiology.

The results of the present study indicate that the distribution of aminoglycosides - resistant *A. baumannii* isolates among the four hospitals was relatively similar. The resistance rates were (50%) at Al-Hilla Teaching Hospital, (51.72%) at Imam Al-Sadiq Teaching Hospital, (50%) at Marjan Teaching Hospital, and (70%) at Babylon Teaching Hospital. Although slight variations were observed among the hospitals, statistical analysis showed no significant difference in resistance prevalence between them ($P > 0.05$). This similarity may be attributed to comparable hospital environments, similar antimicrobial usage practices, and infection control strategies across these healthcare facilities, which can lead to closely related resistance patterns. The findings of the present study are consistent with previous reports indicating that *A. baumannii* is a major cause of hospital- acquired infections, particularly in intensive care units and among critically ill patients. Systematic reviews have shown that this pathogen exhibits significant antimicrobial resistance and persists in healthcare environments, contributing to its widespread occurrence in hospitals within the same geographical

regions (Ayobami *et al.*, 2019). Furthermore, Almasaudi, (2018) emphasized that *Acinetobacter* species, especially *A. baumannii*, are recognized as important nosocomial pathogens with multiple mechanisms of resistance and environmental survival, which enhance their persistence and facilitate transmission among patients hospitals. These observations highlight that the prevalence and distribution of *A. baumannii* in hospitals are strongly influenced by hospital-associated risk factors and infection control practices. However, the results of the present study differ from some other investigations that reported noticeable variations in the prevalence of these bacteria among hospitals. Several studies conducted in Iraq have demonstrated that the distribution of *A. baumannii* varies according to the type of clinical and environmental specimens, with isolates recovered from sources such as sputum, wounds, urine, and hospital environments (Zidan *et al.*, 2022; Obaid, 2025).

In addition, a high prevalence of MDR isolates, including aminoglycoside- resistant to these the bacteria, has been reported in hospitals (Kishk *et al.*, 2021). These findings indicate that variability in isolation sources and resistance patterns exists across healthcare settings. The present study showed that 29 resistant isolates (54.72%), were recovered from inpatients, while 11 isolates (50.00%) were obtained from outpatients, with no statistically significant difference between the two groups ($P > 0.05$). This opportunistic pathogen is commonly associated with hospital-acquired infections, particularly among hospitalized patients who are exposed to prolonged hospital stays, invasive procedures, and extensive antibiotic use. Previous research in Baghdad, Iraq, reported high rates of imipenem-resistant to these the bacteria recovered from both hospitalized patients and the hospital environment (Hussein *et al.*, 2025). Similarly, study conducted in Najaf, Iraq, demonstrated that isolates from clinical specimens exhibited

extensive MDR and carried multiple resistance genes (Jubair *et al.*, 2025). In addition, research in a burn hospital in Al-Sulaymaniyah, Kurdistan Region of Iraq, showed the dissemination of MDR and XDR to these the bacteria among patients and hospital wards (Shali *et al.*, 2022). Furthermore, analysis of isolates from hospitals in Babylon and Al Najaf, Iraq, revealed a high prevalence of carbapenem resistance genes, confirming the challenge of antimicrobial resistance in multiple Iraqi hospitals (Witwit *et al.*, 2026).

Table (4-6): Demographic Characteristics of Patients with Aminoglycoside - Resistant *A. baumannii* Isolates (N=40)

Patient Profile	Status	No. of <i>A. baumannii</i> (%)	No. of Resistant <i>A. baumannii</i> (%)
	<1	4 (5.33)	1 (25.00)
Age Group (Years)	1–20	10 (13.33)	4 (40.00)
	21–40	15 (20.00)	8 (53.33)
	41–60	26 (34.67)	16 (61.54)
	>60	20 (26.67)	11 (55.00)
Total		75 (100.00)	40 (53.33)
Chi-Square	$X^2=2.73$, $df=4$, $P > 0.05$ No significant		
Sex	Female	40 (53.33)	21 (52.50)
	Male	35 (46.67)	19 (54.29)
Total		75 (100.00)	40 (53.33)
Chi-Square	$X^2=0.02$, $df=1$, $P > 0.05$ No significant		
Hospital	Al-Hilla Teaching Hospital	20 (26.67)	15 (50.00)
	Imam Al-Sadiq Teaching Hospital	29 (38.67)	15 (51.72)
	Marjan Teaching Hospital	16 (21.33)	8 (50.00)
	Babylon Teaching Hospital	10 (13.33)	7 (70.00)
Total		75 (100.00)	40 (53.33)
Chi-Square	$X^2=1.31$, $df=3$, $P > 0.05$ No significant		
Hospitalization	Inpatient	53 (70.67)	29 (54.72)
	Outpatient	22 (29.33)	11 (50.00)
Total		75 (100.00)	40 (53.33)
Chi-Square	$X^2=0.14$, $df=1$, $P > 0.05$ No significant		

4.5. Multiple Antibiotic Resistance Phenotypes

According to the classification proposed by Magiorakos *et al.* (2012), multiple antibiotic resistance isolates are categorized as MDR, XDR, and

PDR. The distribution of multiple antibiotic resistance phenotypes among aminoglycoside-resistant isolates recovered in the present study is illustrated in Figure (4-3). Among the aminoglycoside-resistant isolates, the vast majority, 37 isolates (92.50%), exhibited (XDR) phenotypes, indicating non-susceptibility to at least one agent in seven or more of the nine antimicrobial classes tested. In contrast, three isolates (7.50%) were classified as possible (PDR), demonstrating non-susceptibility to all tested antimicrobial categories, while no (MDR) isolates were detected. These findings indicate an alarmingly high level of antimicrobial resistance among the studied isolates, reflecting the organism's remarkable ability to acquire and accumulate multiple resistance mechanisms within hospital environments. The predominance of XDR isolates and the emergence of PDR phenotypes may be attributed to the excessive or inappropriate use of antimicrobial agents, particularly in hospitals and ICUs, in addition to the organism's capacity to persist for prolonged periods in hospitals and form biofilms that enhance survival and resistance. Furthermore, the dissemination of mobile genetic elements, including plasmids, integrons, and resistance-associated genes encoding antibiotic-modifying enzymes, may contribute significantly to the horizontal transfer and spread of antimicrobial resistance determinants among bacterial populations. The presence of these highly resistant phenotypes represents a major therapeutic challenge, as they substantially limit available treatment options and may increase the risk of treatment failure, morbidity, and mortality among infected patients. Similar findings have been reported in previous studies in Tabriz, Iran by Aliakbarzade *et al.* (2014).

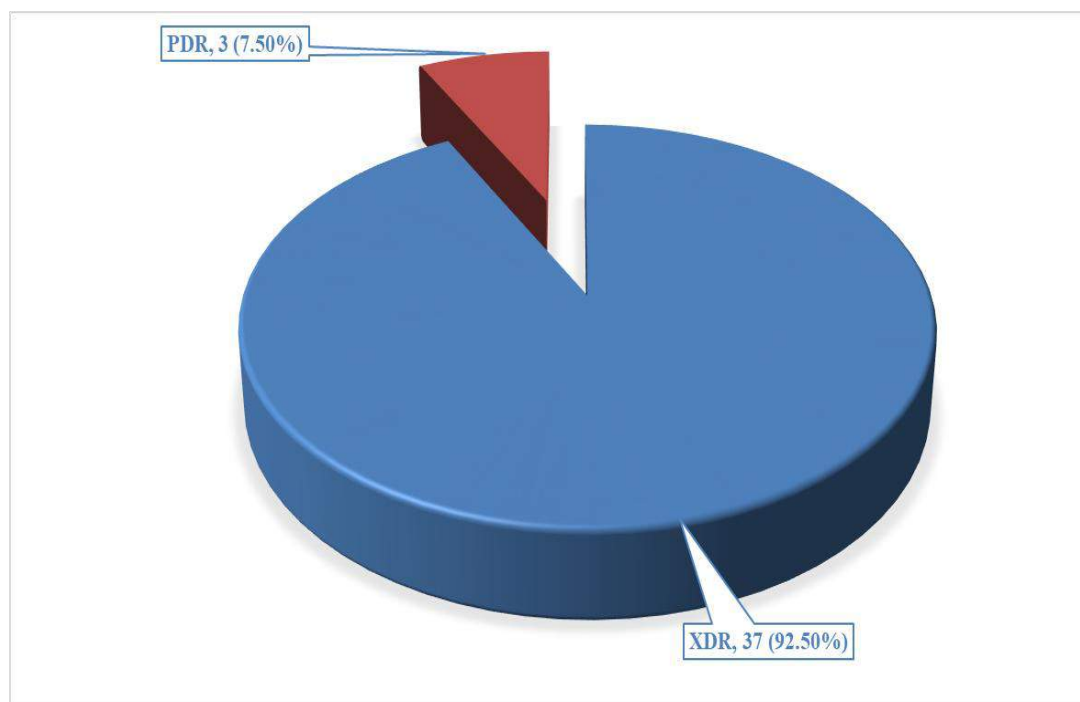


Figure (4-3): Multiple Antibiotic Resistant Phenotypes of aminoglycoside Resistant *A. baumannii* Isolates (N=40).

4.6. Multiple Antibiotics Resistance (MAR) Index

In the present study, the MAR index was calculated based on the antibiogram results of aminoglycoside-resistant *A. baumannii* isolates. The frequency distribution of the MAR index is presented in Figure (4-4). Analysis of the data revealed that most isolates exhibited high MAR index values exceeding 0.2, indicating extensive exposure to antimicrobial agents and strong selective pressure within the clinical environment under investigation. Specifically, three isolates (7.50%) recorded a MAR index of 1.00 (AB-9, AB-19, and AB-34), one isolate (2.50%) showed 0.67 (AB-14), and another isolate (2.50%) demonstrated 0.75 (AB-15). Four isolates (10.00%) had a MAR index of 0.79 (AB-1, AB-2, AB-8, and AB-16), while two isolates (5.00%) recorded 0.83 (AB-5 and AB-6). Ten isolates (25.00%) exhibited a MAR index of 0.88 (AB-3, AB-7, AB-11, AB-12, AB-13, AB-20, AB-21, AB-26, AB-29, and AB-36), eight isolates (20.00%) had 0.92 (AB-4, AB-10, AB-22, AB-23, AB-24, AB-27, AB-30, and AB-31), and

eleven isolates (27.50%) showed a MAR index of 0.96 (AB-17, AB-18, AB-25, AB-28, AB-32, AB-33, AB-35, AB-37, AB-38, AB-39, and AB-40), representing the second-highest level of resistance observed in this study. These findings indicate a substantial prevalence of multiple resistance among aminoglycoside-resistant *A. baumannii* isolates. Regional data from Iraq support the high prevalence of multiple resistant *A. baumannii* in clinical isolates. The present results are consistent with a clinical study at Dr. Soetomo General Academic Hospital in Surabaya, Indonesia, which included *A. baumannii* among the studied organisms and reported MAR index values ranging from 0.22 to 0.91, indicating significant antibiotic exposure within the hospital environment (Puspita *et al.*, 2021). Similarly, a recent investigation in northwest China on MDR *A. baumannii* isolates recovered from hospitalized patients at the First Hospital of Lanzhou University reported MAR index values ranging from 0.3 to 0.8. Among these isolates, 36% had a MAR index of 0.7, (28%) had 0.6, (28%) had 0.5, and (6%) had 0.8, highlighting a substantial presence of highly resistant strains in hospitals (Hu *et al.*, 2024).

In contrast, other clinical studies have documented a wider range of MAR index values among *A. baumannii* isolates derived from different infection sites. For instance, isolates obtained from cerebrospinal fluid, respiratory, and urinary samples exhibited MAR index values ranging from approximately 0.29 to 1.0 with higher values in isolates from central nervous system infections and lower values more common among body fluid samples, reflecting variable antibiotic exposure and selective pressure depending on the infection type (Rajab *et al.*, 2025).

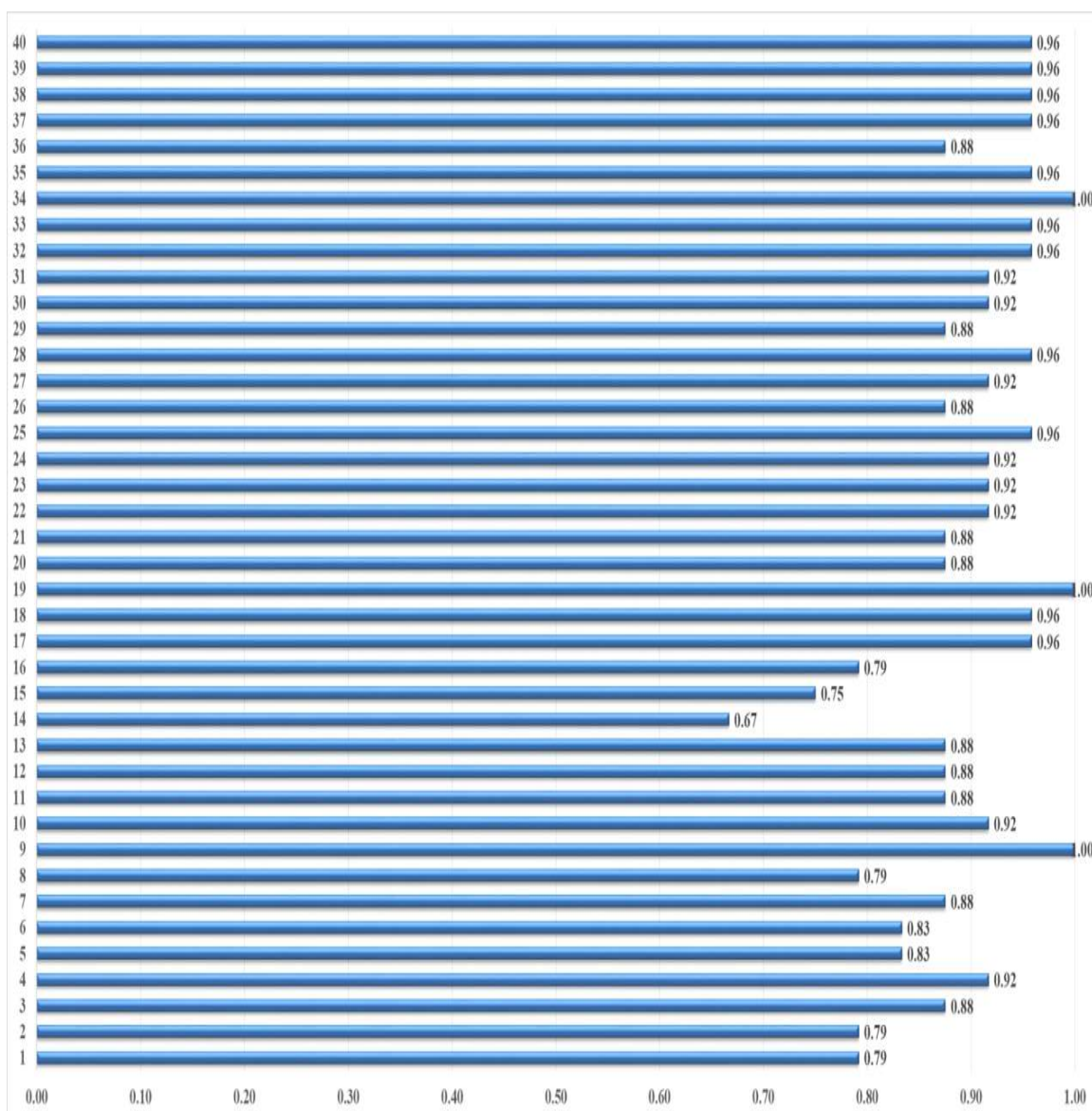


Figure (4-4): Multiple Antibiotic Resistance (MAR) Index of aminoglycoside - Resistant *A. baumannii* Isolates (N = 40) .

4.7. MultidrugResistance Patterns

The *in vitro* antimicrobial susceptibility testing of the 40 aminoglycoside-resistant *A. baumannii* isolates revealed considerable diversity in antimicrobial resistance profiles, with a total of 28 distinct resistance patterns identified, as shown in Table (4-7). The identification of a relatively large number of resistance patterns among 40 isolates indicates substantial heterogeneity in the resistance phenotypes of the studied isolates. Pattern 1 was observed in three isolates (9, 19, and 34),

which were classified as (PDR), accounting for 7.5% of the total aminoglycoside-resistant isolates. The remaining 37 isolates (92.5%) were classified as (XDR) and were distributed among 27 different resistance patterns. Among the identified resistance patterns, pattern 4 was the most frequently observed, occurring in six isolates (15%), followed by pattern 3, which was detected in three isolates (7.5%). Patterns 7, 12, and 13 were each observed in two isolates (5%). The remaining resistance patterns were represented by one isolate each (2.5%), demonstrating substantial diversity in the resistance profiles of the studied *A. baumannii* isolates. The considerable diversity of resistance patterns observed in the present study is consistent with findings from other regional investigations. Mirzaei *et al.* (2020) reported the occurrence of highly resistant phenotypes among clinical isolates of *A. baumannii* in Iran. Similarly, variations in the distribution of MDR, XDR, and PDR phenotypes have been reported in Pakistan, indicating differences in resistance patterns among *A. baumannii* isolates from different healthcare settings (Jabeen *et al.*, 2022). The occurrence of PDR *A. baumannii* has also been reported in Iraq, supporting the presence of highly resistant isolates in healthcare settings (Jubair *et al.*, 2025).

The identification of 28 distinct resistance patterns among 40 isolates demonstrates considerable phenotypic heterogeneity within the studied *A. baumannii* population. This diversity may be associated with differences in the acquisition and accumulation of antimicrobial resistance determinants among individual isolates. The predominance of XDR phenotypes, together with the occurrence of PDR isolates, demonstrates the high level and diversity of antimicrobial resistance among the aminoglycoside-resistant *A. baumannii* isolates investigated in the present study.

Table (4-7) Antibiotic resistance profiles and multidrug resist. Phenotypes of *A. baumannii*

No. of pattern	Antibiotic resistant pattern	No. of antibiotics N=24	No. of classes n=9	Type of resistance	No. of isolates (%)	Isolate No.
1	CN, TOB, AK, NET, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, DO, M, TE	24	9	PDR	3(7.5%)	9,19, 34
2	CN, TOB, AK, NET, K, IPM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, DO, M, TE	23	8	XDR	1(2.5%)	18
3	CN, TOB, AK, NET, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, DO, TE	23	8	XDR	3(7.5%)	25,33,35
4	CN, TOB, AK, NET, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, TE	22	8	XDR	6(15%)	17,28,32,37,39,40
5	TOB, AK, NET, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, DO, TE	22	7	XDR	1(2.5%)	4
6	CN, TOB, AK, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, DO, TE	22	7	XDR	1(2.5%)	22
7	CN, TOB, AK, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, TE	21	7	XDR	2(5%)	24,31
8	CN, TOB, AK, NET, K, IPM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, PI, PB CT, M, TE	21	6	XDR	1(2.5%)	10
9	CN, TOB, NET, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTR, CAZ, CPM, TS, PI, PB CT, DO, TE	21	6	XDR	1(2.5%)	12

10	TOB, AK, NET, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, TE	21	7	XDR	1(2.5%)	20
11	CN, TOB, NET, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, TE	21	7	XDR	1(2.5%)	23
12	CN, TOB, AK, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, TE	21	7	XDR	2(5%)	27,38
13	CN, TOB, AK, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB, DO, TE	21	7	XDR	2(5%)	26,29
14	CN, TOB, AK, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, M,	21	7	XDR	1(2.5%)	30
15	TOB, AK, NET, K, IPM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, CT, M, TE	20	5	XDR	1(2.5%)	3
16	CN, TOB, AK, NET, IPM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, M,	20	6	XDR	1(2.5%)	5
17	TOB, AK, NET, K, IPM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT, TE	20	6	XDR	1(2.5%)	13
18	TOB, AK, NET, K, IPM, MEM, DOR, CIP, LE, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB, M, TE	20	5	XDR	1(2.5%)	21
19	CN, TOB, AK, NET, K, IPM, MEM, DOR, CIP, LE, TPZ, AS, TCC, CTR, CAZ, CPM, PI, DO, M, TE	20	6	XDR	1(2.5%)	36
20	TOB, AK, NET, K, IPM, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, PI, CT, M, TE	19	4	XDR	1(2.5%)	7
21	TOB, AK, NET, K, IPM, DOR, CIP, LE, TP2, AS, TCC, CTX, CTR, CAZ, CPM, TS, PB CT, TE	19	5	XDR	1(2.5%)	8

22	TOB, AK, NET, K, IPM, DOR, CIP, LE, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, CT, TE	18	5	XDR	1(2.5%)	1
23	CN, TOB, AK, NET, DOR, CIP, LE, TPZ, AS, TCC, CTX, CTR, CAZ, CPM, TS, PI, PB CT	18	6	XDR	1(2.5%)	16
24	TOB, AK, NET, K, IPM, DOR, CIP, LE, TPZ, TCC, CTX, CTR, CAZ, CPM, PI, CT, TE	17	3	XDR	1(2.5%)	2
25	CN, TOB, AK, NET, K, IPM, CIP, LE, TPZ, AS, TCC, CTR, CAZ, CPM, TS, CT, TE	17	4	XDR	1(2.5%)	11
26	TOB, AK, NET, K, IPM, LE, TPZ, AS, TCC, CTR, CAZ, CPM, TS, PB CT, DO, TE	17	4	XDR	1(2.5%)	15
27	CN, AK, NET, K, DOR, CIP, LE, AS, TCC, CTR, CPM, TS, PI, PB CT,	15	4	XDR	1(2.5%)	6
28	TOB, AK, NET, K, IPM, CIP, LE, TPZ, AS, TCC, CTR, CAZ, CPM, TS, PB	15	3	XDR	1(2.5%)	14

4.8. Genotypic Identification of Aminoglycoside-Resistant *A. baumannii* Isolates

Molecular identification of the forty aminoglycoside-resistant *A. baumannii* isolates was performed using the species-specific gene *bla*_{OXA-51-like} and the *16S rRNA* gene. All isolates tested positive for both genes, confirming their classification as *A. baumannii*. As shown in Figure (4-5), (4-6) PCR amplification produced clear and distinct DNA bands, with approximate sizes of 353 bp for *bla*_{OXA-51} and 545 bp for *16S rRNA*. The presence of *bla*_{OXA-51-like} in all isolates serves as a reliable intrinsic genetic marker for identifying *A. baumannii*, while the *16S rRNA* gene further confirmed the bacterial identity and supported molecular classification. These results are consistent with a previous study conducted in Brazil, which demonstrated the effectiveness of *16S rRNA* gene-based approaches for bacterial identification in hospital environments (Rampelotto *et al.*, 2019).

Genes such as *bla*_{OXA-51-like} are considered highly specific molecular markers for accurate species-level identification and phylogenetic analysis of *A. baumannii* (Janda and Abbott, 2007). Studies from Iraq and the surrounding region have also reported the use of *bla*_{OXA-51} for identifying *A. baumannii* isolates. For instance, Al-Kadmy (2018) employed this gene for species confirmation, while Ridha and Ali (2019) and Kadhom and Ali (2022) similarly reported its reliability in genotypic identification of clinical isolates. These findings align with the results of the present study, confirming the suitability of *bla*_{OXA-51-like} and *16S rRNA* for accurate identification of aminoglycoside-resistant *A. baumannii*.

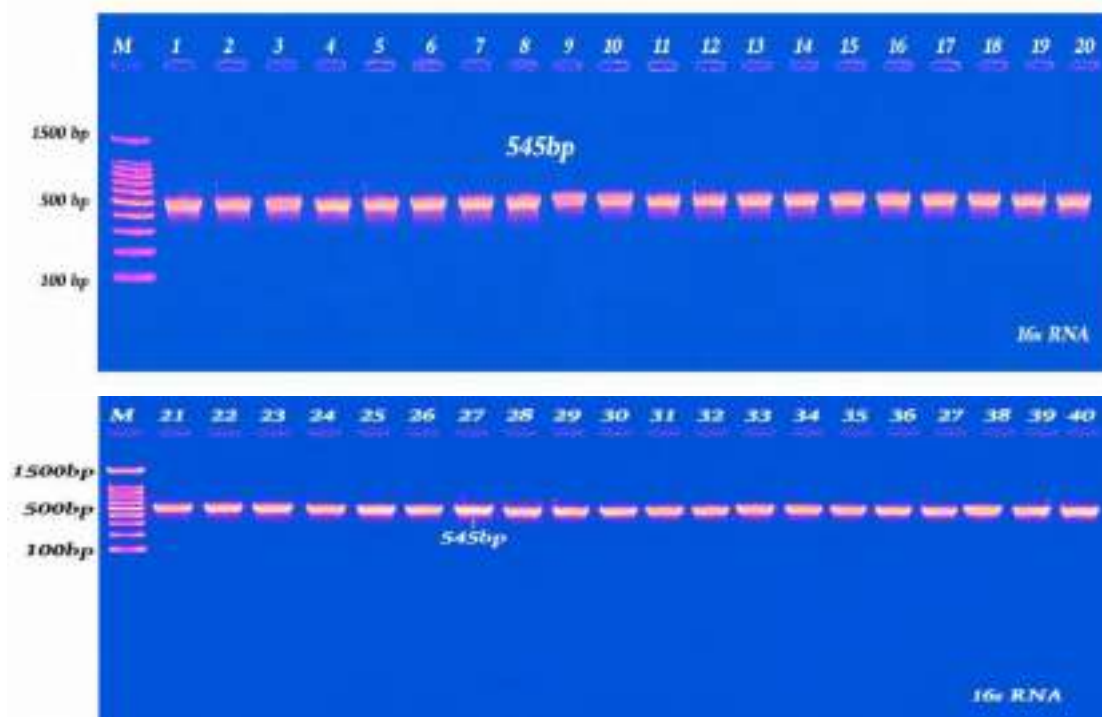


Figure (4-5): Electrophoresis of the PCR Products of *16S rRNA* (545 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (1-40): Positive Results of the *16S rRNA* .

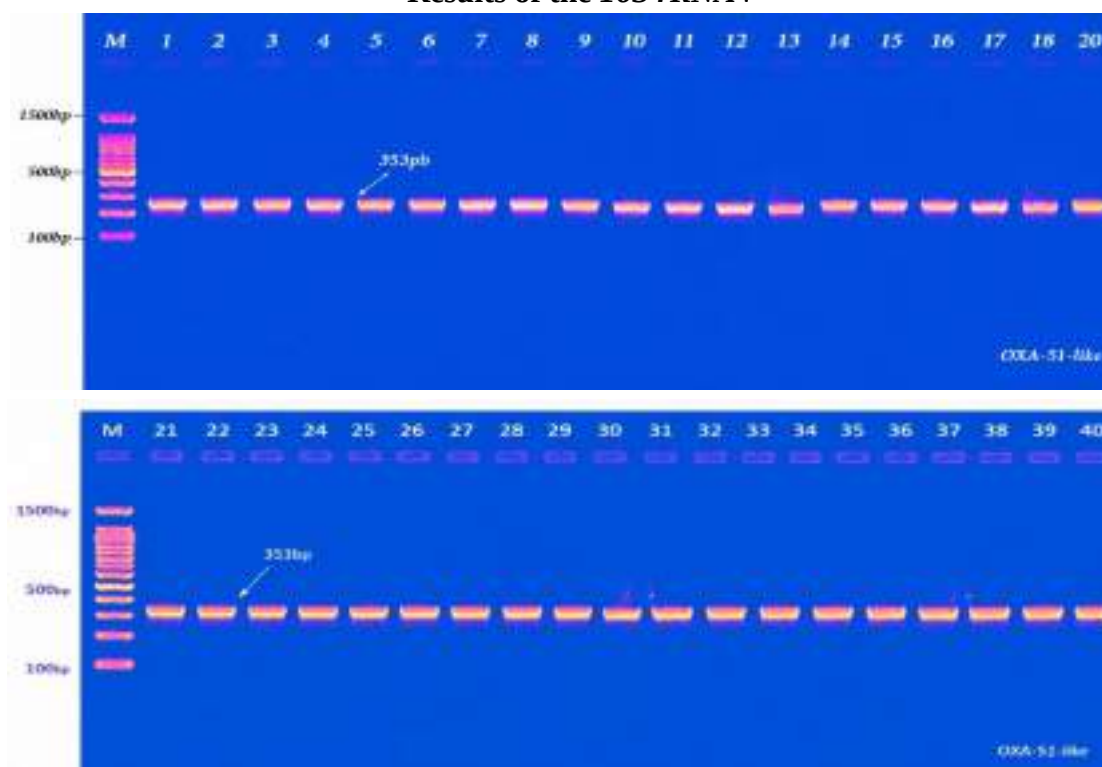


Figure (4-6): Electrophoresis of the PCR Products of *bla*_{OXA-51-like} (353 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (1-40): Positive Results of the *bla*_{OXA-51-like}.

4.9. Sequencing of 16S rRNA Gene

The 16S rRNA gene sequencing analysis was performed to molecularly confirm the identity of the studied *A. baumannii* isolates. All investigated isolates demonstrated a high degree of sequence similarity with reference sequences retrieved from the GenBank database, confirming the reliability of the phenotypic and initial molecular identification methods used in the present study. These findings are consistent with those reported by Khosravi *et al.* (2015), who demonstrated that 16S rRNA gene sequencing is an effective molecular method for accurate identification of *A. baumannii* isolates. The highly conserved nature of the 16S rRNA gene further supports its usefulness as a reliable molecular marker for bacterial identification and taxonomic confirmation. To strengthen the genetic characterization and ensure representation of the circulating strains, seven representative sequences were submitted to the GenBank database and assigned accession numbers PX915552.1, PX915553.1, PX915571.1, PX915573.1, PX915580.1, PX915581.1, and PX915582.1. (Appendix 1).

These deposited sequences provide publicly accessible molecular references that may facilitate future comparative, phylogenetic, and epidemiological investigations of *A. baumannii* strains. Phylogenetic analysis based on partial 16S rRNA gene sequences revealed a distinct clustering pattern among the studied isolates as shown in Figure (4-17). The majority of isolates (AB35, AB10, AB19, AB34, AB8, AB24, AB1, AB26, and AB9) clustered together within a major clade characterized by short branch lengths and minimal genetic distances. This finding indicates a high level of sequence conservation and close evolutionary relatedness among these isolates. The observed clustering pattern may reflect the circulation of genetically related strains within the same clinical environment, suggesting possible clonal

relatedness and dissemination of *A. baumannii* isolates within the hospital setting (Karthikeyan *et al.*, 2026).

In contrast, isolates AB21, AB12, and AB17 formed a separate divergent cluster with comparatively longer branch lengths, indicating greater genetic distances relative to the major cluster. The presence of these genetically distinct isolates may suggest the introduction of multiple lineages into the clinical environment or the occurrence of independent infection sources. Similar observations have previously been associated with the emergence of genetically diverse *A. baumannii* populations in healthcare settings (Wright *et al.*, 2014).

Additionally, isolate AB13 showed close clustering with reference GenBank strains, indicating strong genetic conservation with globally reported *A. baumannii* sequences and suggesting close evolutionary relatedness to well-characterized international lineages. This finding may indicate the circulation of strains sharing common ancestral origins with globally distributed isolates. Furthermore, the representative GenBank-submitted isolates designated Iraq(1, 8, 9, 10, 13, 34, and 35) were distributed across the phylogenetic tree according to their respective genetic relationships, supporting the overall diversity pattern observed among the studied isolates. The phylogenetic analysis demonstrated a predominantly low level of genetic divergence among most isolates, alongside limited but distinct genetic variation within a smaller subset of strains. This pattern reflects the coexistence of a dominant closely related lineage together with minor genetically distinct variants, indicating both clonal dissemination and microevolutionary changes within the studied *A. baumannii* population. These findings provide important insights into the molecular epidemiology, evolutionary dynamics, and dissemination

patterns of this clinically significant nosocomial pathogen among the studied isolates. These findings are consistent with Duan *et al.* (2024).

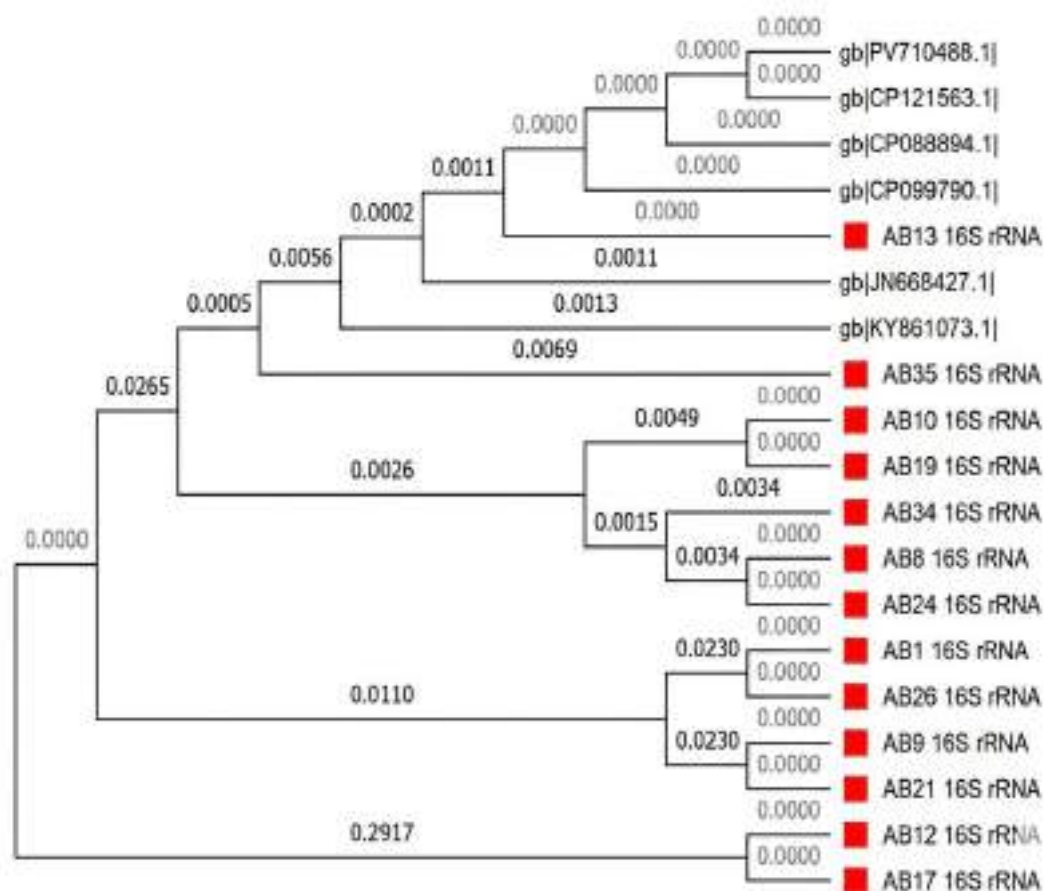


Figure (4-7): Phylogenetic Tree of Partial 16S rRNA Gene sequences among Local and Reference *A. baumannii* isolates

4.10. Prevalence of Aminoglycoside-Modifying Enzyme (AME) Genes and 16S rRNA Methylase Genes

The present study demonstrated a high prevalence of (AME) genes and 16S rRNA methylase genes among 40 aminoglycoside-resistant *A. baumannii* clinical isolates. Among the AME genes, *aac(6')-Ib* was the most frequently detected gene, followed by *aph(3')-VI* (*aphA6*) and *ant(4')-IIb*. Among the 16S rRNA methylase genes, *rmtB*, *rmtD*, and *armA* were identified in variable proportions. These findings indicate that aminoglycoside resistance in the studied isolates

is mediated through combined mechanisms involving enzymatic drug modification and ribosomal target protection, contributing to elevated resistance levels. Genetic analysis of aminoglycoside-resistant *A. baumannii* isolates revealed the presence of several resistance-associated genes, including [*aacC1*, *aac(3)-II*, *aac(6'')-Ib*, *aph(3')-VI* (*aphA6*), *aadA1*, and *ant(4')-IIB*], suggesting a complex genetic background supporting aminoglycoside resistance. The coexistence of multiple AME genes within single isolates may reflect cumulative enzymatic activity resulting from selective antimicrobial pressure in hospital environments, consistent with findings reported by Asadollahi *et al.* (2011). In the present study, the *aac(6'')-Ib* gene was detected in (50%) of resistant isolates Figure (4-8). This gene is frequently associated with plasmids and integrons that facilitate horizontal gene transfer and rapid dissemination of resistance determinants. Comparable findings were reported in China (Chen *et al.*, 2022), whereas higher prevalence rates were documented in Saudi Arabia (El-Badawy *et al.*, 2021), highlighting geographical variation related to antimicrobial usage practices and infection control measures. The *aacC1* gene was detected in 20% of isolates Figure (4-9), which is consistent with Mortazavi *et al.* (2020). Similarly, the *aac(3)-II* gene was identified in (15%) of isolates Figure (4-10), comparable to the findings of Wang *et al.* (2016), although higher rates were reported in Ahvaz, Iran (Shooshtari *et al.*, 2020). These differences may reflect regional variability in the distribution of AME genes and differences in antimicrobial selective pressure. Regarding phosphotransferase genes, the *aph(3')-VI* (*aphA6*) gene was detected in 47.5% of isolates Figure (4-11), which is consistent with a local Iraqi study reporting a prevalence of (47%) (Fadhil & Farhan, 2025).

The high prevalence of this gene suggests its important role in mediating aminoglycoside resistance, particularly against amikacin, among clinical isolates in the local environment. The *aadA1* gene was detected at a low frequency (7.5%) Figure (4-12) . Whereas the *aadB* gene was not detected in any isolate. This finding suggests limited circulation of these determinants within the studied isolates compared with reports from neighboring regions (Kishk *et al.*, 2021; Khurshid *et al.*, 2020). In contrast, the *ant(4')-IIb* gene was identified in 45% of isolates Figure (4-13), indicating its important contribution to aminoglycoside inactivation through adenylation mechanisms. Several isolates exhibited coexistence of multiple AME genes, including *aac(6')-Ib*, *aph(3')-VI* (*aphA6*), *ant(4')-IIb*, *aacC1*, *aac(3) II*, and *aadA1*, indicating the accumulation of multiple resistance determinants within individual isolates. In contrast, the absence of detectable AME genes in a limited number of isolates suggests that additional resistance mechanisms, such as efflux pumps, reduced membrane permeability, or ribosomal alterations, may also contribute to aminoglycoside resistance. Analysis of 16S rRNA methylase genes revealed the presence of *armA*, *rmtB*, and *rmtD*, indicating the occurrence of high-level aminoglycoside resistance mediated through methylation of the ribosomal binding site, which interferes with aminoglycoside binding and reduces antibiotic activity. The *rmtB* gene was detected in (30%) of isolates. Figure (4-14) . Although earlier reports described limited evidence regarding its prevalence in *A. baumannii*, its detection in the present study suggests an increasing role of ribosomal target modification as a resistance mechanism, similar to findings reported in Vietnam (Tada *et al.*, 2013). In contrast, a study conducted in Tehran, Iran failed to detect this gene (Gholami *et al.*, 2017), reflecting geographical variation associated with local

epidemiological and antimicrobial usage factors. The *armA* gene was identified in (12.5%) of isolates Figure (4-15), consistent with reports from Pakistan and other regional studies (Bakour *et al.*, 2014; Salimizand *et al.*, 2018). The distribution of this gene supports its dissemination as a mobile genetic element-associated resistance determinant among clinical isolates. The *rmtD* gene was detected in the present study in six isolates (15%) of *A. baumannii* resistant to aminoglycosides using (PCR) Figure (4-16) . The identity of the positive isolates was further confirmed by sequencing analysis, which validated the PCR amplification results. Although this gene has been investigated in previous studies involving *A. baumannii*, including a large-scale study conducted in the United Kingdom and Ireland involving 550 clinical isolates, no positive detection of *rmtD* was reported in that study (Taylor *et al.*, 2022), where *armA* was identified as the predominant 16 S rRNA methylase gene. This variation suggests a geographical difference in the distribution of aminoglycoside resistance determinants, which may be influenced by factors such as antimicrobial selective pressure and the dissemination of mobile genetic elements. The detection of *rmtD* in the present study indicates its presence within the circulating clinical strains in the studied setting and highlights its potential contribution to aminoglycoside resistance in this local population. The findings of the present study demonstrate the widespread dissemination of aminoglycoside resistance determinants among clinical *A. baumannii* isolates. The coexistence of AME genes and 16S rRNA methylase genes within the same isolates reflects the complexity of resistance mechanisms and may contribute to reduced therapeutic efficacy of aminoglycosides (Zhang *et al.*, 2023).

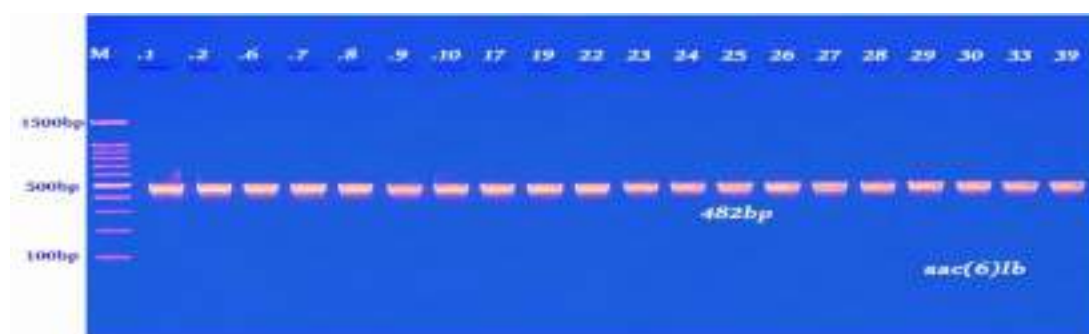


Figure (4-8): Electrophoresis of the PCR Products of *aac(6')-Ib* (482 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (1-39): Positive Results of the *aac(6')-Ib*.



Figure (4-9): Electrophoresis of the PCR Products of *aacC1* (465 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (5-34): Positive Results of the *aacC1*.



Figure (4-10): Electrophoresis of the PCR Products of *aac(3)-II* (370 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (3-28): Positive Results of the *aac(3)-II*.



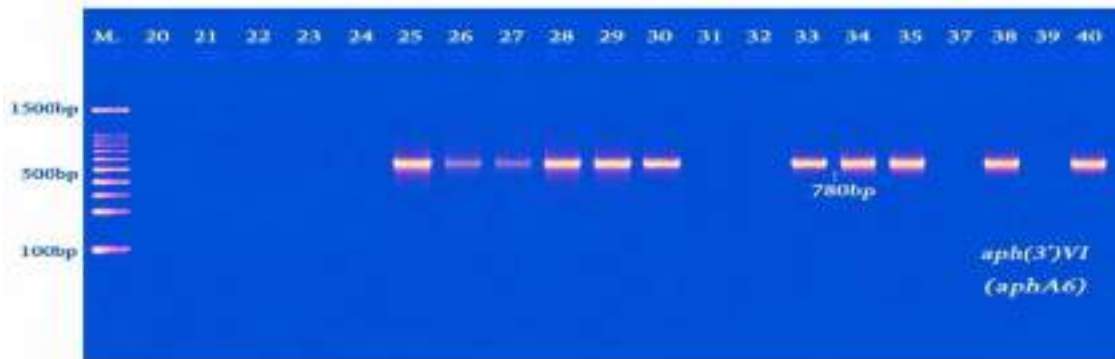


Figure (4-11): Electrophoresis of the PCR Products of *aph(3')-VI (aphA6)* (780 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (1-40): Positive Results of the *aph(3')-VI (aphA6)*.



Figure (4-12): Electrophoresis of the PCR Products of *aadA1* (792 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (5-13): Positive Results of the *aadA1*.

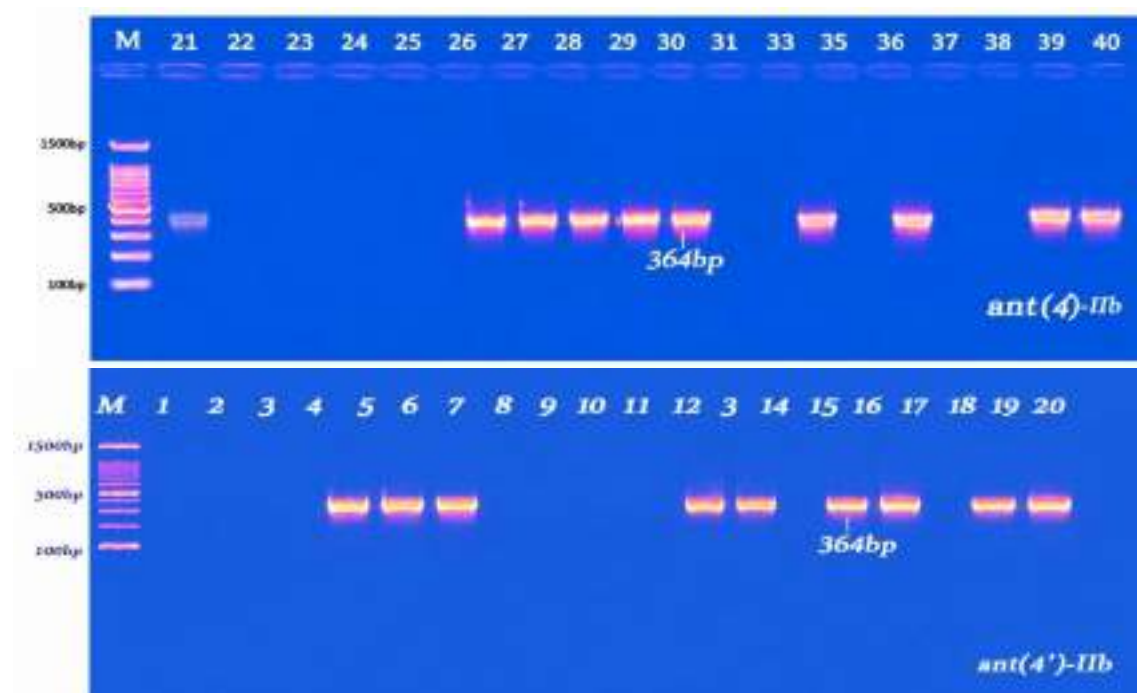
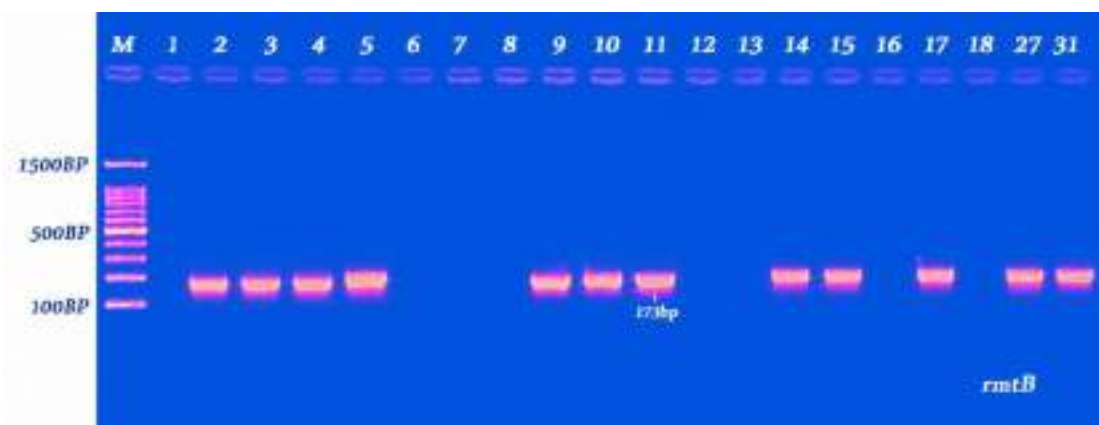


Figure (4-13): Electrophoresis of the PCR Products of *ant(4')-IIb* (364 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (1-40): Positive Results of the *ant(4')-IIb*.



Figure(4-14): Electrophoresis of the PCR Products of *rmtB* (173 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (1-31): Positive Results of the *rmtB*.



Figure (4-15): Electrophoresis of the PCR Products of *armA* (315 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (14-27): Positive Results of the *armA*.

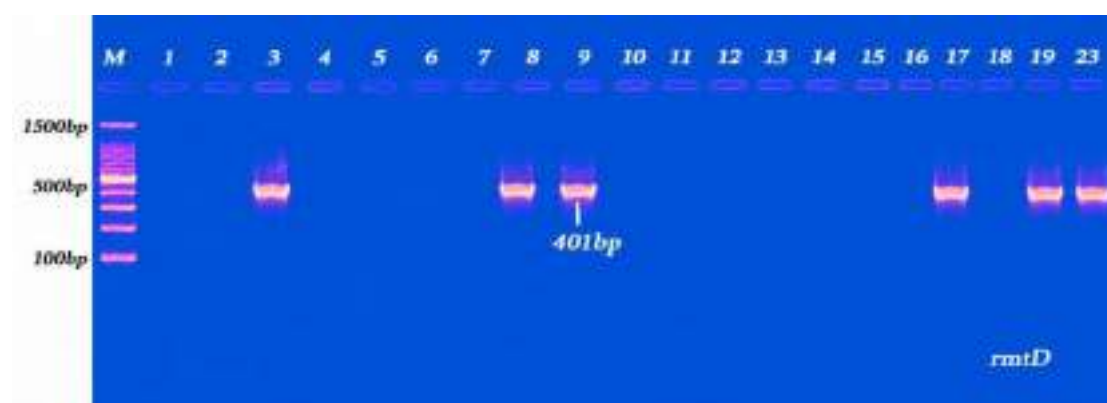


Figure (4-16): Electrophoresis of the PCR Products of *rmtD* (401 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (3-23): Positive Results of the *rmtD*.

4.11. Association Between Aminoglycoside Resistance Genes, Phenotypic Resistance, and MAR Index in *A. baumannii*

The detected genes belonged to two major groups AMEs and 16s rRNA methylase genes, indicating that aminoglycoside resistance in these isolates is mediated through a combination of enzymatic drug inactivation and ribosomal target modification. This reflects the adaptive ability of the organism under strong antimicrobial selective pressure. Among the AME genes, and as shown in Table(4-8). The *aac(6')-Ib* was the most frequently detected (50.0%), followed by *aph(3')-VI* (*aphA6*) (47.5%), *ant(4')-IIb* (45.0%), and *aacC1* (20.0%). Lower frequencies were observed for *aac(3)-II* (15.0%) and *aadA1* (7.5%), whereas *aadB* was not detected in any isolate. This distribution indicates that enzymatic inactivation, particularly acetylation and phosphorylation mechanisms, represents the dominant pathway of aminoglycoside resistance in the studied isolates. The high prevalence of *aac(6')-Ib* is likely associated with its location on mobile genetic elements such as plasmids and integrons, facilitating horizontal gene transfer. Similarly, the widespread occurrence of *aphA6* may be attributed to its strong association with high-level resistance to amikacin, which exerts continuous selective pressure in clinical settings. The absence of *aadB* suggests limited circulation of this determinant in the studied population compared with other regions. Among the 16S rRNA methylase genes, *rmtB* was the most prevalent (30.0%), followed by *rmtD* (15.0%) and *armA* (12.5%). These genes are clinically significant as they confer high-level resistance by methylating the aminoglycoside binding site on the 16S rRNA, thereby preventing antibiotic binding to the ribosome. The detection of *rmtD* in this study is particularly notable. Its presence indicates dissemination within the local circulating strains. This finding suggests geographical variation in the distribution of this determinant. Phenotypic

analysis revealed that all isolates exhibited resistance to at least four aminoglycosides (gentamicin, tobramycin, amikacin, netilmicin, and kanamycin), indicating extensive resistance within this antimicrobial class. Most isolates were classified as extensively drug-resistant (XDR), while a smaller proportion exhibited pandrug-resistant (PDR) phenotypes. The Multiple Antibiotic Resistance (MAR) index ranged from 0.67 to 1.00, reflecting high antimicrobial exposure and strong selective pressure in the hospital environment. A small number of isolates showed intermediate susceptibility (IN) to certain agents. Integration of genotypic and phenotypic data demonstrated that isolates harboring multiple resistance genes tended to exhibit higher MAR index values and more severe resistance profiles, supporting the accumulation of resistance determinants within single strains. Comparative analysis with previous studies showed variability in gene distribution. A study from Iran reported higher frequencies of *aphA6* and *aacC1* (Aliakbarzade *et al.*, 2014), while Aris *et al.* (2019) highlighted the key role of *aphA6* in amikacin resistance among global clone 1 isolates. In contrast, Rizk and Abou El Khier (2019) reported different distributions, with *aadB* being more prevalent in some regions, which differs from the current findings where this gene was not detected. Furthermore, Hu *et al.* (2024) reported MAR index values ranging from 0.3 to 0.8 in multidrug-resistant *A. baumannii*, which are lower than those observed in the present study, indicating stronger antimicrobial pressure in the current clinical setting. High MAR values reflect intensive antibiotic usage and contribute to the selection of resistant strains. At the molecular level, *aphA6* is often associated with mobile genetic elements such as *TnaphA6*, facilitating horizontal gene transfer among bacterial populations. The coexistence of multiple resistance genes within individual isolates reflects genetic accumulation driven by horizontal gene transfer and clonal expansion, contributing to the emergence of XDR and PDR phenotypes.

These results confirm the combined role of enzymatic inactivation and ribosomal protection in mediating aminoglycoside resistance in *A. baumannii* (Martínez-Trejo *et al.*, 2022).

Table (4-8) :Distribution of Aminoglycoside Resistance Genes and Their Association with Phenotypic Resistance Patterns and MAR Index in *A. baumannii* Clinical Isolates(N=40).

Type of Gene	Number of isolates	Isolates number	Antibiotic resistance to aminoglycosides					MAR index	Multiple Antibiotic Resistance phenotypes
			CN	TOB	AK	NET	K		
<i>aacC1</i>	8 (20.00)	12	R	R	S	R	R	0.88	XDR
		15	S	R	R	R	R	0.75	XDR
		16	R	R	R	R	S	0.79	XDR
		17	R	R	R	R	R	0.96	XDR
		19	R	R	R	R	R	1.00	PDR
		28	R	R	R	R	R	0.96	XDR
		29	R	R	R	R	R	0.88	XDR
		34	R	R	R	R	R	1.00	PDR
<i>aac(3)-II</i>	6 (15.00)	3	S	R	R	R	R	0.88	XDR
		4	R	R	R	R	R	0.92	XDR
		5	R	R	R	R	S	0.83	XDR
		10	R	R	R	R	R	0.92	XDR
		21	S	R	R	R	R	0.88	XDR
		28	R	R	R	R	R	0.96	XDR
<i>aac(6')-Ib</i>	20(50.00)	1	S	R	R	R	R	0.79	XDR
		2	S	R	R	R	R	0.79	XDR
		6	R	R	R	R	R	0.83	XDR
		7	S	R	R	R	R	0.88	XDR
		8	S	R	R	R	R	0.79	XDR
		9	R	R	R	R	R	1.00	PDR
		10	R	R	R	R	R	0.92	XDR
		17	R	R	S	R	R	0.96	XDR
		19	R	R	R	R	R	1.00	PDR
		22	S	R	R	R	R	0.92	XDR
		23	R	R	S	R	R	0.92	XDR
		24	R	R	R	R	R	0.92	XDR
		25	R	R	R	R	R	0.96	XDR
		26	R	R	R	S	R	0.88	XDR
		27	R	R	R	S	R	0.92	XDR
		28	R	R	R	R	R	0.96	XDR
		29	R	R	R	R	R	0.88	XDR
		30	R	R	R	S	R	0.92	XDR
		33	R	R	R	R	R	0.96	XDR
		39	R	R	R	R	R	0.96	XDR
		1	S	R	R	R	R	0.79	XDR

<i>aph(3')-VI</i> (<i>aphA6</i>)	19 (47.50)	4	R	R	R	R	R	0.92	XDR
		7	S	R	R	R	R	0.88	XDR
		8	S	R	R	R	R	0.79	XDR
		11	R	R	R	R	R	0.88	XDR
		12	R	R	S	R	R	0.88	XDR
		14	S	R	R	R	R	0.67	XDR
		16	R	R	R	R	S	0.79	XDR
		18	R	R	R	R	R	0.96	XDR
		19	R	R	R	R	R	1.00	PDR
		25	R	R	R	R	R	0.96	XDR
		29	R	R	R	R	R	0.88	XDR
		30	R	R	R	S	R	0.92	XDR
		33	R	R	R	R	R	0.96	XDR
		34	R	R	R	R	R	1.00	PDR
		35	R	R	R	R	R	0.96	XDR
		36	R	R	R	R	R	0.88	XDR
		38	R	R	R	IN	R	0.96	XDR
		40	R	R	R	R	R	0.96	XDR
<i>aadA1</i>	3(7.50)	5	R	R	R	R	S	0.83	XDR
		10	R	R	R	R	R	0.92	XDR
		13	S	R	R	R	R	0.88	XDR
<i>aadB</i>	0 (0.00)	---	---	---	---	---	--	---	---
<i>ant</i> (4')-IIb	18 (45.00)	4	R	R	R	R	R	0.92	XDR
		5	R	R	R	R	S	0.83	XDR
		9	R	R	R	R	R	1.00	PDR
		12	R	R	S	R	R	0.88	XDR
		13	S	R	R	R	R	0.88	XDR
		15	S	R	R	R	R	0.75	XDR
		16	R	R	R	R	S	0.79	XDR
		18	R	R	R	R	R	0.96	XDR
		19	R	R	R	R	R	1.00	PDR
		27	R	R	R	S	R	0.92	XDR
		28	R	R	R	R	R	0.96	XDR
		29	R	R	R	R	R	0.88	XDR
		30	R	R	R	S	R	0.92	XDR
		31	R	R	R	IN	R	0.92	XDR
		34	R	R	R	R	R	1.00	PDR
		36	R	R	R	R	R	0.88	XDR
		39	R	R	R	R	R	0.96	XDR
		40	R	R	R	R	R	0.96	XDR
<i>armA</i>	5 (12.50)	14	S	R	R	R	R	0.67	XDR
		20	S	R	R	R	R	0.88	XDR
		21	S	R	R	R	R	0.88	XDR
		26	R	R	R	S	R	0.88	XDR
		27	R	R	R	S	R	0.92	XDR
<i>rmtD</i>	6 (15.00)	3	S	R	R	R	R	0.88	XDR
		8	S	R	R	R	R	0.79	XDR
		9	R	R	R	R	R	1.00	PDR

<i>rmtB</i>	12 (30.00)	17	R	R	R	R	R	0.96	XDR
		19	R	R	R	R	R	1.00	PDR
		23	R	R	S	R	R	0.92	XDR
		2	S	R	R	R	R	0.79	XDR
		3	S	R	R	R	R	0.88	XDR
		4	R	R	R	R	R	0.92	XDR
		5	R	R	R	R	S	0.83	XDR
		9	R	R	R	R	R	1.00	PDR
		10	R	R	R	R	R	0.92	XDR
		11	R	R	R	R	R	0.88	XDR
		14	S	R	R	R	R	0.67	XDR
		15	S	R	R	R	R	0.75	XDR
		17	R	R	R	R	R	0.96	XDR
		27	R	R	R	S	R	0.92	XDR
		31	R	R	R	IN	R	0.92	XDR

K,kanamycin, NET,netilmicin, AK, amikacin, TOB, tobramycin and CN, gentamicin .

4.12. Occurrence of AME and 16S rRNA Methylase Genes According to the Aminoglycoside Resistance Phenotype

The association between aminoglycoside resistance phenotypes and the distribution of aminoglycoside resistance genes among *A. baumannii* isolates is presented in Table (4-9). Analysis of antimicrobial susceptibility patterns among the 40 aminoglycoside-resistant isolates revealed five distinct resistance phenotypes against the tested aminoglycosides. The most predominant phenotype was simultaneous resistance to all tested aminoglycosides, including kanamycin, netilmicin, amikacin, tobramycin, and gentamicin, which was observed in 18 isolates (45%). This phenotype was associated with diverse combinations of aminoglycoside-modifying enzyme (AME) genes together with 16S rRNA methylase genes, particularly *rmtB* and *rmtD*, indicating the accumulation of multiple resistance determinants within highly resistant isolates. The second most frequent phenotype involved resistance to kanamycin, netilmicin, amikacin, and tobramycin and was detected in 11 isolates (27.5%). These isolates exhibited variable genetic profiles comprising different combinations of AME genes in addition to

methylase genes such as *rmtB*, *rmtD*, and *armA*. Resistance to netilmicin, amikacin, tobramycin, and gentamicin was identified in two isolates (5%), each demonstrating distinct combinations of AME genes associated with the *rmtB* gene. In addition, resistance to kanamycin, netilmicin, tobramycin, and gentamicin was observed in three isolates (7.5%), mainly associated with AME genes including *aac(6')-Ib*, *aph(3')-VI* (*aphA6*), and *ant(4')-IIb*. Furthermore, a resistance phenotype involving kanamycin, amikacin, tobramycin, and gentamicin was detected in six isolates (15%). Some of these isolates carried only AME genes, whereas others additionally harbored methylase genes such as *armA* and *rmtB*. Overall, the findings demonstrated marked heterogeneity in the distribution of AME and 16S rRNA methylase genes among different aminoglycoside resistance phenotypes. The presence of resistance genes did not always completely correspond with the observed phenotypic resistance patterns, suggesting possible variation in gene expression levels or the involvement of additional resistance mechanisms contributing to aminoglycoside resistance. The coexistence of multiple AME genes and 16S rRNA methylase genes within highly resistant isolates may explain the broad resistance patterns observed against aminoglycoside agents. Similar findings have been reported in Iraqi studies, which documented frequent detection of *aac(6')-Ib*, *aph(3')-VI* (*aphA6*), and *armA* among clinical *A. baumannii* isolates associated with elevated aminoglycoside resistance profiles in hospital environments (Kadhim and Al Draghi, 2025). The coexistence of multiple resistance determinants among different resistance phenotypes reflects the complex genetic background underlying aminoglycoside resistance in clinical *A. baumannii* isolates (Sutimbekova *et al.*, 2026).

Table (4-9): Resistance Patterns and Genetic Profiles of Aminoglycoside-Resistant *A. baumannii* (n = 40)

Aminoglycoside Resistance pattern	Aminoglycoside genetic profiles	Isolates code No.	No. (%) of isolate
K, NET, AK, TOB, CN	<i>aac(3)-II, aph (3')-VI (aphA6), ant (4')-IIB, rmtB</i>	4	18(45)
	<i>rmtD aac(6')-Ib, ant (4')IIB, rmtB,</i>	9	
	<i>aac(3)-II, aac(6')-Ib, aadA1, rmtB</i>	10	
	<i>aph (3')-VI (aphA6), rmtB</i>	11	
	<i>aacC1, aac(6')-Ib, rmtD, rmtB</i>	17	
	<i>aph (3')-VI (aphA6), ant (4')- IIB</i>	18, 36	
	<i>aacC1, aac(6')-Ib, aph (3')-VI (aphA6), ant (4')-IIB, rmtD</i>	19	
	<i>aac(6')-Ib, aph (3')-VI (aphA6)</i>	25	
	<i>aacC1, aac(3)-II, aac(6')-Ib, aph (3')-VI (aphA6), ant (4')-IIB</i>	28	
	<i>aacC1, aac(6')-Ib, ant (4')-IIB</i>	29	
	-----	32, 37	
	<i>aac(6')-Ib, Aph (3')-VI (aphA6)</i>	33	
	<i>aacC1, Aph (3')-VI (aphA6), ant (4')-IIB,</i>	34	
	<i>aph (3')-VI (aphA6)</i>	35	
	<i>aac(6')-Ib, ant (4')-IIB,</i>	39	
	<i>aph (3')-VI (aphA6), ant (4')-IIB</i>	40	
K, NET, AK, TOB	<i>aac(6')-Ib, aph (3')-VI (aphA6)</i>	1	11(27.5)
	<i>aac(6')-Ib, rmtB</i>	2	

	<i>aac(3)-II, rmtD, rmtB</i>	3	
	<i>aac(6')-Ib, aph (3')-VI (aphA6)</i>	7	
	<i>aac(6')-Ib, aph (3')-VI (aphA6), rmtD</i>	8	
	<i>ant (4')-IIb, aadA1</i>	13	
	<i>aadA1, armA, rmtB</i>	14	
	<i>aacC1, rmtB</i>	15	
	<i>armA</i>	20	
	<i>aac(3)-II, ant (4')-IIb, armA</i>	21	
	<i>aac(6')-Ib</i>	22	
NET, AK, TOB, CN	<i>aac(3)-II, aadA1, ant (4')-IIb, rmtB</i>	5	2(5)
	<i>aacC1, aph (3')-VI (aphA6), ant (4')- IIb</i>	16	
K, NET, TOB, CN	<i>aac(6')-Ib</i>	6	3(7.5)
	<i>aacC1, aph (3')-VI (aphA6), ant (4')- IIb</i>	12	
	<i>aac(6')-Ib, rmtD</i>	23	
K, AK, TOB, CN	<i>aac(6')-Ib</i>	24	6(15)
	<i>aac(6')-Ib, armA</i>	26	
	<i>aac(6')-Ib, aph (3')-VI (aphA6)</i>	27	
	<i>aac(6')-Ib, ant (4')-IIb</i>	30	
	<i>ant (4')-IIb, rmtB</i>	31	
	<i>aph (3')-VI (aphA6)</i>	38	

K,kanamycin, NET,netilmicin, AK, amikacin, TOB, tobramycin and CN, gentamicin .

4.13. Class 1 Integron (*intI1*) Gene and Antimicrobial Resistance in *A. baumannii*

The class 1 integron gene (*intI1*), used as a molecular marker indicating the presence of class 1 integron structures in *A. baumannii*, was detected in 9 (22.50%) of the 40 aminoglycoside-resistant *A. baumannii* isolates, as shown in Figure (4-17). This finding indicates the presence of class 1 integron-associated elements among the studied isolates and suggests that *intI1*-positive isolates represent a subset of resistant strains that may contribute to the dissemination of antimicrobial resistance determinants.

Among the nine *intI1*-positive isolates, aminoglycoside resistance genes showed a heterogeneous distribution, as presented in Table (4-10). Regarding the 16S rRNA methylase genes, *rmtD* was detected in 1 isolate (11.11%), whereas *armA* was detected in 2 isolates (22.22%). The *rmtB* gene was not detected (0.00%) among the *intI1*-positive isolates. Among the aminoglycoside-modifying enzyme (AME) genes, *aac(6')-Ib* was the most frequently detected gene, occurring in 6 isolates (66.67%), followed by *aph(3')-VI* (*aphA6*) in 5 isolates (55.56%) and *ant(4')-IIb* in 3 isolates (33.33%). The *aacC1* gene was detected in 1 isolate (11.11%), whereas *aac(3)-II* was detected in 1 isolate (11.11%). The *aadA1* gene was not detected (0.00%) among the *intI1*-positive isolates. The total number of detected aminoglycoside resistance genes exceeded the total number of *intI1*-positive isolates because individual isolates could harbor more than one resistance gene simultaneously. This finding demonstrates the coexistence of multiple aminoglycoside resistance determinants within some *intI1*-positive isolates and indicates heterogeneity in their genetic

resistance profiles. In relation to antimicrobial resistance phenotypes, 8 (88.89%) of the *intI1*-positive isolates exhibited an extensively drug-resistant (XDR) phenotype, whereas 1 (11.11%) exhibited a pandrug-resistant (PDR) phenotype. Thus, the majority of *intI1*-positive isolates exhibited an XDR phenotype. The coexistence of multiple aminoglycoside resistance genes within *intI1*-positive isolates highlights the potential role of class 1 integrons in the accumulation and dissemination of antimicrobial resistance determinants. However, the detection of *intI1* in 9 (22.50%) of the aminoglycoside-resistant isolates suggests that class 1 integrons alone may not fully account for the distribution of resistance determinants among the studied isolates. Other mobile genetic elements, including plasmids and transposons, may also contribute to the acquisition and dissemination of resistance genes. When compared with previous studies, these findings are consistent with reports from Iraq demonstrating the presence of class 1 integrons associated with antimicrobial resistance determinants among clinical *A. baumannii* isolates (Alabdali, 2022). Variations in the prevalence of class 1 integrons among regional and international studies may reflect differences in antimicrobial use, infection control practices, geographical distribution, and circulating resistant clones. The present study indicates that class 1 integrons may contribute to the dissemination of aminoglycoside resistance in *A. baumannii*, although they may act in conjunction with other genetic mechanisms that collectively shape the resistance profiles observed in clinical isolates (Makke et al., 2020).



Figure (4-17): Electrophoresis of the PCR Products of *IntI1* (160 bp) from *A. baumannii* Isolates. Lane (L): DNA Ladder (100-1500 bp). Lanes (1-40): Positive Results of *IntI1*.

Table (4-10): Relationship Between (*intI1*), Aminoglycoside Genes, and Multiple Resistance Patterns

Integron Class	No. of Positive Isolates (%)	No. of Aminoglycosides Gene (%)									Multiple Antibiotic Resistance Patterns	
		Aminoglycoside-Modifying Enzymes (AMEs)						16S rRNA methylases				
		<i>aacC1</i>	<i>aac(3)-II</i>	<i>aac(6')-Ib</i>	<i>aph(3')-VI (aphA6)</i>	<i>aadA1</i>	<i>ant(4')-IIB</i>	<i>armA</i>	<i>rmtD</i>	<i>rmtB</i>	XDR	PDR
class 1 integron gene (<i>intI1</i>)	9(22.50)	1 (11.11%)	1 (11.11%)	6 (66.67%)	5 (55.56%)	0(0.00%)	3 (33.33%)	2 (22.22%)	1 (11.11%)	0 (0.00%)	8 (88.89%)	1 (11.11%)

Note: *intI1* positivity was calculated among 40 aminoglycoside-resistant isolates, whereas gene and resistance phenotype percentages were calculated among the nine *intI1*-positive isolates. Individual isolates may carry multiple resistance genes.

4.14. DNA Sequencing Analysis:

4.14.1. Sequence and Phylogenetic Study of *rmtD* Gene in *A. baumannii*

A. baumannii *rmtD* gene sequences aligned, revealing clear genetic relationships in isolates analyzed, and the dendrogram shows two major groupings as shown in Figure (4-18). In the first group, all reference (global strains) of AB *rmtD* gene, including, CP024612.1, CP130628.2, CP195695.1, CP031444.1, AP029033.1, CP169833.1, and CP031743.1, and one local isolate AB23. The other major grouping, or cluster, contains the rest of local isolates (AB3, AB8, AB9, and AB17) which are completely distanced from reference-associated group.

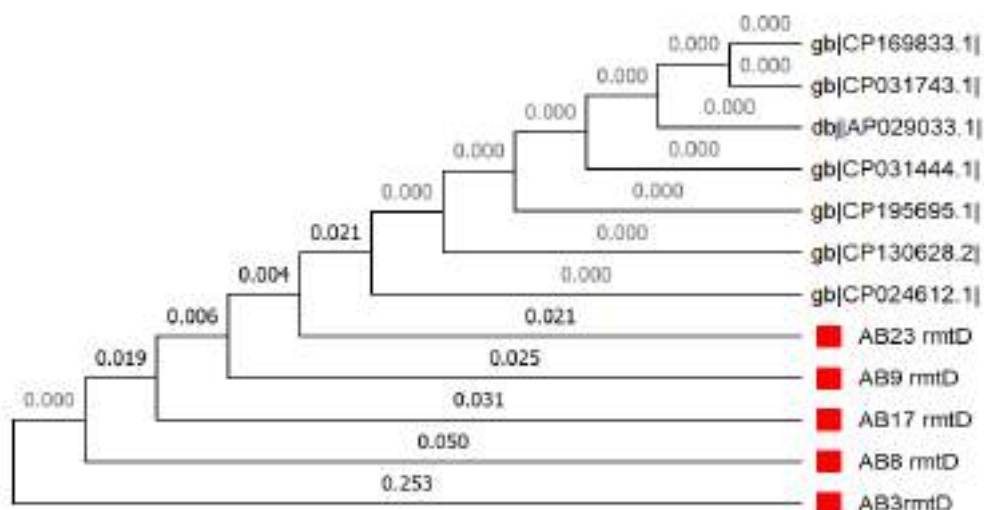


Figure (4-18): Phylogenetic Tree of Partial *rmtD* gene Sequences among Local and Reference *A. baumannii* isolates

The phylogenetic analysis of the *rmtD* gene in local *Acinetobacter baumannii* isolates compared with global reference strains, using MEGA software and the UPGMA algorithm (Zueter *et al.*, 2025). demonstrated clear genetic divergence leading to the formation of two major clusters. Isolate AB23 clustered closely with global reference strains, indicating a high level of genetic similarity and evolutionary proximity, whereas isolates AB3, AB8, AB9, and AB17 formed distinct branches, reflecting notable genetic divergence. Sequence analysis revealed substantial heterogeneity in genetic variation among the studied isolates. The most divergent isolate, AB3, exhibited the lowest sequence identity (72.1%) accompanied by a high number of frameshift-associated mutations and a nonsense mutation, suggesting severe disruption of the open reading frame and a potential loss or alteration of protein function. In contrast, AB8 showed moderate genetic variation (90.5% identity) characterized exclusively by missense mutations, indicating a limited impact on protein structure. Similarly, AB17 demonstrated 93.2% identity with the presence of frameshift-associated mutations, which may contribute to partial structural modifications of the encoded protein. Isolates AB9 and AB23 exhibited the highest levels of

genetic conservation, with sequence identities exceeding (95%) and a predominance of synonymous substitutions or minor missense mutations indicating strong preservation of gene function. Among them, AB23 showed the closest genetic relationship to the reference strains. The findings indicate the presence of marked genetic heterogeneity within local populations, ranging from highly conserved to highly divergent genotypes. The occurrence of structurally disruptive mutations, such as frameshifts and nonsense mutations in certain isolates, suggests variability in the genetic stability of the *rmtD* gene and its potential functional implications. This study represents one of the limited local reports documenting the molecular detection and sequence analysis of the *rmtD* gene in clinical isolates of *A. baumannii*, providing important insights into its genetic diversity and its role in the molecular epidemiology of antimicrobial resistance. There is high conservation of the *rmtD* gene among the global *A. baumannii* strains, but there is a wide variation with the local isolates. This variation led to at least a distinct cluster. The variation is indicative of locally confined genetic divergence among the isolates with AB23 showing a high resemblance to the global strains and AB3 having high divergence. There is a genetic profile for the *rmtD* gene that is divergent and conserved in the local *A. baumannii* isolates.

Table (4-11): Nucleotide Variation and Sequence Identity of the *rmtD* Gene among Local *A. baumannii* Isolates

Isolate	Total Differences	Identity (%)	Synonymous	Missense	Nonsense	Frameshift-associated
AB3	62	72.1%	Low	Very High	1	52
AB8	21	90.5%	Moderate	Moderate	0	0
AB9	11	95.0%	Moderate	Low	0	0
AB17	15	93.2%	Low	Moderate	0	12
AB23	10	95.5%	High	Low	0	6

4.14.2. Phylogenetic Analysis of the *armA* Gene in *A. baumannii*

The phylogenetic analysis of the *armA* gene in *A. baumannii* revealed a clear genetic separation into two major clusters, strongly supported by high bootstrap values reaching 100% at the principal nodes, confirming the robustness of the constructed phylogenetic tree as shown in Figure (4-19). Cluster I comprised all global reference strains retrieved from GenBank (dbj AP027038.1, gb|CP121625.1, gb|CP040087.1, gb|CP096734.1, and gb CP115629.1). This clustering reflects a high level of global conservation of the *armA* gene and indicates minimal evolutionary divergence among worldwide strains. Within this cluster, a subtle internal sub-structure was observed, where AP027038.1 and CP121625.1 exhibited the closest phylogenetic relationship, likely representing a recent common ancestor. In contrast, CP040087.1, CP096734.1, and CP115629.1 showed slight divergence, most likely due to the accumulation of minor nucleotide substitutions over time. In contrast, Cluster II included the local clinical isolates AB14, AB25, and AB26, which formed a distinct and well-separated lineage from the global reference strains. This clear separation indicates a genetic distinction between local and global populations of the *armA* gene. Although the local isolates clustered together, they still exhibited minor intra-cluster variation, suggesting limited sequence polymorphism among them. The findings indicate that the *armA* gene is highly conserved globally, while showing a clear phylogenetic distinction between local clinical isolates and global reference strains, reflecting regional genetic differentiation without major disruption of the overall evolutionary structure of the gene.

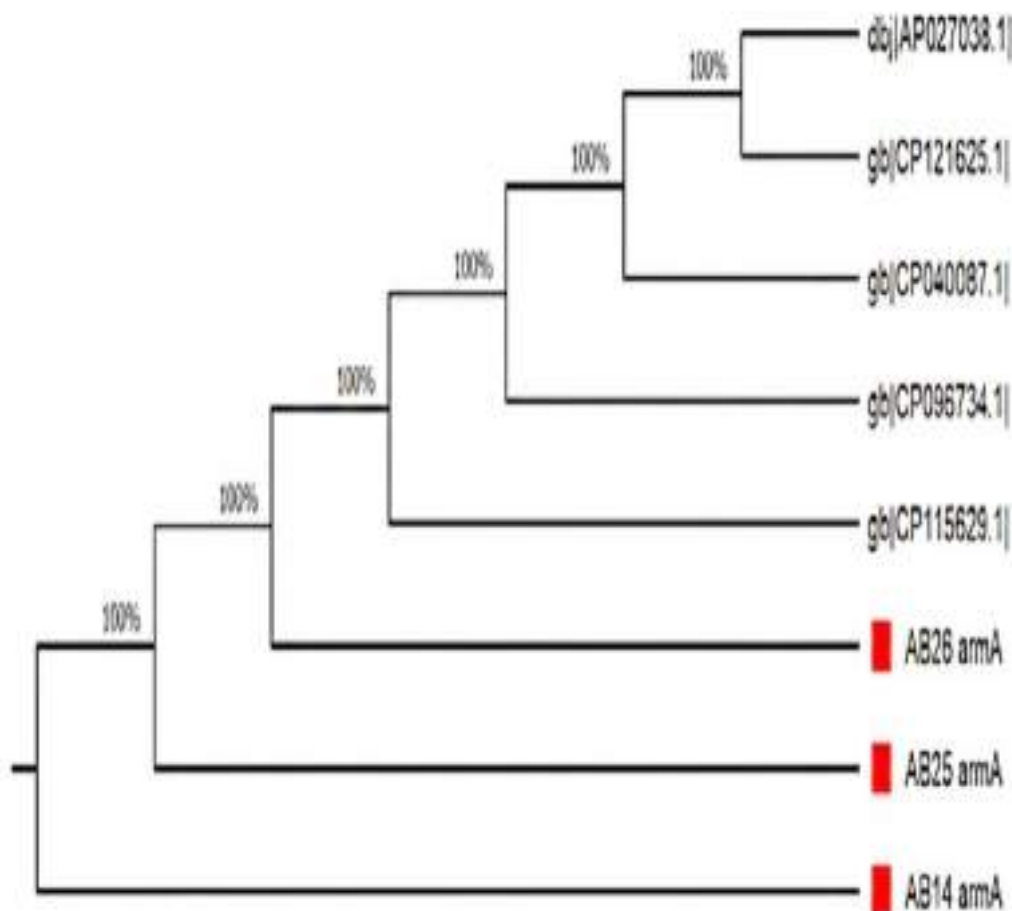


Figure (4-19): Phylogenetic tree of partial *armA* gene sequences among local and reference *A. baumannii* isolates.

The phylogenetic analysis of the local isolates AB14, AB25, and AB26 in comparison with global reference strains was performed using MEGA software and the UPGMA clustering algorithm, revealing a clear genetic clustering pattern based on the similarity distance matrix derived from sequence alignment. The results showed that AB25 and AB26 were more closely related to each other, suggesting a recent common ancestor or a shared source of infection, whereas AB14 appeared more genetically distant, which may reflect the accumulation of additional mutations or exposure to different selective pressures leading to greater evolutionary divergence. The

findings indicate that the *armA* gene in these local isolates has undergone regional evolutionary divergence, likely driven by continuous antibiotic selective pressure and localized transmission dynamics. The tight clustering of the local isolates supports the hypothesis of a common epidemiological origin, while their separation from global reference strains suggests post-horizontal gene transfer (HGT) adaptation followed by independent evolution within the local environment. These results emphasize the importance of continuous surveillance of the molecular evolution and dissemination of aminoglycoside resistance genes in *A. baumannii* to better understand the dynamics of antimicrobial resistance spread (Cho *et al.*, 2009).

Table (4-12): Comparative Nucleotide and Amino Acid Variation Analysis of the *armA* Gene in *A. baumannii* Isolates Relative to the Reference Sequence

Position (bp)	Isolate	Reference Base	Mutated Base	Mutation Type	Codon Effect	Amino Acid Effect	Mutation Classification
7	AB26	T	–	Deletion	Frame disruption	Likely frameshift	Frameshift mutation

4.14.3. Sequencing and Phylogenetic Analysis of the *aac(3)-II* Gene in *A. baumannii*

Studying the evolutionary pattern of the phylogenetic analysis of the *aac3* gene in *A. baumannii* shows that the analyzed sequences have a divided structure in the evolutionary pattern with a clear delineation of the sequences into two primary clusters with additional internal incremental sub-clustering in Figure (4-20). In comparison to the

reference strains, the dendrogram's structure has both uninterrupted and disrupted lineages amongst the local isolates (AB3, AB4, and AB5).

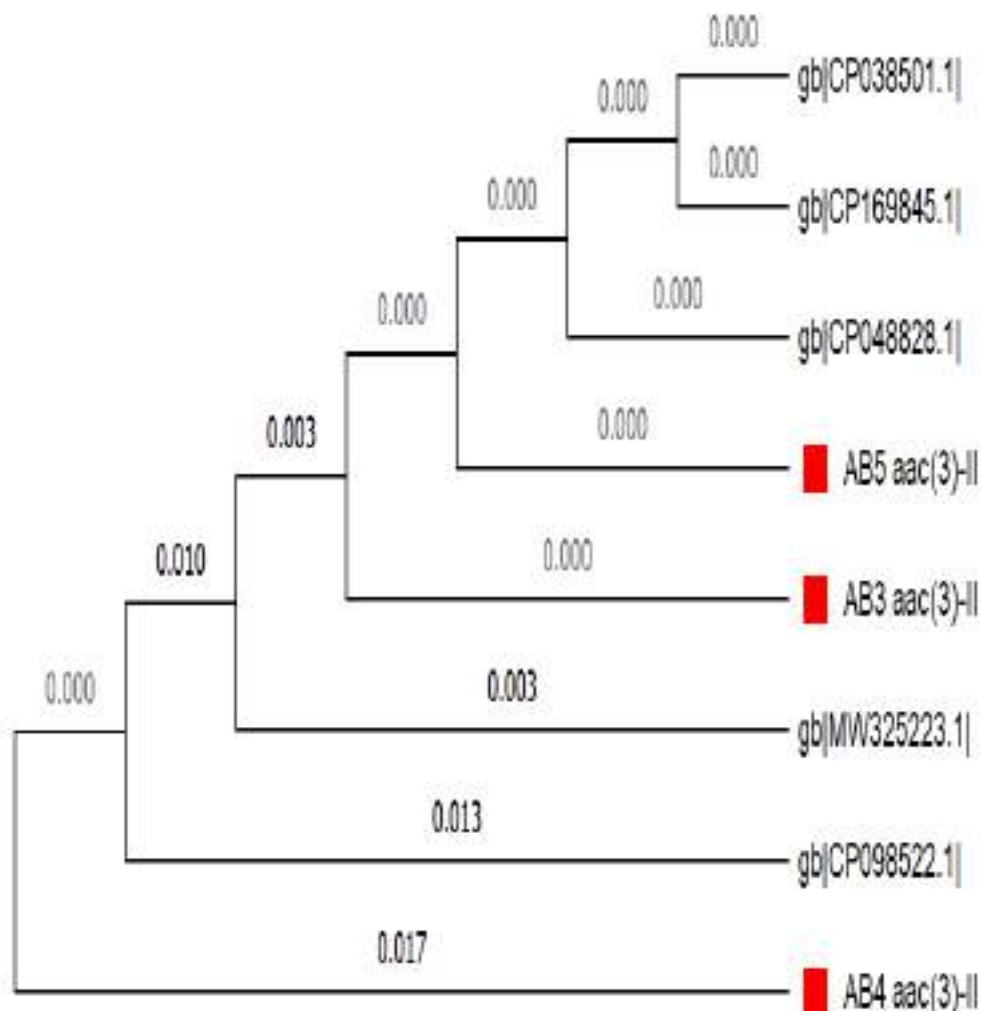


Figure (4-20): Phylogenetic tree of partial *aac(3)-II* gene sequences among local and reference *A.baumannii* isolates

The phylogenetic analysis of the *aac(3)-II* gene in local *A. baumannii* isolates compared with global reference strains was performed using MEGA software and the UPGMA clustering algorithm, revealing two distinct evolutionary clusters based on sequence similarity and genetic distance matrices. Cluster I included the local isolates AB3 and AB5 together with most global reference strains (gb/CP048828.11, gb CP169845.1, gb|CP038501.1). This

cluster showed a high level of genetic conservation, with extremely short branch lengths ranging from 0.000 to 0.003, indicating minimal sequence divergence. The reference sequences were highly consistent and tightly grouped, while AB5 showed virtually no divergence from the reference strains, suggesting near-complete sequence conservation. AB3 exhibited only slight divergence, indicating very limited nucleotide variation relative to both AB5 and the global lineage. In contrast, Cluster II consisted of the local isolate AB4 together with two reference sequences (gb|MW325223.1| and gb|CP098522.1), forming a distinct and more divergent evolutionary lineage. Within this cluster, AB4 demonstrated the greatest genetic distance, with a branch length of 0.017, indicating marked divergence from the other sequences. The reference sequences in this cluster showed moderate divergence (0.003-0.013) relative to Cluster I, while remaining closer to each other than to AB4, confirming the distinct evolutionary position of AB4 within this group. The dendrogram reveals two clearly defined phylogenetic patterns for the *aac3* gene. Cluster I represents a conserved lineage comprising global reference strains and the closely related local isolates AB3 and AB5, whereas Cluster II represents a divergent lineage characterized by AB4 and distinct reference sequences. These findings suggest that AB3 and AB5 carry globally conserved variants of the *aac(3)-II* gene, while AB4 represents a genetically distinct variant, likely resulting from mutation accumulation, local adaptive evolution, or horizontal gene transfer events. These findings are supported by Álvarez D. *et al.* (2020), who highlighted the crucial role of horizontal gene transfer and the accessory genome in the evolutionary dynamics of *A. baumannii*. In conclusion, the *aac(3)-II* gene exhibits both

conserved and divergent evolutionary patterns in *A. baumannii*, reflecting geographic and genetic variability. The predominance of conserved lineages alongside the emergence of a divergent variant highlights the dynamic nature of aminoglycoside resistance determinants and their molecular evolution across different regions. The sequence alignment and identity analysis of the *aac(3)-II* gene revealed a high level of genetic conservation among the studied isolates, with the exception of one isolate that showed increased variability. In particular, isolates AB3 and AB5 demonstrated complete sequence identity (100%) with the reference strain, with no detected nucleotide substitutions, insertions, or deletions, indicating full conservation of the gene sequence in these isolates. Isolate AB4 showed the highest genetic divergence among the studied isolates, with a sequence identity of 97% (311/320) and multiple mutations including one deletion and eight nucleotide substitutions. The deletion at position 9 is predicted to cause a frameshift effect, potentially altering the downstream coding sequence. The substitution mutations resulted in several missense amino acid changes, while only one was synonymous, indicating a predominantly non-silent mutation profile. These genetic alterations confirm a higher level of variability in AB4 compared to the other isolates. The findings are consistent with the phylogenetic analysis, where AB4 appeared as the most divergent lineage, whereas AB3 and AB5 remained closely related to the reference strains with minimal variation. The *aac(3)-II* gene is largely conserved across isolates, with AB4 representing a distinct variant shaped by localized evolutionary changes.

Table (4-13): Comparative Analysis of Nucleotide and Amino Acid Variations in the *aac(3)-II* Gene of *A. baumannii* Isolates Relative to the Reference Sequence.

Position (bp)	Isolate	Reference Base	Mutated Base	Mutation Type	Codon Effect	Amino Acid Effect	Mutation Classification
9	AB4	A	–	Deletion	Frameshift	Downstream	Frameshift
17	AB4	G	A	Substitution	GTT → ATT	Val → Ile	Missense
33	AB4	G	A	Substitution	GCT → ACT	Ala → Thr	Missense
67	AB4	C	A	Substitution	GCC → GAC	Ala → Asp	Missense
119	AB4	C	A	Substitution	GGC → GAC	Gly → Asp	Missense
150	AB4	A	T	Substitution	ATA → ATT	Ile → Ile	Synonymous
188	AB4	G	A	Substitution	GTG → ATG	Val → Met	Missense
236	AB4	C	T	Substitution	CCA → CTA	Pro → Leu	Missense
238	AB4	T	C	Substitution	TCC → CCC	Ser → Pro	Missense

4.14.4. Sequencing and Phylogenetic Analysis of the *aac(6')-Ib* Gene in *A. baumannii*

The assessment of phylogenesis of the *aac(6')-Ib* gene in *A. baumannii* revealed that the sequences under analysis get categorized into 2 primary clusters, with one of the principal groups further categorized into subclusters in Figure (4-21). The analysis of the dendrogram topology indicated that the local samples of the investigated *A. baumannii* isolates formed an identifiable group in Cluster 2; however, the local isolates clustered differently in 2 separate phylogenetic regions, thus, confirming the existence of at least 2 identifiable and related, yet separable, sequence patterns present in the investigated samples

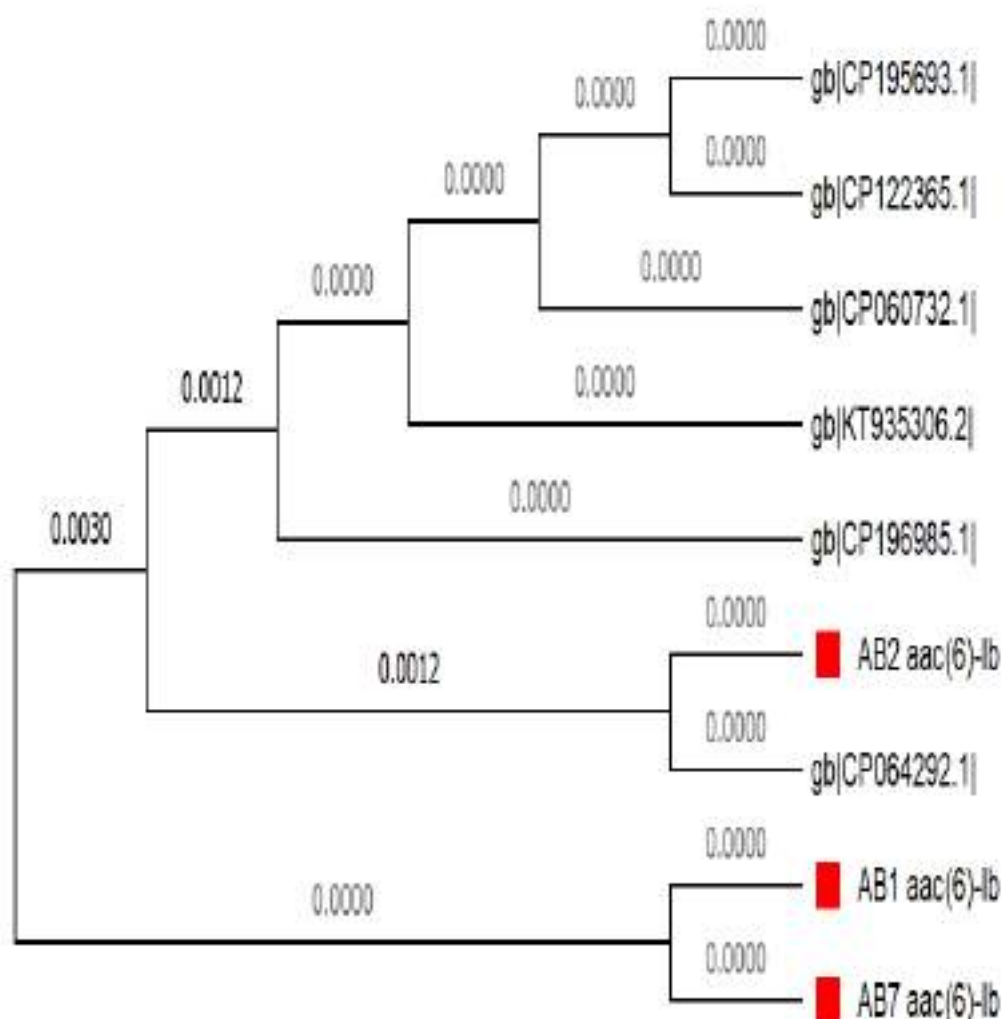


Figure (4-21): Phylogenetic Tree of Partial *aac(6')-Ib* Gene Sequences among Local and Reference *A. baumannii* Isolates

The phylogenetic analysis of the *aac(6')-Ib* gene in local *A. baumannii* isolates compared with global reference strains, using MEGA software and the UPGMA algorithm, revealed the presence of two major evolutionary clusters (Gopikrishnan, 2025). Cluster I included the majority of the global reference strains together with the local isolate AB2, including gb|CP195693.1, gb|CP122365.1, gb|CP060732.1, gb|KT935306.2, gb|CP196985.1, AB2 aac6, and gb|CP064292.1. The branching pattern within this cluster demonstrated a high level of sequence conservation and minimal genetic divergence. The reference strains gb|CP195693.1 and gb|

CP122365.1 showed the closest phylogenetic relationship, while AB2 clustered closely with gb/CP064292.1 with an almost zero branch length, indicating nearly complete sequence identity and a highly conserved genetic profile. In contrast, Cluster II consisted of the local isolates AB1 *aac6* and AB7 *aac6*, which formed a closely related terminal pair with no detectable branch distance, suggesting identical or nearly identical sequences within the analyzed region. However, this cluster was clearly separated from Cluster I, indicating that AB1 and AB7 represent a distinct local evolutionary lineage of the *aac(6')-Ib* gene.

The phylogenetic findings indicate the coexistence of conserved and locally divergent variants of the *aac(6')-Ib* gene among *A. baumannii* isolates. While AB2 remained closely associated with globally circulating strains, AB1 and AB7 appeared to represent an independent local lineage, possibly reflecting localized evolutionary adaptation. Sequence alignment and identity analysis demonstrated a high degree of conservation of the *aac6* gene among the studied isolates. AB1 and AB2 showed 99% sequence identity (411/412) with the reference strain, with only a single nucleotide substitution at position 210 (A→G), resulting in a missense mutation without any insertion or deletion events. In contrast, AB7 showed 100% sequence identity (412/412) with the reference sequence and no detectable mutations. These findings confirm that the *aac6* gene is highly conserved, with only minimal sequence variation detected among the studied isolates. These findings are supported by Harmer *et al.* (2023), who reported that *aac(6')* genes are generally conserved with limited sequence variation among bacterial isolates.

Table (4-14): Comparative Nucleotide and Amino Acid Variation Analysis of the *aac(6')*-Ib Gene in *A. baumannii* Isolates Relative to the Reference Sequence.

Isolate	Nucleotide position (bp)	Reference codon/base	Mutant codon/base	Mutation type	Amino acid change	Mutation classification
AB1	210	A at the third base of codon CCA	G at the third base of codon CCG	Substitution	No amino acid change confirmed from the nucleotide triplet itself; however, the alignment annotation indicates an amino acid difference at this site	Missense according to the alignment annotation
AB2	210	A at the third base of codon CCA	G at the third base of codon CCG	Substitution	No amino acid change confirmed from the nucleotide triplet itself; however, the alignment annotation indicates an amino acid difference at this site	Missense according to the alignment annotation
AB7*	—	—	—	No mutation detected	No amino acid change	Conserved sequence

4.14.5. Sequencing and Phylogenetic Analysis of the *aadA1* Gene in *A. baumannii*

The phylogenetic analysis of the *aadA1* gene in *A. baumannii* revealed a distinct clustering phenomenon where the investigated sequences were segregated into two primary clusters in Figure (4-22). This observation is bolstered by specific branch length measurements and consistent nodal values. The dendrogram illustrates significant genetic differentiation between the majority of the reference strains and the selected local isolates, suggesting two distinct evolutionary lineages. Similar phylogenetic observations were previously reported by Djahanschiri *et al.* (2022).

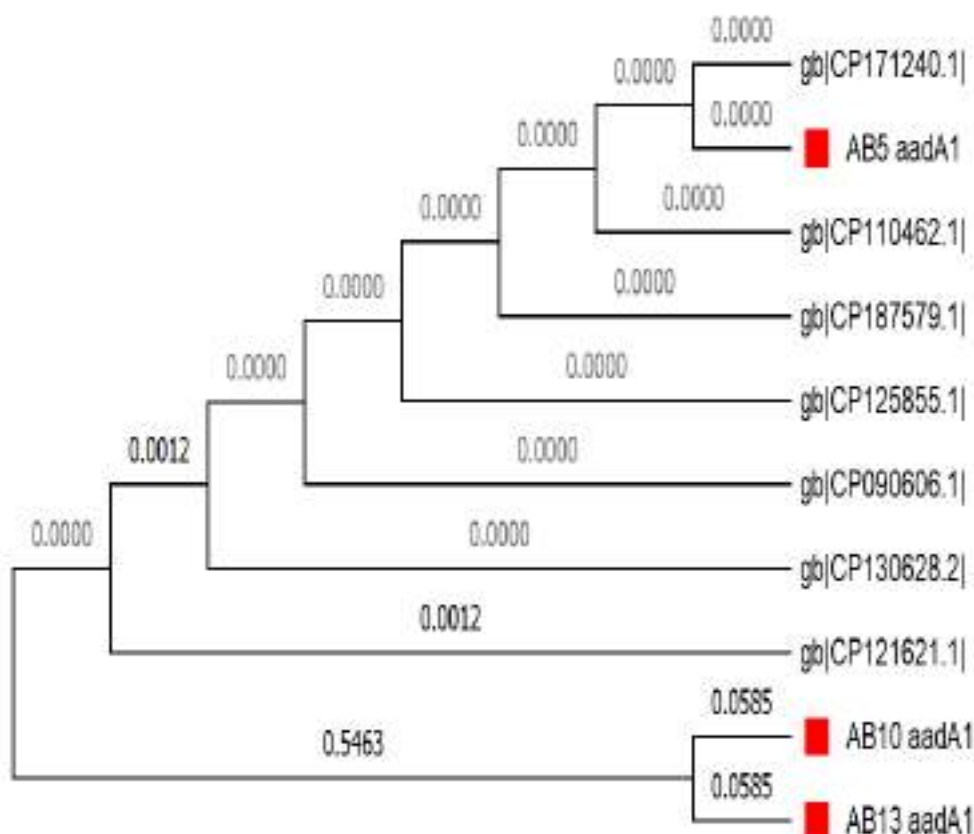


Figure (4-22): Phylogenetic Tree of Partial *aadA1* Gene Sequences among Local and Reference *Acinetobacter baumannii* Isolates

The phylogenetic relationships between local isolates and global reference strains were analyzed using partial *aadA1* gene sequences with the UPGMA method in MEGA software (Liu *et al.*, 2020). Two major clusters were identified. Cluster I included the local isolate AB5 with the reference strains (gb|CP171240.1, gb|CP110462.1, gb|CP187579.1, gb|CP125855.1, gb|CP090606.1, gb|CP130628.2, and gb|CP121621.1). This cluster showed very short branch lengths (0.000-0.001), indicating minimal genetic variation, high conservation of the *aadA1* gene, and a close relationship between AB5 and gb|CP171240.1], suggesting recent common ancestry. Cluster II contained only the local isolates AB10 and AB13, forming a distinct sub-lineage separated from Cluster I by a longer branch

length (0.546). The equal branch length between AB10 and AB13 (0.058) indicated close relatedness and a recent common ancestor, while their separation from the reference cluster reflected significant genetic divergence caused by nucleotide variations in the *aadA1* gene.

The dendrogram therefore demonstrated a large conserved cluster containing the reference strains and AB5, and a smaller divergent cluster containing AB10 and AB13. The long evolutionary distance between these groups suggests that AB10 and AB13 represent locally evolved *aadA1* variants, possibly associated with mutation, evolutionary pressure, or horizontal gene transfer. Their close relationship also supports a possible common origin or transmission within the same geo-clinical environment. The study confirmed the conserved nature of the *aadA1* gene in most *A. baumannii* strains while identifying distinct local variants useful for tracking aminoglycoside resistance spread.

The *aadA1* gene sequences of isolates AB10 and AB13 showed identical mutation patterns compared with the reference sequence, with 88% identity (164/187) and 22 gap positions (1196). The detected variations included 22 deletions and one nucleotide substitution at position 121 (T→C), while no insertions were observed as shown in Table (4-15). Most deletions were concentrated at positions 56, 84, 102-110, 146, 151, 153–154, and 160-166, indicating major sequence gaps relative to the reference. These extensive deletions are likely to disrupt the coding sequence and alter the downstream amino acid composition. The similarity between AB10 and AB13 suggests that both isolates carry the same divergent *aadA1* gene variant.

Table (4-15) : Comparative Nucleotide and Amino acid Variation Analysis of the *aadA1* Gene in *A. baumannii* Isolates.

Position	Isolate(s)	Reference base	Mutated base	Mutation type	Amino acid effect	Mutation classification
56	AB10, AB13	G	–	Deletion	Downstream amino acid sequence affected from this point if coding frame is maintained through the indel	Frameshift-associated deletion
84	AB10, AB13	T	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
102	AB10, AB13	C	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
103	AB10, AB13	T	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
104	AB10, AB13	C	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
105	AB10, AB13	C	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
106	AB10, AB13	A	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
107	AB10, AB13	A	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
108	AB10, AB13	A	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
109	AB10, AB13	T	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
110	AB10, AB13	T	–	Deletion	Downstream amino acid sequence affected	Frameshift-associated deletion
121	AB10, AB13	T	C	Substitution	Exact amino acid substitution cannot be confirmed from this BLAST-style excerpt alone because the hit is on the plus/minus strand and the indels disrupt straightforward codon tracking	Substitution

146	AB10, AB13	G	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
151	AB10, AB13	C	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
153	AB10, AB13	T	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
154	AB10, AB13	G	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
160	AB10, AB13	C	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
161	AB10, AB13	A	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
162	AB10, AB13	A	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
163	AB10, AB13	T	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
164	AB10, AB13	G	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
165	AB10, AB13	G	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion
166	AB10, AB13	T	—	Deletion	Downstream amino acid sequence affected	Frameshift- associated deletion

4.14.6. Sequencing and Phylogenetic Analysis of the *ant(4')-IIb* Gene in *A. baumannii*

Phylogenetic analysis of the *ant(4')-IIb* gene in *A. baumannii* clustered the analyzed sequences into two major clusters Figure (4-23) indicating a significant geographic separation between the local isolates and the reference strains. The dendrogram indicated that the majority of GenBank reference sequences, such as gb CP159763.1, gb|CP121591.1, gb|CP195695.1, gb|CP008706.1, gb|CP130628.2, gb|CP175832.1, and gb|CP199060.1, formed a single major lineage, whereas local isolates AB27 *ant* and AB28 *ant* diverged. This suggests that the local isolates

differ in the *ant* gene region as compared to the reference strains. The dendrogram indicated that the local isolates differ significantly from the reference strains, as local isolates AB27 *ant* and AB28 *ant* diverged from this major lineage.

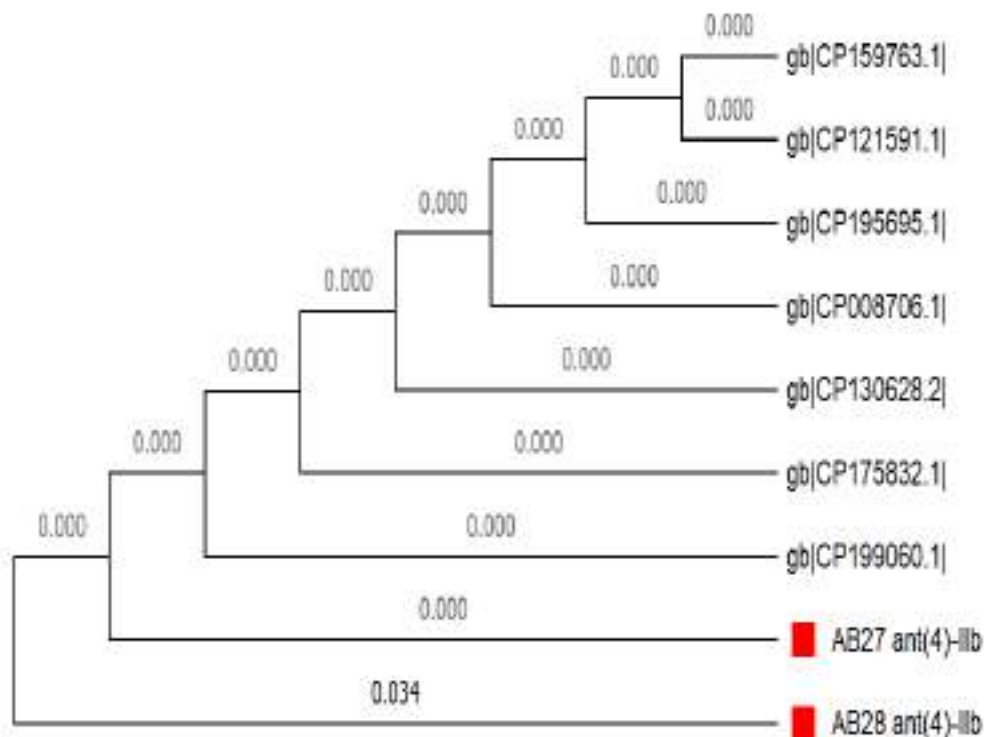


Figure (4-23): Phylogenetic Tree of Partial *ant(4')-IIb* Gene Sequences among Local and Reference *A. baumannii* Isolates

Phylogenetic relationships between the local isolates (AB27 and AB28) and global reference strains were inferred using partial sequences of the *ant(4')-IIb* gene. The analysis was performed using MEGA software based on a UPGMA (unweighted pair group method with arithmetic mean) clustering approach derived from the pairwise similarity/distance matrix generated through sequence alignment. The results revealed two main clusters. Cluster I comprised all reference strains and exhibited a stepwise hierarchical structure with very short branch lengths and node values of 0.000, indicating extremely low genetic divergence and a high level of sequence conservation. Within this cluster the pair gb|CP159763.1| and

gb|CP121591.1| showed the closest relationship (~15% similarity), followed sequentially by gb|CP195695.1| and gb|CP008706.1|, reflecting a gradual pattern of relatedness among the reference sequences.

Cluster II included the local isolates AB27 and AB28, which were positioned on separate branches. AB27 was more closely related to the reference lineage and showed a branch value of 0.000, indicating minimal divergence. In contrast, AB28 exhibited a greater genetic distance (0.034), representing the most divergent isolate in the analysis and suggesting a distinct phylogenetic position relative to both AB27 and the reference strains. The dendrogram demonstrated two major groupings: a highly conserved reference cluster and a more divergent local cluster. Unlike the reference sequences, AB27 and AB28 did not form a tightly defined subcluster, with AB28 showing a more independent branching pattern and greater evolutionary separation.

Sequence identity analysis further demonstrated that AB27 was 100% identical to the reference strain with no nucleotide substitutions, insertions, or deletions, indicating complete conservation at both nucleotide and amino acid levels. In contrast, AB28 showed 97% sequence identity due to two nucleotide substitutions, confirming its higher level of genetic variation.

Table (4-16): Nucleotide and Amino acid Variations Detected in the *ant* gene of *A. baumannii* Isolates (AB27 and AB28) Compared with the Reference Sequence.

Position (bp)	Isolate	Reference Base	Mutated Base	Mutation Type	Codon Change	Amino Acid Change	Mutation Classification
6	AB28	A	T	Substitution	ATG → TTG	Met(M) → Leu(L)	Missense
17	AB28	A	G	Substitution	GCA → GCG	Ala(A) → Ala(A)	Synonymous

4.15. ERIC-PCR Dendrogram Analysis of *A. baumannii*

The aim of building the ERIC-PCR dendrogram is to determine the degree of genetic relatedness of the *A. baumannii* isolates studied (AB series). (Falah *et al.*, 2019).

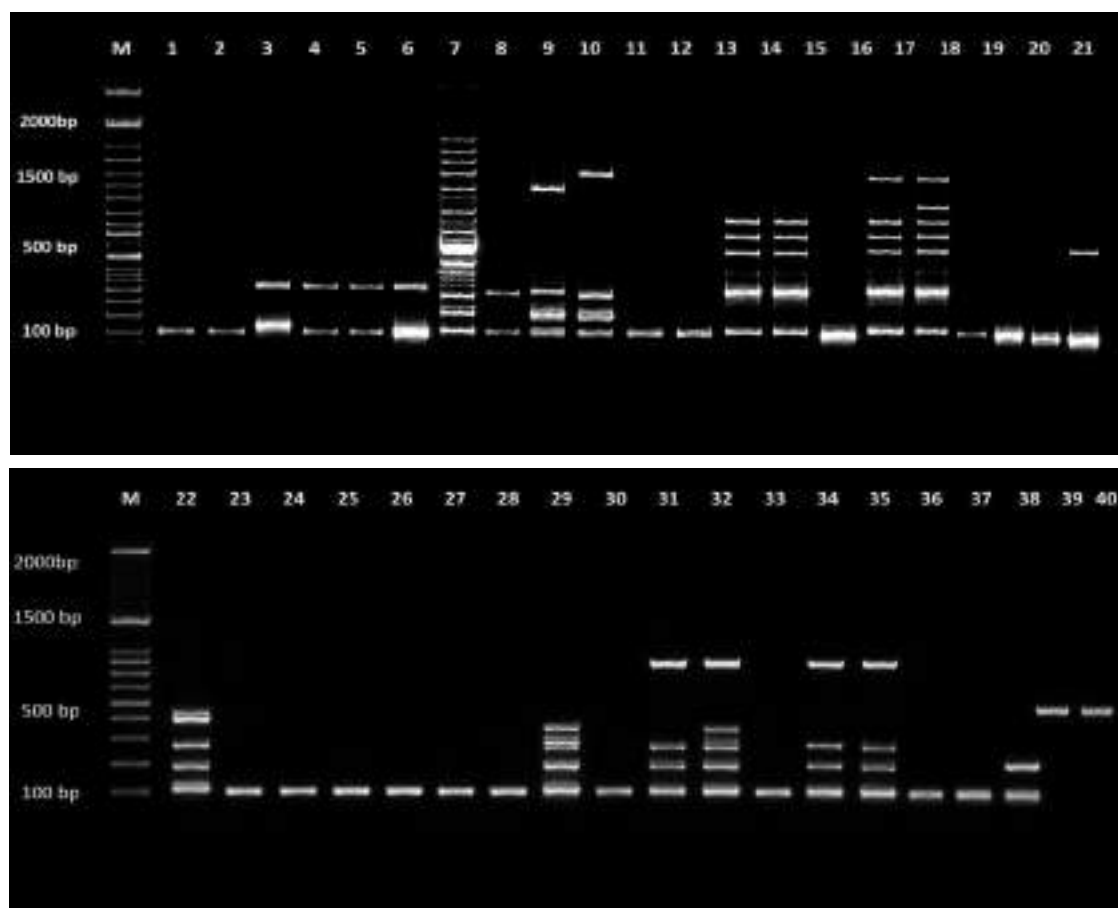


Figure (4-24): *A. baumannii* patterns obtained by PCR Using Agarose Gel Electrophoresis The Numbers indicating the Isolates and Lane M is molecular size DNA Ladder (100-2000bp).

A similarity coefficient was used in the analysis where the value of 1 means there is high genetic similarity while the value of 0 means there is high genetic diversity. A heterogeneous population structure was shown by the Dendrogram since the isolates formed several clusters and not a single predominant lineage. Two major groups of isolates were formed with a similarity coefficient value of 30-35%, suggesting that the sampled environment has at least two populations that are genetically distinct and circulating. The first major cluster (Cluster I) consists of isolates AB39,

AB40, AB8, AB6, AB3, AB4, AB5, AB11, AB2, AB1, AB12, AB15, AB18, AB19, AB20, AB23, AB24, AB25, AB26, AB27, AB28, AB30, AB33, AB36, and AB37, in Figure (4-25). This cluster has similarity levels in the range of 45-55%, which means that the isolates in the cluster share a common ancestor, however, the isolates exhibit diversification. The several subclusters within the cluster suggests that there are microevolutionary processes that are occurring, which could be a result of mutations, genetic exchange, or antibiotic exposure that exerts selective pressure. The broad distribution of isolates within the cluster indicates *A. baumannii* genetic diversity and adaptability. The second major cluster (Cluster II) consisted of isolates AB21, AB9, AB10, AB22, AB29, AB32, AB31, AB34, AB35, AB38, AB7, AB13, AB14, AB16, and AB17. In relation to cluster I, this group displayed a greater internal similarity, in the range of 60 to 85%, pointing to a greater genetic relatedness to each other. Within this cluster, some subgroups, especially isolates AB13 to AB17, had very high similarity values of 75% and above, suggestive of a clonal expansion. Such clustering may point to the hospital acquired infections (nosocomial infections) or a common source of infection in the particular clinical environment.

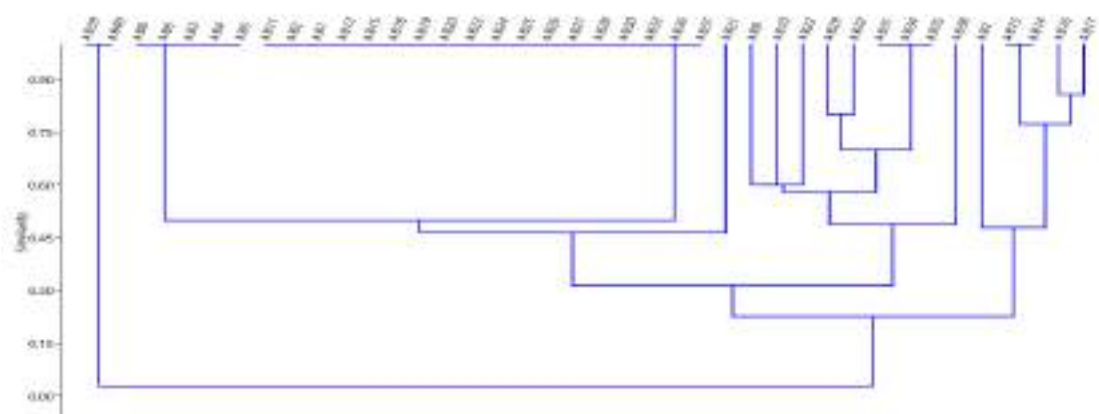


Figure (4-25): ERIC-PCR Dendrogram Illustrating the Genetic Relationships among *A. baumannii* Isolates. The dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) based on the Jaccard similarity coefficient.

Some isolate pairs or groups had very high similarity values of more than 80 to 90%, marking them as clonal and thus pointing to recent transmission, and a shared ecological or clinical situation. From an epidemiological point of view, these highly similar groups are especially important as they are very likely to represent strains related to an outbreak. as previously described in molecular epidemiological studies (Aljindan *et al.*, 2018) . In contrast, a group of isolates had very low similarity values (0 to 20%), reflecting the high genetic divergence. Such genetically distinct isolates are likely to be independent and to have originated from various different environmental or geographical sources, thereby increasing the overall population diversity.

4.15.1.ERIC-PCR Correlation With Source of Infection

In this study, the combined use of ERIC-PCR genotyping and epidemiological data (the hospital and specimen source) has provided insights into the transmission routes and potential sources of infection of *A. baumannii*. Similar findings regarding the usefulness of ERIC-PCR in epidemiological tracking of *A. baumannii* isolates were reported by Abdullah, (2024). The dendrogram showed that high genetic similarity of isolates was related to high association with certain hospitals and specimen types, indicating the presence of structured transmission dynamics and not random association. A notable case was the grouping of isolates from Imam Al-Sadiq Teaching Hospital, especially the ones from burn specimens. This hospital had a large contribution to the burn related isolates, including isolates 6, 7, 9, 12, 13, 22, 25, 27, 30, and many of these were in the closely related subclusters in the dendrogram. The similarity of these isolates pointed out the presence of one or more dominant clonal lineages in the circulation burn unit. This is in line with the well-documented

phenomenon of *A. baumannii*. The compromised skin barrier, the prolonged hospital stay, and the aggressive antibiotic use that accompany the burn unit, facilitate the transmission and persistence of the *A. baumannii*. as previously reported by Sabbir and Elma (2024).

On the other hand, the Al-Hilla Teaching Hospital isolates were mostly derived from wound specimens, such as isolates 2, 5, 8, 15, 20, 21, 28, and 31. These wound isolates demonstrated moderate genetic similarity and were more likely to be clustered in the same level of the hierarchy when compared to the burn isolates from Imam Al-Sadiq Teaching Hospital. This indicates the likely presence of endemic strains with local transmission that facilitates the circulation of these strains in the hospital. The diversity seen in this group may be due to repeated and related strain introductions as opposed to microevolution, small outbreaks, or single-point outbreaks consistent with the findings of Cain and Hamidian ,(2023). In the case of isolates from Marjan Teaching Hospital, a distinct pattern was noted due to the presence of diverse specimen types, including sputum, blood, burn, and urine (isolates 10, 11, 18, 19, 26, 32, 38, and 39). Compared to the other specimens, these isolates fell into several distinct clusters in the dendrogram and lacked any cohesive group. This lack of close clustering indicates that these isolates are genetically more diverse than those from other hospitals. This suggests that multiple unrelated strains are responsible for the infections observed in this hospital, rather than a single dominant clone. Multiple pathways such as diverse infection origins, environmental reservoirs, community-acquired strains, or interpatient transmission leading to sustained clonal expansion may explain the observed pattern which is consistent with the role of genetic and resistance determinants in the dissemination and evolution of *A. baumannii* as described by

Hamidian and Hall (2018). Likewise, isolates from Babylon Teaching Hospital, especially blood samples (isolates 14, 17, 23, 24, 29, and 35) lack close clustering and are distributed across various branches of the dendrogram. This means that bloodstream infections in this location are not linked to a single outbreak strain and instead, come from many genetically different isolates. The presence of isolates from sputum (for example, isolate 33) and their separation from bloodstream isolates, provide more evidence for the strain diversity in the hospital. Another critical finding is that some isolates from different specimens which are genetically connected, such as isolates from different specimens including sputum, blood, and related burns and wounds. This indicates that some *A. baumannii* strains can tireless and infect several body systems, thus showing their opportunistic and flexibility. This is consistent with previous ICU-based studies on the clinical importance and distribution of *A. baumannii* (Sehree *et al.*, 2021). On the other hand, genetically different isolates from the same hospital and same type of specimens, show that newly introduced strains are captured repeatedly in a continuous manner. This may be related to contamination, healthcare personnel, or the transfer of a patient to other healthcare systems. The findings from the molecules and epidemiological studies suggest that *A. baumannii* infections in this study are the result of a combination of clonal dissemination and genetic diversity. In the high-risk environments, especially the burn units, the close relatedness of the isolate's points to the strong presence of clonal dissemination. In contrast, other areas of the hospital display a greater diversity of strains. This dual pattern supports the implementation of stringent infection control practices in conjunction with the continued molecular monitoring to identify and curb the transmission of the dominant clone and to assess the influx of new strains.

4.15.2. Integration of Correlation with ERIC-PCR Clustering

There is considerable potential for combining the ERIC-PCR dendrogram with band-specific correlation matrices, particularly with respect to genetic analysis of *A. baumannii* isolates regarding both isolate and molecular marker levels. Major clusters with varying similarity levels and correlation values among certain ERIC-PCR bands were observed in the dendrogram as shown in Figure (4-26).

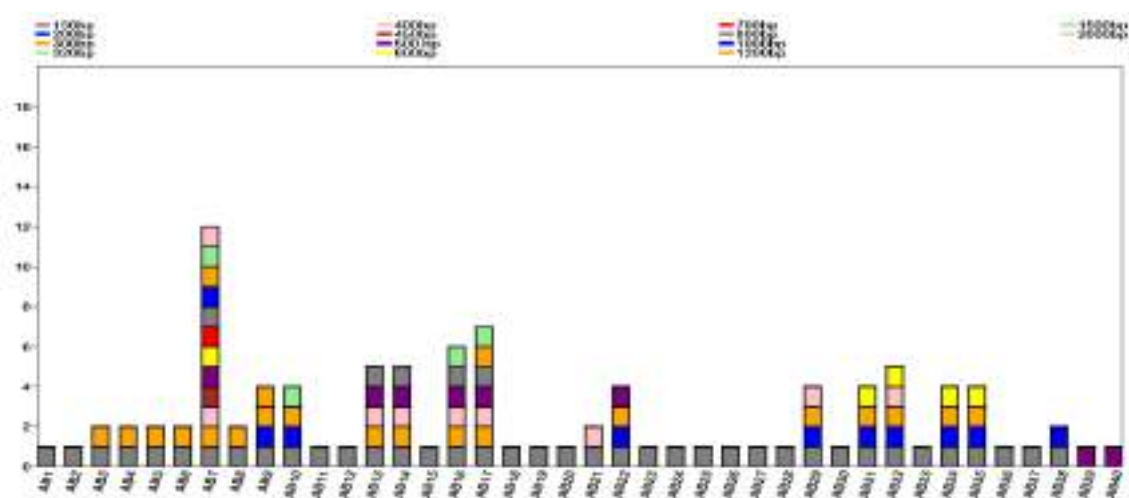


Figure (4-26): Frequency of ERIC-PCR Banding Patterns among *A. baumannii* Isolates. The stacked bar chart represents the presence and frequency of amplified DNA fragments (150–2000 bp) for each isolate (AB1–AB40). Each color corresponds to a specific band size, illustrating the molecular fingerprint of individual isolates. Isolates with a higher number of bands, particularly those sharing multiple ERIC-PCR fragments, reflect greater genetic similarity and contribute to clustering in the dendrogram. In contrast, variation in band composition and the absence of specific fragments among isolates indicate genetic diversity, supporting the differentiation into distinct clusters observed in the ERIC-PCR analysis.

Bands of 700 bp, 1000 bp, and 2000 bp exhibited perfect positive correlations ($r = 1$) with each other, with extremely low P-values (6.7416×10^{-186}), indicating highly significant associations. These highly correlated fragments suggest that the isolates carrying these fragments share a common genetic background and contribute to the formation of closely related groups within the ERIC-PCR dendrogram. The strong correlations among these bands are likely associated with the clonal clustering observed.

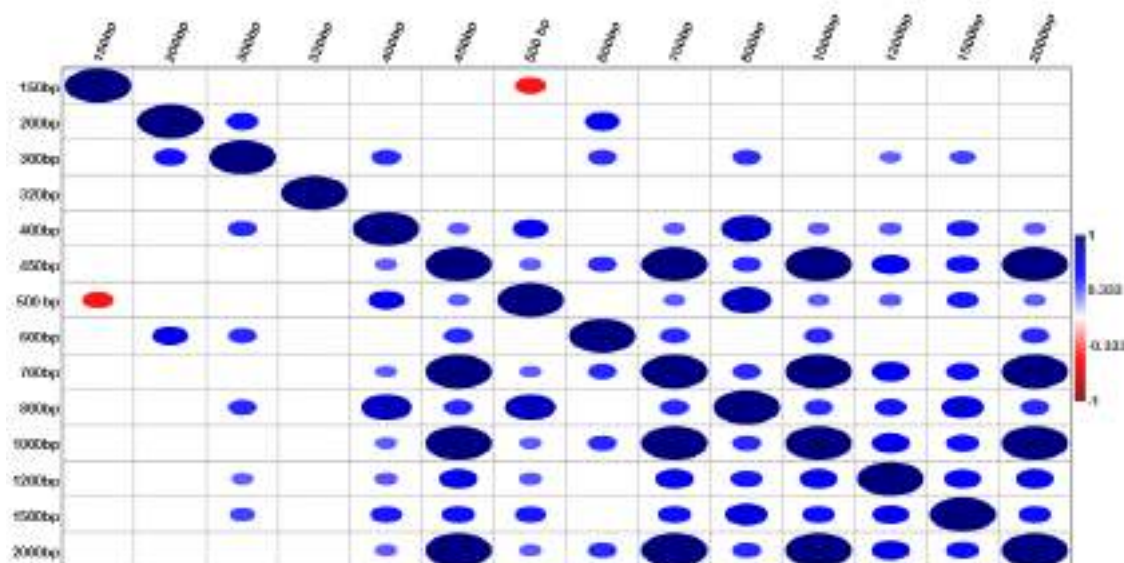


Figure (4-27): ERIC-PCR dendrogram band-based correlation matrix for *A. baumannii* isolates. The dendrogram depicts the genetic relation of isolates using fingerprinting from ERIC-PCR, while the correlation matrix visualizes the relationships concerning the amplified DNA fragments (bp). Fragments (bands) with high correlation ($r \approx 1$) represent conserved genetic markers that caused band clustering (clonal clustering); in contrast, weak or negative correlations indicate genetic variability and band diversity among the isolates. This approach indicates the presence of clonal lineages and segregation in the strains of the studied population.

On the contrary, some bands showed weak or even negative correlations (e.g., 500 bp with 150 bp: $r \approx -0.45$) which may be the result of genetic variability and different bands, and how these are distributed among the isolates. This variability accounts for the dispersion of some isolates across different branches of the dendrogram, especially those isolates originating from different infection sources like sputum, blood, and urine. Bands like 300 bp, 400 bp, and 600 bp showed a degree of variability with other bands or markers, and this also means that their presence and absence within some isolates (and not others) is a factor for clustering isolates into different groups. Some p-values are extremely low (e.g., 6.7×10^{-186}) which indicates that those correlations are highly significant and not because of random coincidence. The presence or absence of these specific bands within a cluster is a form of genetic diversity. In general, the correlation matrix together with the dendrogram shows that clusters are

explained by a common banding pattern while the genetic diversity is explained by the gain or loss of certain ERIC fragments. With that said, *A. baumannii* isolates show clearly defined clonal relatedness and significant genomic variability. This variability explains the epidemiological complexities associated with the organism, which is consistent with previous findings using ERIC typing that demonstrated similar patterns of genetic diversity and clonal relationships among clinical *A. baumannii* isolates (Nasrabadi *et al.*, 2026).

CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The following conclusions can be drawn based on the findings:

1. Aminoglycoside-resistant *A. baumannii* is more prevalent among hospitalized patients, indicating a strong association with healthcare-associated infections in Babylon Province.
2. These isolates exhibit high resistance levels to a wide range of antimicrobial agents
3. The alarming rates of XDR and PDR highlighted in this study pose a significant therapeutic challenge for patient management.
4. All *A. baumannii* isolates exhibited high MAR index values (>0.2), indicating strong antimicrobial pressure, confirming an extremely high level of antimicrobial resistance in Babylon hospitals, with some isolates showing MAR index values of 1.00 and 0.67.
5. The majority of aminoglycoside-resistant *A. baumannii* isolates carried multiple resistance determinants, including aminoglycoside-modifying enzyme (AME) genes and 16S rRNA methylase genes.
6. The current study demonstrated the presence of class 1 integron among aminoglycoside-resistant isolates, suggesting its potential role in the dissemination of antibiotic resistance.
7. Clonal dissemination contributes to the spread of aminoglycoside and other antibiotic resistance genes among *A. baumannii* isolates in Babylon hospitals.

Recommendations

1. Establishing and enforcing comprehensive antimicrobial stewardship programs in all healthcare facilities in Iraq.
2. Developing systematic national surveillance programs using molecular techniques is essential to monitor the spread of aminoglycoside resistance genes and class 1 integrons in *A. baumannii*.
3. Further studies using advanced molecular techniques such as multilocus sequence typing (MLST) are recommended to determine the source of dissemination and to better understand the epidemiology of aminoglycoside resistance genes in *A. baumannii* isolates.
4. Extending surveillance efforts to include other clinically significant non-fermenting Gram-negative bacteria beyond *A. baumannii* to better understand the burden of aminoglycoside resistance.

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Appendixes

Appendix (1): GenBank Submission Records of Seven *A. baumannii* 16S rRNA Gene Sequences

Acinetobacter baumannii strain B.S.and.F.S.Iraq9 16S ribosomal RNA gene, partial sequence

GenBank PX915571.1

[FASTA](#) [Graphics](#)[Go to:](#)

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 VERSION PX915571.1
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 SOURCE Acinetobacter baumannii
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 Moraxellales; Moraxellaceae; Acinetobacter; Acinetobacter
 calcoaceticus/baumannii complex.
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 AUTHORS Taame,B.S. and Abd,F.S.
 TITLE Dissemination and Molecular Characterization of Aminoglycoside
 Resistant Acinetobacter baumannii Isolates from Basal Hospitals
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 536)
 AUTHORS Taame,B.S. and Abd,F.S.
 TITLE Direct Submission
 JOURNAL Submitted (24-JAN-2026) College of Science, University of
 Al-Qadisiyah, 38 street, Babylon, Babylon 51000, Iraq
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 361 gaggcaacca tgactgact ttgcctacg ttgtacatt ccagagatga attgttgct
 421 tggggaatc agattctac atctgctgc ctactctcag ctgtgtgtt gagatgctt
 481 gtcagctga gctgagcat ttacatttt actaatgac cccttttcg aaat

Figure A1. GenBank record of *A. baumannii* isolate Iraq9

Acinetobacter baumannii strain B.S.and.F.S.Iraq13 16S ribosomal RNA gene, partial sequence

GenBank: PX915580.1

[FASTA](#) [Graphics](#)[Go to](#)

LOCUS PX915580 537 bp DNA linear BCT 29-JAN-2026
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 VERSION PX915580.1
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 SOURCE *Acinetobacter baumannii*
 ORGANISM *Acinetobacter baumannii*
 Bacteria; Pseudomonadati; Pseudomonadota; Gammaproteobacteria; Moraxellales; Moraxellaceae; *Acinetobacter*; *Acinetobacter calcoaceticus/baumannii* complex.
 REFERENCE 1 (bases 1 to 537)
 AUTHORS Tsama,B.S. and Abd,F.S.
 TITLE Dissemination and Molecular Characterization of Aminoglycoside Resistant *Acinetobacter baumannii* Isolates from Babil Hospitals
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 537)
 AUTHORS Tsama,B.S. and Abd,F.S.
 TITLE Direct Submission
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 361 acgcnagaa ccttacctg ccttgacata ctgaaactt tccagagtg gattggtgc
 421 tttaggaatc tagatacgg tactacatg ctgtgtcg ctggttgtt gacatgttg
 481 gtttagtccc gacgagcgc aaccctttt cttacttgc aaatttttc gaaaaat

Figure A1. GenBank record of *A. baumannii* isolate Iraq13

Acinetobacter baumannii strain B.S.and.F.S.Iraq8 16S ribosomal RNA gene, partial sequence

GenBank: PX915553.1

FASTA Graphics

Go to

LOCUS PX915553 537 bp DNA linear OCT 29-JAN-2026
 DEFINITION Acinetobacter baumannii strain B.S.and.F.S.Iraq8 16S ribosomal RNA gene, partial sequence.
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 VERSION PX915553.1
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 SOURCE Acinetobacter baumannii
 ORGANISM Acinetobacter baumannii
 bacteria; pseudomonadati; pseudomonadota; gammaproteobacteria; moraxellales; moraxellaceae; Acinetobacter; Acinetobacter calcoaceticus/baumannii complex.
 REFERENCE 1 (bases 1 to 537)
 AUTHORS Taama,B.S. and Abd,F.S.
 TITLE dissemination and molecular characterization of aminoglycoside resistant Acinetobacter baumannii isolates from sabil hospitals
 JOURNAL unpublished
 REFERENCE 2 (bases 1 to 537)
 AUTHORS Taama,B.S. and Abd,F.S.
 TITLE Direct Submission
 JOURNAL Submitted (24-JAN-2026) College of Science, University of Al-Qadisiyah, 10 street, Babylon, Babylon 51000, Iraq
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 481 ttatgtccg actgagagc ttaccttgc attaatgcc caatcttga aaaaat

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Figure A1. GenBank record of *A. baumannii* isolate Iraq8

Acinetobacter baumannii strain B.S.and.F.S.Iraq34 16S ribosomal RNA gene, partial sequence

GenBank: PX915581.1

[FASTA](#) [Graphics](#)[Go to](#)

LOCUS PX915581 532 bp DNA linear 8CT 29-JAN-2026
 DEFINITION Acinetobacter baumannii strain B.S.and.F.S.Iraq34 16S ribosomal RNA gene, partial sequence.
 ACCESSION PX915581
 VERSION PX915581.1
 KEYWORDS
 SOURCE Acinetobacter baumannii
 ORGANISM Acinetobacter baumannii
 Bacteria; Pseudomonadota; Pseudomonadota; Gammaproteobacteria; Moraxellales; Moraxellaceae; Acinetobacter; Acinetobacter calcoaceticus/baumannii complex.
 REFERENCE 1 (bases 1 to 532)
 AUTHORS Teama,B.S. and Abd,F.S.
 TITLE : Dissemination and Molecular Characterization of Aminoglycoside Resistant Acinetobacter baumannii Isolates From Basra Hospitals
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 532)
 AUTHORS Teama,B.S. and Abd,F.S.
 TITLE direct submission
 JOURNAL submitted (24-JAN-2026) college of science, university of Al-Qadisiyah, 18 street, Babylon, Babylon 51000, Iraq
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Figure A1. GenBank record of *A. baumannii* isolate Iraq 34

Acinetobacter baumannii strain B.S.and.F.S.Iraq1 16S ribosomal RNA gene, partial sequence

GenBank: PX915552.1

[FASTA](#) [Graphics](#)[Go to:](#)

LOCUS PX915552 539 bp DNA linear BCT 29-JAN-2026
 DEFINITION *Acinetobacter baumannii* strain B.S.and.F.S.Iraq1 16S ribosomal RNA gene, partial sequence.
 ACCESSION PX915552
 VERSION PX915552.1
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 ORGANISM *Acinetobacter baumannii*
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 REFERENCE 1 (bases 1 to 539)
 AUTHORS Taama,B.S. and Abd,F.S.
 TITLE Dissemination and Molecular Characterization of Aminoglycoside Resistant *Acinetobacter baumannii* Isolates from Babil Hospitals
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 539)
 AUTHORS Taama,B.S. and Abd,F.S.
 TITLE Direct Submission
 JOURNAL Submitted (24-JAN-2026) College of Science, University of Al-Qadisiyah, 18 street, Babylon, Babylon 51000, Iraq
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 Sequencing Technology :: Sanger dideoxy sequencing
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Figure A1. GenBank record of *A. baumannii* isolate Iraq 1

Acinetobacter baumannii strain B.S.and.F.S.Iraq10 16S ribosomal RNA gene, partial sequence

GenBank: PX915573.1

[FASTA](#) [Graphics](#)[Go to...](#)

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VERSION     PX915573.1
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            calcoaceticus/baumannii complex.
REFERENCE   1 (bases 1 to 537)
  AUTHORS   Teama,B.S. and Abd,F.S.
  TITLE     Dissemination and Molecular Characterization of Aminoglycoside
            Resistant Acinetobacter baumannii Isolates from Babil Hospitals
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 537)
  AUTHORS   Teama,B.S. and Abd,F.S.
  TITLE     Direct submission
  JOURNAL   submitted (24-JAN-2026) college of science, university of
            Al-Qadisiyah, 30 street, Babylon, Babylon 51000, Iraq
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301  caaatgatt gctggggcc cgcacagcg gggagcatg tggttatct cgtgcacg
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421  ggaatctag attctaggc ctgcatagt gtgtcagct cgtgtctga gatgttgt
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Figure A1. GenBank record of *A. baumannii* isolate Iraq10

Acinetobacter baumannii strain B.S.and.F.S.Iraq35 16S ribosomal RNA gene, partial sequence

GenBank: PX915582.1

[FASTA](#) [Graphics](#)[Go to](#)

LOCUS PX915582 537 bp DNA linear OCT 29-2025
 DEFINITION Acinetobacter baumannii strain B.S.and.F.S.Iraq35 16S ribosomal RNA gene, partial sequence.
 ACCESSION PX915582
 VERSION PX915582.1
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 ORGANISM Acinetobacter baumannii
 Bacteria; Pseudomonadati; Pseudomonadota; Gammaproteobacteria; Moraxellales; Moraxellaceae; Acinetobacter; Acinetobacter calcoaceticus/Baumannii complex.
 REFERENCE 1 (bases 1 to 537)
 AUTHORS Teama,B.S. and Abd,F.S.
 TITLE Dissemination and Molecular Characterization of Aminoglycoside Resistant Acinetobacter baumannii Isolates from Bahil Hospitals
 JOURNAL Unpublished
 REFERENCE 2 (bases 1 to 537)
 AUTHORS Teama,B.S. and Abd,F.S.
 TITLE Direct Submission
 JOURNAL Submitted (24-10-2025) College of Science, University of Al-Qadisiyah, 10 street, Babylon, Babylon 51000, Iraq
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Figure A1. GenBank record of *A. baumannii* isolate Iraq35

Appendix

Appendix (2): Nucleotide multiple sequence alignment of the genes in clinical *A. baumannii* isolates compared with reference strains.

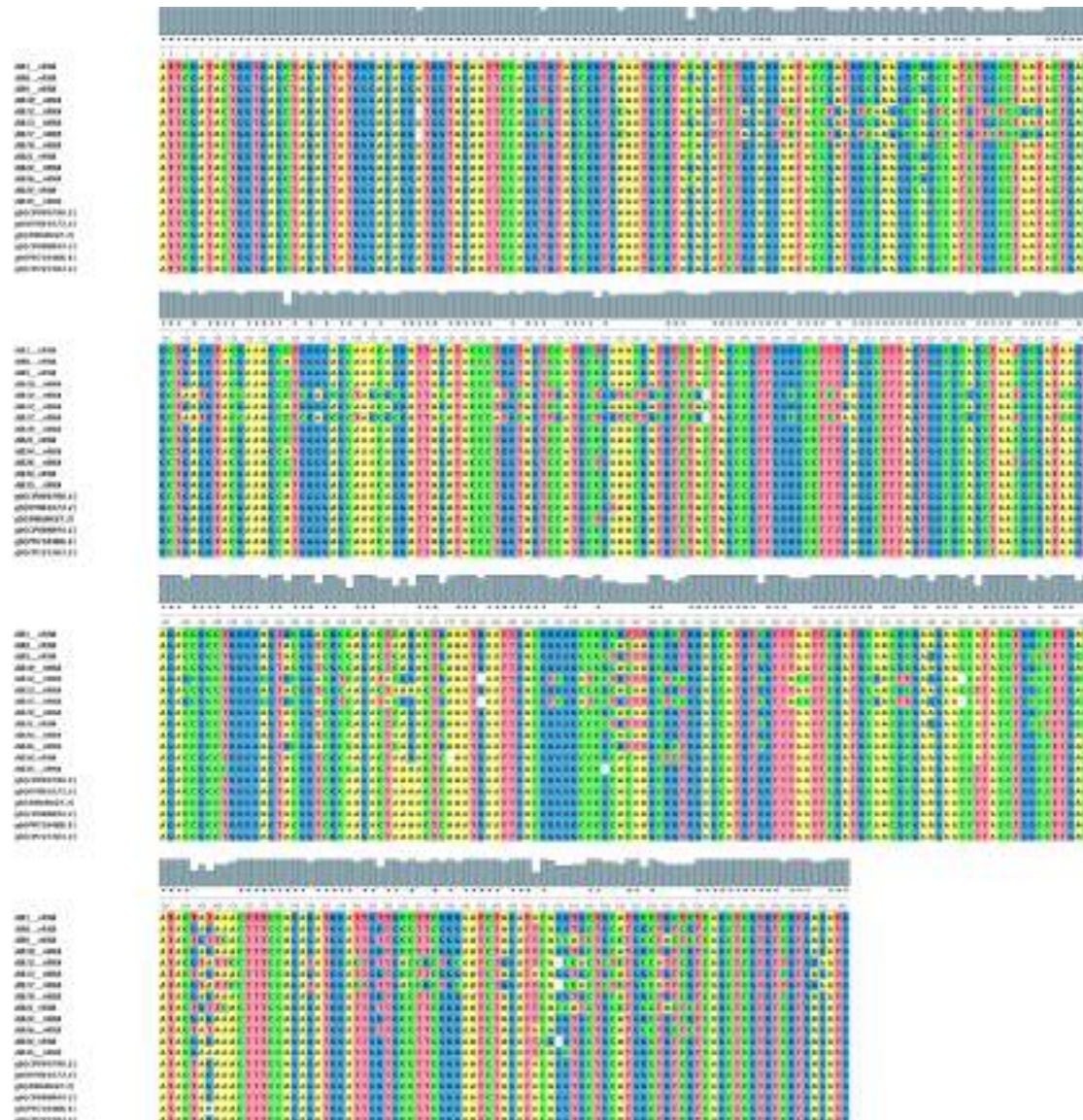


Figure A2: Nucleotide multiple sequence alignment of the 16S rRNA gene

Genomic tracks for the 1000 Genomes Project, showing coverage and variant calls for the region 10:11,111,111-11,111,111. The tracks include a reference sequence, a coverage track, and variant call tracks for various populations (AFR, EUR, CHB, CHS, JPT, KHV, YRI, CEU, GBR, IBS, FIN, LWK, PUR, BWA, CHB, CHS, JPT, KHV, YRI, CEU, GBR, IBS, FIN, LWK, PUR, BWA). The variant call tracks show the presence of specific variants across the populations, with colors indicating the variant type (e.g., A, C, G, T).

Figure A2:Nucleotide multiple sequence alignment of the aac(3)-II gene

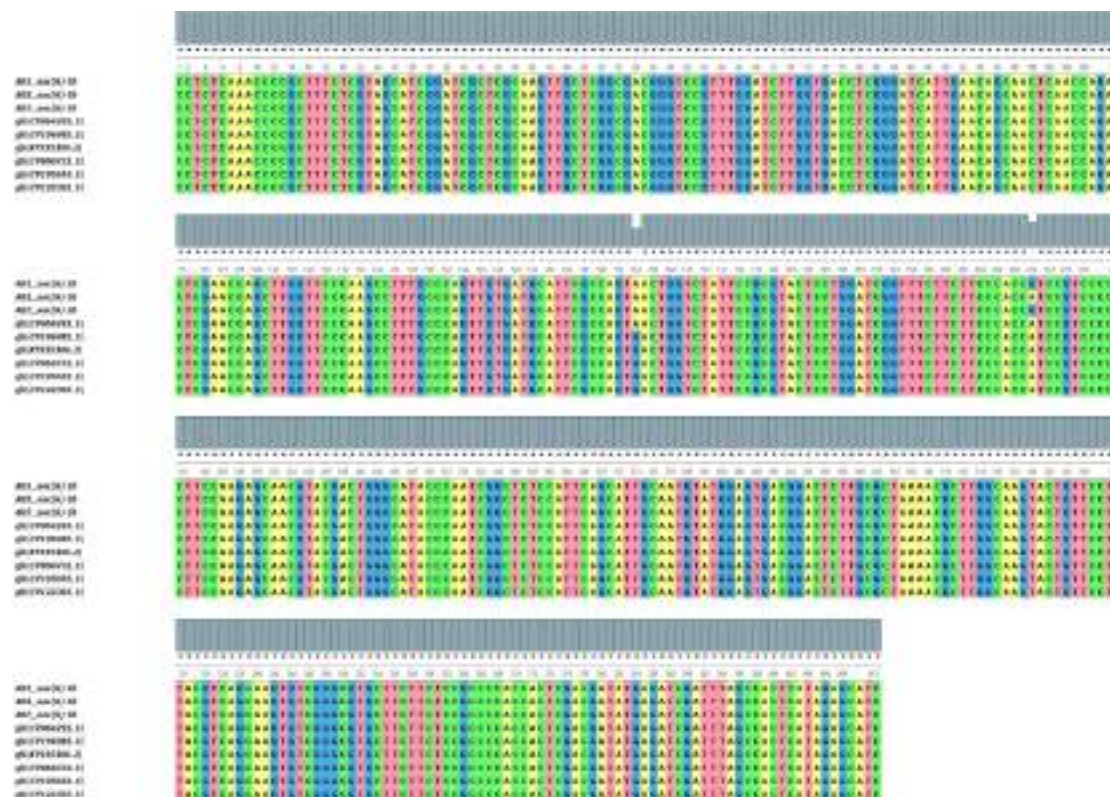


Figure A2:Nucleotide multiple sequence alignment of the aac(6')-Ib gene

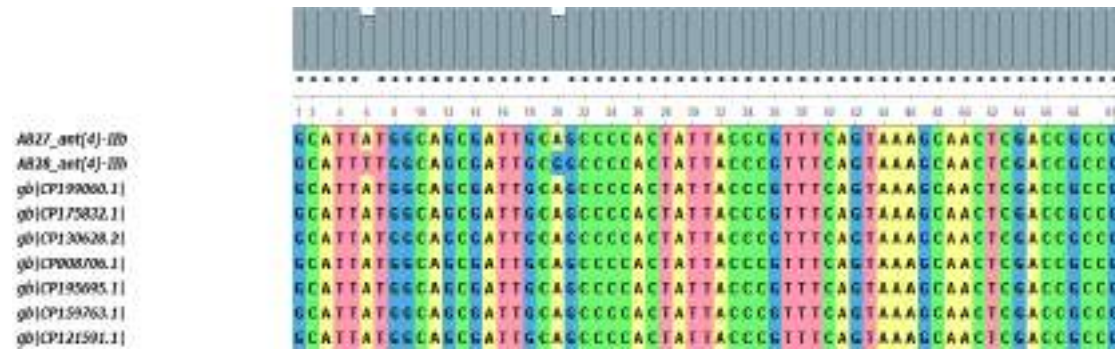
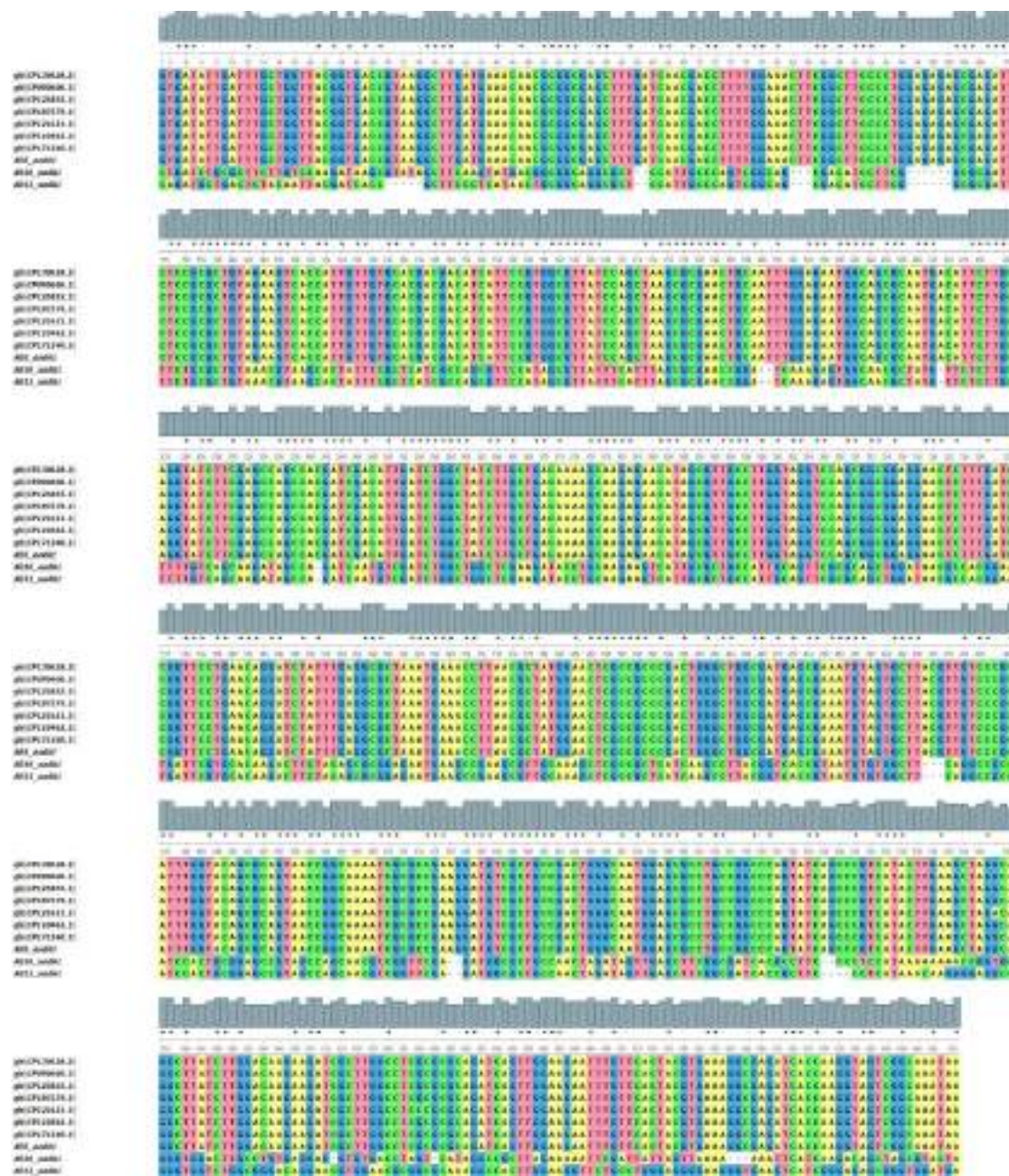


Figure A2: Nucleotide multiple sequence alignment of the ant (4')-IIB gene



Appendix

Figure A2: Nucleotide multiple sequence alignment of the *aadA1* gene

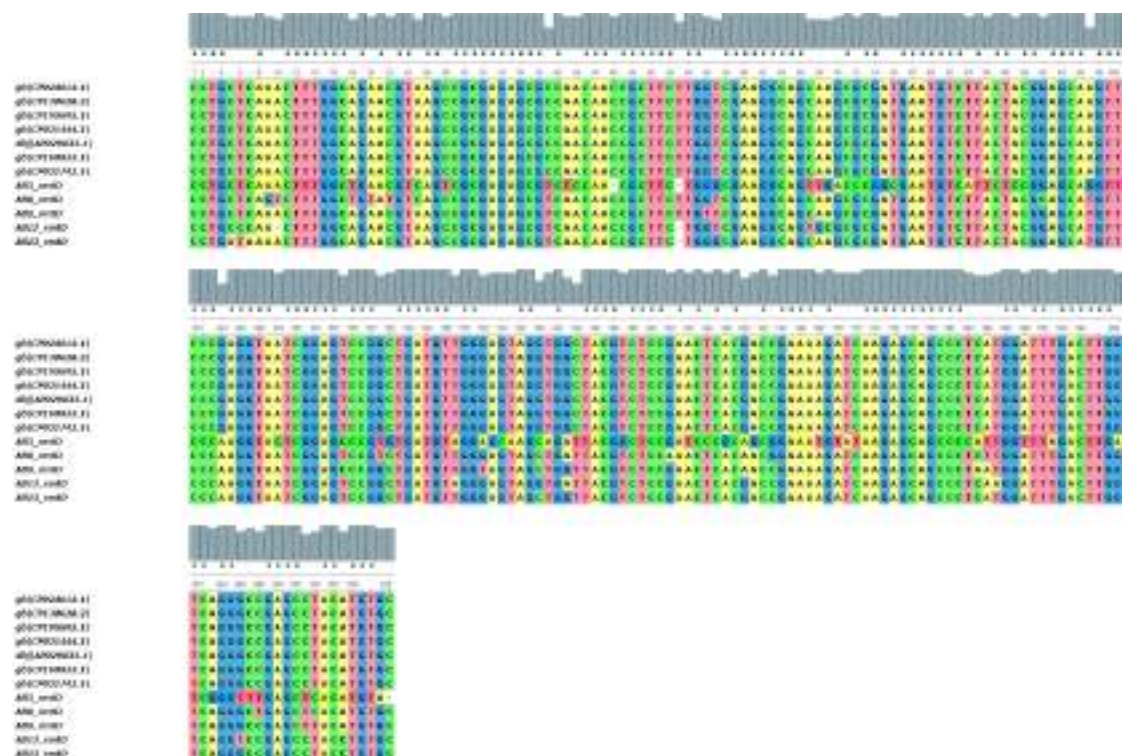


Figure A2: Nucleotide multiple sequence alignment of the *rmtD* gene

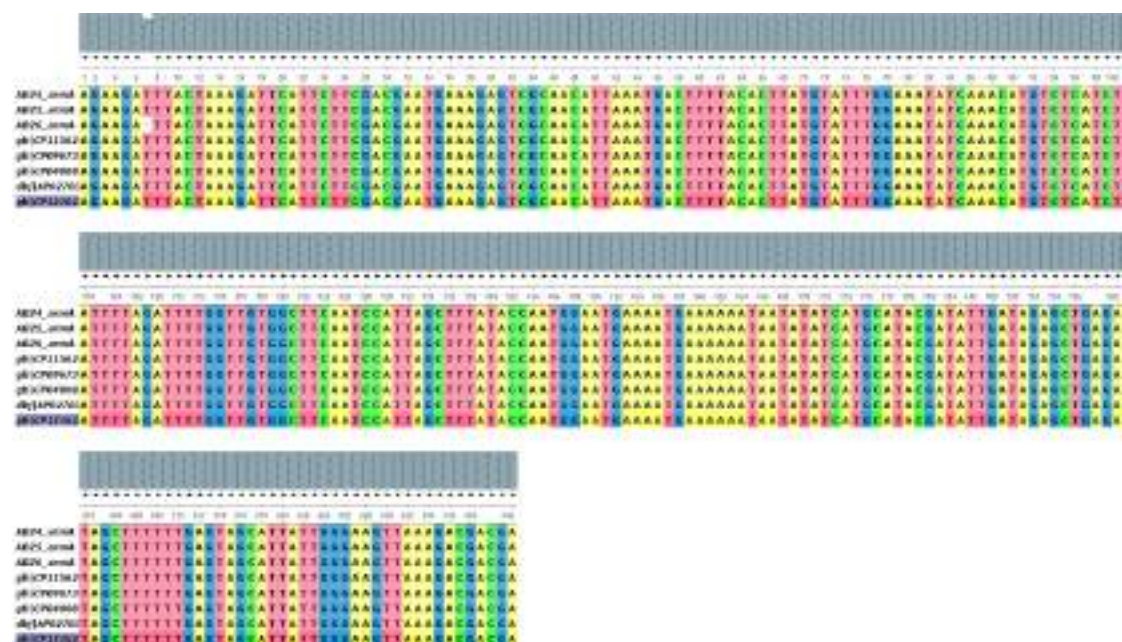


Figure A2: Nucleotide multiple sequence alignment of the *armA* gene

الملخص :

تُعد بكتريا *Acinetobacter baumannii* من الممرضات الانتهازية المهمة وأحد الأسباب الرئيسية للعدوى المرتبطة بالرعاية الصحية، ولا سيما بين المرضى ذوي الحالات الحرجة والمرضى المثبطين مناعياً. وقد عُزلت بصورة متزايدة من مصادر سريرية مختلفة، بما في ذلك جروح الحروق، والدم، والقشع، ومسحات الجروح، والبول. وقد أصبح ظهور عزلات *A. baumannii* المقاومة للأمينوغليكوزيدات مصدر قلق كبير في البيئات السريرية، إذ يحد من الخيارات العلاجية ويعقّد إدارة العدوى. أُجريت هذه الدراسة لتحديد حدوث وخصائص عزلات *A. baumannii* المقاومة للأمينوغليكوزيدات والمعزولة من عينات سريرية مختلفة في مستشفيات محافظة بابل، العراق.

أُجريت هذه الدراسة المقطعية على ما مجموعه 519 عينة سريرية جُمعت عشوائياً من المرضى الراقدين في المستشفى (المرضى الداخليين) وغير الراقدين (المرضى الخارجيين) المراجعين لأربعة مستشفيات رئيسية في محافظة بابل خلال مدة عشرة أشهر من عام 2025. تم تشخيص جميع عزلات *A. baumannii* اعتماداً على الخصائص المظهرية واستخدام الاختبارات الكيميائية الحيوية التقليدية وتأكيداً باستعمال نظام VITEK-2.

تم تقييم الحساسية للمضادات الحيوية باستخدام طريقة انتشار الأقراص تجاه مجموعة من مضادات الأمينوغليكوزيدات والمضادات الأخرى، فضلاً عن تحديد التركيز المثبط الأدنى (MIC) لكل من الكوليسيتين والبوليميكسين B.

تم عزل *A. baumannii* من 75 عينة (14.45%). وكُشف عن مقاومة الأمينوغليكوزيدات في 40 عينة (53.33%)، صُنفت منها 37 عينة (92.50%) على أنها واسعة المقاومة للمضادات الحيوية (XDR)، في حين عُدت 3 عزلات (7.50%) مقاومة محتملة لجميع المضادات الحيوية (PDR). ولوحظت قيم مرتفعة لمؤشر المقاومة المتعددة للمضادات الحيوية (MAR) تراوحت بين 0.67 و1.00 بين جميع عزلات *A. baumannii* المقاومة للأمينوغليكوزيدات، حيث تجاوزت جميع العزلات الحد الحرج البالغ 0.2. وتشير هذه النتائج إلى تعرض واسع للعوامل المضادة للميكروبات وارتفاع معدل انتشار المقاومة المتعددة للمضادات الحيوية بين العزلات المدروسة وتم التأكيد الجزيئي على مستوى النوع باستخدام تفاعل

البلمرة المتسلسل (PCR) باستهداف جيني *bla*OXA-51-like و *SrRNA16*، أعقب ذلك إجراء تسلسل جين *S rRNA 16* للتشخيص الجزيئي والتأكيد النهائي لعزلات *A. baumannii*. كما تم الكشف عن جينات مقاومة الأمينوغليكوزيدات، بما في ذلك سبعة جينات مشفرة للإنزيمات المعدلة للأمينوغليكوزيدات (AMEs) وثلاثة جينات مشفرة لميثيلازات *S rRNA16*، بالإضافة إلى جين الإنتغرون من الصنف الأول *intI* باستخدام تقنيات تفاعل البلمرة المتسلسل التقليدية.

أظهر التحري عن جينات مقاومة الأمينوغليكوزيدات انتشاراً مرتفعاً لجينات الإنزيمات المعدلة للأمينوغليكوزيدات (AMEs) فمن بين جينات إنزيمات الأسيتيل ترانسفيراز للأمينوغليكوزيدات (AACs)، كان الجين *aac(6')-Ib* الأكثر شيوعاً بنسبة (50.00%)، تلاه الجين *aacC1* بنسبة (20.00%) والجين *aac(3)-II* بنسبة (15.00%). أما بالنسبة لجينات إنزيمات الفوسفوترانسفيراز للأمينوغليكوزيدات (APHs)، فقد كُشف عن الجين *aph(3')-VI* (*aphA6*) في (47.50%) من العزلات. وفيما يتعلق بجينات إنزيمات النوكليوتيد ترانسفيراز للأمينوغليكوزيدات (ANTs)، كان الجين *ant(4')-Iib* الأكثر شيوعاً بنسبة (45.00%)، يليه الجين *aadA1* بنسبة (7.50%)، في حين كانت جميع العزلات سالبة للجين *aadB*. إضافة إلى ذلك، كُشف عن جينات ميثيلازات *S rRNA16*، والتي شملت *rmtB* بنسبة (30.00%) و *rmtD* بنسبة (15.00%) و *armA* بنسبة (12.50%). كما كُشف عن الإنتغرونات من الصنف الأول في (22.50%) من العزلات استناداً إلى تضخيم الجين *intI1*.

علاوة على ذلك، أُجري تحليل التسلسل الجيني لعزلات مختارة حاملة لجينات مقاومة الأمينوغليكوزيدات، بما في ذلك الجينات المشفرة للإنزيمات المعدلة للأمينوغليكوزيدات (AMEs) وميثيلازات *S rRNA. 16* بالإضافة إلى ذلك، تم تحديد القرابة الوراثية بين 40 عزلة مقاومة للأمينوغليكوزيدات باستخدام تقنية تفاعل البلمرة المتسلسل لتسلسلات الإجماع المتكررة بين الجينات المعوية (ERIC-PCR).

في الختام فإن الانتشار المرتفع لمحددات مقاومة الأمينوغليكوزيدات، إلى جانب أنماط المقاومة واسعة النطاق (XDR) والمقاومة المحتملة لجميع المضادات الحيوية (PDR)، يسلط الضوء على التهديد الخطير الذي تمثله *A. baumannii* في مستشفيات بابل. كما يشير الارتفاع في قيم مؤشر المقاومة المتعددة للمضادات الحيوية (MAR) إلى وجود ضغط مرتفع

ناتج عن استخدام المضادات الحيوية. وأظهر تحليل القرابة الوراثية باستخدام تقنية-ERIC PCR احتمال وجود انتشار استتسالي بين العزلات. لذا فإن الكشف السريع والتطبيق الصارم لإجراءات مكافحة العدوى يعدان ضروريين للحد من المزيد من الانتشار. ووفقاً لحدود علمنا، تُعد هذه الدراسة أول تقرير يوثق محددات مقاومة الأمينوغليكوزيدات في عزلات *A. baumannii* في محافظة بابل، العراق.



جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة القادسية - كلية العلوم
قسم علوم الحياة

الانتشار والتوصيف الجزيئي لعزلات *Acinetobacter baumannii*

المقاومة للأمينوغليكوزيدات في محافظة بابل

أطروحة

مقدمة إلى مجلس كلية العلوم/جامعة القادسية وهي جزء من متطلبات نيل درجة

الدكتوراه فلسفة/علوم الحياة

من قبل

بيداء شهيد طعمة

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بإشراف

أ. د. فراس سرحان عبد المياحي

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