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The Impact of the adeE and adeY Genes on the Antibiotic Resistance Mechanisms of Acinetobacter baumannii

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Abstract: *Current study identified Acinetobacter baumannii isolates from burn wound samples using biochemical and molecular techniques focusing on the rpoB gene. All isolates showed variable virulence factors, including biofilm formation (100%), gelatinase activity (90%), and mannose-resistant hemagglutination (72%). Antibiotic susceptibility testing revealed high resistance rates to Rifampin (96%), Tetracycline (92%), and third-generation cephalosporins such as Cefotaxime (73%) and Ceftriaxone (81%). Lower resistance rates were observed for Levofloxacin (31%) and Gatifloxacin (27%), suggesting potential effectiveness. PCR analysis confirmed the presence of AdeE and AdeY efflux pump genes in several isolates. However, statistical analysis, including Chi-square/Fisher's tests and t-tests, demonstrated no significant correlation between the presence of these genes and antibiotic resistance patterns ($p > 0.05$). This indicates that while efflux pump genes are present, their presence alone does not predict antibiotic resistance in the tested isolates. The findings highlight the complex mechanisms of resistance in A. baumannii and the need for continued surveillance and molecular diagnostics to guide effective infection control.*

Keywords: *isolates, Acinetobacter, biofilm*

1. INTRODUCTION

Burn injuries rank as one of the worst traumatic events because patients need extensive periods of hospital care combined with intensive medical treatments as well as surgical operations. Burn trauma patients face severe complications because extensive burns make their skin vulnerable to infection through both protective measures and weakened immune response. Acinetobacter baumannii stands as one of the most hazardous multidrug-resistant pathogens which infects burn wounds among numerous other possible infectious species (Peleg et al., 2021; Nowak & Paluchowska, 2023).

During the past twenty years Acinetobacter baumannii has displayed enhanced clinical importance especially when infecting critical care patients who receive treatment at burn units and intensive care facilities. Acinetobacter baumannii maintains survival throughout long periods of time on dry hospital surfaces which explains why it gained its reputation. The bacterium demonstrates long-term survival in challenging environments through its natural resistance qualities while establishing strong biofilms that resist both defense mechanisms of the host body and current antimicrobial treatments (Lee et al., 2022). Biofilm development elevates the pathogenic potential of this virus because it prolongs infections and complications the treatment process especially in patients with major burn injuries and compromised immunity.

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A. baumannii demonstrates strong environmental adaptability together with its exceptional ability to develop antibiotic resistance against nearly every known antibiotic including carbapenems that healthcare professionals use as their final treatment options. XDR and pan-drug-resistant strains of bacteria across the healthcare environment have made therapeutic management difficult for burn patients who develop infections. A. baumannii strains lead to extended hospitalizations as well as elevated medical expenses while increasing surgical intervention need and resulting in substantial enhancement of patient fatality rates (Zarrilli et al., 2023).

The growing number of A. baumannii infections in burn patients requires immediate heightened monitoring systems along with strict infection prevention protocols and new antibiotic development. The effective treatment of bacteria needs both proper antibiotic usage with preventive measures to stop their spread in healthcare settings.

Antibiotic resistance has transformed into a major international health problem and constitutes a major hurdle for the successful treatment of infectious diseases. The world faces a pressing concern about E. coli bacterium infections because E. coli strains show decreased responsiveness to available antibiotics which researchers from different nations have identified as a concerning pattern. ESBL development in bacteria serves as a major resistance factor that prevents treatment success. The specific enzyme possesses degradation capabilities for all third-generation cephalosporins as well as aztreonam while clavulanic acid successfully blocks its activity. The antibiotic resistance of bacteria that produce ESBL becomes problematic because they resist many different classes of pharmaceutical treatments. Alongside this need comes the requirement to conduct regular antimicrobial resistance checks to learn about resistance mechanisms and determine optimal antibiotic treatments (Chinemerem et al., 2022; Baker et al., 2022; Ahmed et al., 2024).

Among immunocompromised patients Acinetobacter baumannii exists as a pleomorphic type of opportunistic Gram-negative bacterium. The beginning of the twenty-first century has produced a substantial increase in fear about A. baumannii. Categorization as the "red alert" pathogen is due to its multi-drug resistance properties which lead to infections of both hospital and community-based populations (Murugaiyan et al., 2024).

Various diseases such as bacteremia along with pneumonia and meningitis and wound infections tend to develop from A. baumannii found within battle zones according to Javadi (2024) and Nguyen and Joshi (2021). Hospital environments face major difficulties due to A. baumannii which medical professionals identify as one among six dangerous multidrug-resistant (MDR) pathogens across global healthcare facilities. Different countries demonstrate

varying A. baumannii resistance patterns which have sharply increased throughout the years. Anti-gentamycin and anti-ceftazidime resistance rates span between 0% and the high figure of 81%. Tests have demonstrated that the resistance rates for amikacin span between 10% and 51% and ciprofloxacin shows resistance levels of 19% to 81%. The resistance of Piperacillin-tazobactam lies between 36% and 75% in different countries that are part of this study. A. baumannii infections have been successfully treated with imipenem and meropenem since they were recognized as the most dependable antimicrobial therapies. Recent studies show that antimicrobial efficacy declines because failure rates can reach 87% based on clinical applications. Colistin and tigecycline remain the minimal available therapeutic choices for handling MDR A. baumannii infections. Research shows that the use of colistin and tigecycline as treatments has become more worrying throughout Europe because resistant cases have grown to between 3% and 6%. Medical practitioners face a significant challenge because Acinetobacter species constantly evolve to become resistant to all available antimicrobial drugs (Ibrahim et al., 2021; Agyepong et al., 2023).

The antibiotic resistance of Acinetobacter baumannii shows an increase through various β-lactamase enzyme activities together with enzyme mechanisms. The resistance-developing role of non-enzymatic factors includes the activity of efflux pumps together with membrane permeability changes and penicillin-binding protein (PBP) sequence mutations. The significant mechanisms responsible for resistance elevation in clinical isolate populations are these initial two factors. Bacterial β-lactamase activity produces resistance to penicillins and cephalosporins as well as carbapenems. The bacterial enzyme β-lactamases produced by A. baumannii resides in either chromosomal and plasmid genetic locations (Huang et al., 2024).

Acinetobacter baumannii displays multidrug resistance when it demonstrates resistance toward various widely employed antibiotics. The approaches healthcare professionals use in treating Acinetobacter infections need to consider both the patient condition and susceptibilities. People fighting Acinetobacter infections already have weak health conditions with the infection producing significant medical complications that could lead to death (Rafailidis et al., 2024).

This research project intends to analyze the pathogenic qualities of A. baumannii combined with its ability to colonize while determining how plasmids act as mediators for these harmful traits and antibacterial resistance. The research aims to increase scientific knowledge about significant pathogenic microorganisms which lack adequate investigation in Iraq (Rahimzadeh et al., 2024).

Clinical analysis of Acinetobacter baumannii requires the combined use of conventional microbiological procedures along with genetic testing of the gyrB and rpoB genes. Research findings have established biofilm production and enzyme gelatinase activity together with factors I and III as key virulence characteristics of this bacteria. Additionally, the study investigates A. baumannii's responses to various antibiotics. PCR technology helps detect AdeE and AdeY genes which signify multidrug resistance in the laboratory. Bacterial plasmid content analysis and plasmid curing tests to study antibiotic resistance ability and virulence gene expression are included in the research design (Zack et al., 2024).

2. MATERIAL AND METHODS

Patients

Three hundred specimens originated from patients with burn injuries. The examined patients sought treatment at one of Al Sadiq Hospital, Hilla General Teaching Hospital, or Merjan Medical City in Hillah City from January 2021 through January 2022. Medical personnel collected wounds swabs by using sterile equipment while putting samples into sterile receptacles intended for microbial analysis. The lab personnel cultured the samples through MacConkey agar and blood agar base plates while maintaining 37 degrees Celsius for 24 hours' aerobic incubation (Mengistu et al., 2023).

Diagnosis of *A. baumannii*

The samples required multiple culture testing on general and specific media platforms involving MacConkey agar with blood agar and CHROM agar. The media helped bacterial species from burn wound specimens to both thrive and differentiate themselves. After bacterial culture researchers conducted Gram staining and manual and automated biochemical tests for preliminary bacterial isolate identification. The confirmation of Acinetobacter baumannii isolates happened through conventional polymerase chain reaction (PCR) which used DNA extracted from bacteria with the Wizard® Genomic DNA Purification Kit A1125 from Promega, Madison, WI USA. The purified genomic DNA from A. baumannii became available to work as a starting material for the PCR process where researchers amplified both the rpoB gene and AdeE AdeY antibiotic resistance genes (Louws et al., 2024).

One reaction mixture of 50 µl contained a primer-set designated to amplify the rpoB gene. The distinct 397 base pair PCR amplicon produced after amplification revealed the

presence of A. baumannii on the electrophoresis 2% agarose gel according to Maybin et al. (2024).

The AdeE and AdeY antibiotic resistance genes against ciprofloxacin and ceftriaxone and amikacin and levofloxacin and tetracycline and gatifloxacin and ceftazidime and gentamicin were amplified through PCR according to Liu et al. (2025). The reaction mixture used for amplification consisted of 25 µl volume and a particular primer set. Two clear bands of 376 bp for AdeE and 587 bp for AdeY materialized in a 2% agarose gel containing the PCR products. The PCR products received proper preparation before being added to the electrophoresis gel to check for resistance markers (Liu et al., 2025).

Antibiotic Susceptibility Test

Research investigators started the incubation at 37ºC for 24 hours using nutrient broth to grow two to four colonies of bacterial isolates from burned wound samples. The bacterial cultures were made turbid by using a 0.5 McFarland standard tube for comparison after incubation. The investigators administered a standardized bacterial suspension throughout Mueller-Hinton agar (MHA) plates by using a sterile cotton swab evenly. A selection of antibiotic discs was applied through the use of sterilized forceps onto the agar surface. The plate surface received antibiotic discs of Tetracycline (Ti) at 75 µg and Rifampin (RA) at 30 µg as well as Levofloxacin (LE) at 5 µg and Gentamycin (Gen) at 10 µg along with Ciprofloxacin (Cip) at 30 µg and Ceftriaxone (CTR) at 5 µg and Ceftoexam (CTX) at 5 µg and Amikacin (AK) at 30 µg. The plate contained approximately 6 to 7 discs. The test plates stayed under 37ºC incubation conditions for twenty-four hours longer. Studies on antimicrobial susceptibility determined the size of inhibition zones using millimeter measurements which followed the interpretation standards set by National Committee for Clinical Laboratory Standards from 2015 (Montes-Robledo et al., 2024; Hurton et al., 2025).

Colonization Factor (hemagglutinating activity)

The investigators placed isolated bacterial colonies within BHI broth containers then let them incubate at 37º C for 24 hours. Research for red blood cells required detailed PBS washing of human blood to create a solution with 4% red blood cells. A lab test pipette dropped broth culture liquid into normal saline solution before adding separate drops of both human blood suspension and 0.1M D-mannose to a clean slides surface. A positive result appears through clot formation after placing the solution at 20º C for five minutes. (Motzer et al., 2025) (Asha et al.2024).

Gelatinase Assay

The assessment of gelatinase activity was conducted utilizing Luria Bertani agar enriched with gelatin. Initially, all isolates were cultivated on Brain Heart Infusion (BHI) agar plates for a period of 24 hours at a temperature of 37 degrees Celsius. After completing the initial growth phase, the isolates were subsequently transferred to LB agar for additional incubation. Following an overnight incubation at 37 degrees Celsius, the plates were then cooled for a duration of 5 hours at 4 degrees Celsius. Colonies that displayed a turbid appearance were interpreted as indicative of a positive result, as documented in the research of (Anand et al.2025)

Biofilm formation of *Acinetobacter*:

Brain Heart Infusion Broth received the inoculated specimen with single colony through a 24-hour incubation at 37˚C under controlled environment. Safranin solution was used to stain the inner tube walls after the growth cycle ended for all cultures. The solvent ethanol was used to break the wall stain attachment while dissolving the stain within one milliliter solution. A spectrophotometer determined biofilm production capability of isolated strains by measuring the optical density of the stain dissolved in ethanol. (Pallavi et al.2025) (Liu et al.2022).

Statistical analysis

The statistical testing using Kendall's tau_b was conducted through SPSS version 21 to identify significance between variables.

3. RESULTS AND DISCUSSION

The research adopted biochemical testing as well as advanced molecular methods to identify Acinetobacter spp. by concentrating on the essential detection and classification target which is the rpoB gene. Accurate Acinetobacter identification during the initial phase depended on inspecting unique bacterial colony structures in conjunction with both cell microscopic analysis and test outcome interpretations from biochemical experiments. The combination of microscopic cell observations and biochemical tests provided authentic and reliable identification of Acinetobacter genus within burn wound samples (de et al., 2024).

Multiple Acinetobacter strains failed to produce fermentation end products on MacConkey agar tests since they did not use lactose for breakdown. Helena Line Repeat then tested the microorganisms on blood agar base where the isolates were unable to perform

hemolysis further confirming their restricted metabolic capacity in carbohydrate metabolism and blood cell destruction mechanisms. The laboratory analyses confirmed that all Acinetobacter species display maximum growth at the normal pathogenic organism temperature of 37ºC. The growth superiority of A. baumannii at 44ºC offers researchers an analytical tool for specific laboratory identification of this bacterium in hospital burn infections. Acinetobacter pathogenicity and ecological behavior in burn-related infections gains more clarity from this essential data which serves clinical microbiology (Moon et al., 2025).

Acinetobacter displayed both positive and negative test results through biochemical analysis where it produced active catalase but showed no activity for oxidase or hydrogen sulfide. The evaluation for citrate metabolism with Simmons medium yielded inconsistent results which indicated poor consistency in citrate breakdown. The organism showed negative results in three essential biochemical tests named indole, methyl red, and Voges-Proskauer among other important tests for bacterial species identification (Subasri and Dharaneedharan, 2025).

Molecular Identification of Acinetobacter spp.

Between 300 positive urine culture cases examined for Follow-up Testing only 22 presentations of A. baumannii were Detected with a frequency of 7.3%. PCR testing protocols provided the basis for reaching this conclusion. (Raheem et al.2024)

The figure shown in (1) provides a complete breakdown of the amplification results and their connection to the rpoB gene. From a total of carefully analyzed samples only 22 isolates resulted in laboratory confirmation of Acinetobacter baumannii. The analysis depends on observing a 397 base pair PCR product to establish specific identification results as a central requirement. (Nguyen and Joshi, 2021)

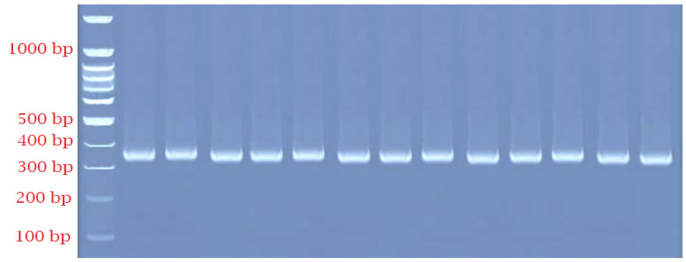

Figure 1. Agarose gel electrophoresis for Identification of *Acinetobacter baumannii* by amplification of *rpo*B gene by PCR (amplicon size 397 bp; electrophoresis carried out at 60V for 80 minutes). Line 1: Ladder DNA (100bp) Line 2 - 14: *A. baumannii* isolates.

Health concerns about A. baumannii have increased because this pathogen developed multidrug-resistance in recent years. This increasing trend has revealed the urgent need to develop both proper surveillance techniques and rapid pathogen identification methods for this concerning bacterial pathogen. Different healthcare settings depend on these actions as they ensure the success of infection control and prevention approaches. Epidemiological surveillance regarding outbreaks requires identifying A. baumannii independently from other Acinetobacter species which are not A. baumannii. Uses of the rpoB gene through PCR analysis serves as a standard diagnostic method to define Acinetobacter species which enhances both the detection precision and preparedness measures. (Li et al.2025).

The ten isolates under investigation showed complete biofilm-forming ability while producing variable biofilm amounts among them. The specific component known as colonization factor I proved resistant to mannose and was detected in 72% of the isolated samples. Throughout growth phases, a high number of 90% isolates produced gelatinase enzymes that required incubation times ranging from one day to 7 days to reach effective levels. The studied isolates display different traits through their adaptive methods of enzyme and biofilm production. (Flemming et al.2023)

The bacteria A. baumannii contains an extensive accumulation of virulence elements which destroy host tissues while simultaneously beating immune system defenses. The bacterium demonstrates three essential traits that enable its pathogenicity: outstanding cell binding abilities and protective polysaccharide capsules to block immune responses and damaging enzymes which break down soft tissues to intensify infection severity. The essential pathogenic factor of the bacterium is its endotoxin lipopolysaccharide from its membrane which significantly increases its disease-inducing capability. A biofilm's viscous nature blocks neutrophil movement thus promoting pathogenicity dramatically when A. baumannii infects with other pathogens. Biofilms represent an important virulence trait in A. baumannii that allows this microorganism to stay present in hospital environments leading to rapid infection spread. The bacteria survive exceptionally well on multiple surfaces including finger tips of humans and various plastics together with dry objects that are commonly disregarded by healthcare. Biofilms developed by the bacterium protect it from drying out and cleaning procedures which allows survival in critical healthcare facilities. A. baumannii builds protective films that enable the bacterium to connect with vulnerable medical patients who have weakened immune systems thus precipitating extensive outbreaks of infections affecting medical devices such as catheters and causing ventilator-related pneumonia. The competence of A. baumannii to develop biofilms on both living tissues and non-living

surfaces greatly improves its capacity to survive under difficult environmental situations including medical devices and hospital equipment as well as other suboptimal conditions. A. baumannii displays remarkable adaptive capabilities that make it a substantial infection risk within healthcare zones where it finds suitable conditions for survival (Lucidi et al.2024).

Table 1. Virulence factor of A. baumannii

No of isolates	gelatinase	Mannose resistance	biofilm production
1	+	-	0.14
2	+	-	0.284
3	+	+	0.98
4	+	-	0.2
5	+	-	0.177
6	+	+	0.197
7	+	+	0.289
8	-	-	0.123
9	+	-	0.752
10	+	-	0.254
11	+	+	0.426
12	+	-	0.341
13	+	+	0.109
14	+	-	0.166
15	+	-	0.141
16	+	-	0.093
17	-	-	0.18
18	+	-	0.152
19	+	-	0.184
20	+	-	0.206
21	+	+	0.33
22	+	-	0.221

: negative result - **+: positive result**

Antibiotic Sensitivity Test:

Research identified the antibiotic resistance pattern of 22 Acinetobacter baumannii isolates using the disc diffusion method and eight antibiotic agents. Before 1966 Bauer and Kirby developed the antibiotic susceptibility assessment technique which became known as the disc diffusion method. (Saikia et al.2024)

The figure (2) displays the results on antibiotic resistance discovered in the study. The treatment challenge becomes evident when a substantial 96% of examined isolates show resistance against Rifampin. A high resistance rate was recorded for Tetracycline because

92% of examined isolates no longer responded to the antibiotic treatment. New treatment approaches need immediate development because Cephotaxime and Ceftriaxone join Rifampin in demonstrating high resistance levels. The resistance rates for these antibiotics stood at 73% and 81%, respectively. Resistances to antibiotics were minimal when using Gatifloxacin since 27% of bacteria demonstrated resistance and Levofloxacin yielded a 31% resistance rate suggesting these drugs could potentially treat selected cases of infection. Current antibiotic research needs support from detailed data analysis about the efficacy of different bacterial isolates. (Guan et al.2024)

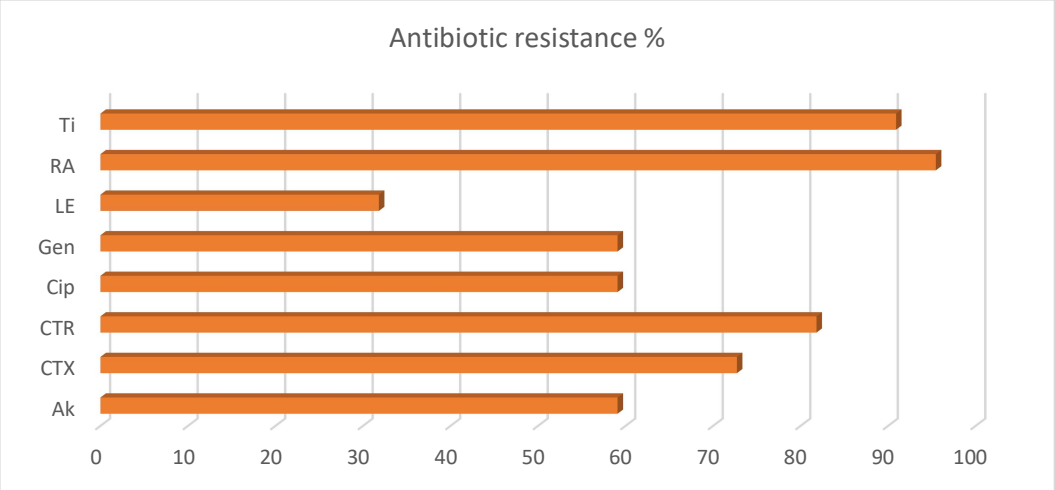

Figure 2. The percentage of antibiotic resistance, Tetracycline Ti 75 µg Rifampin RA30 µg Levofloxacin LE 5 µg Gentamycin Gen 10 µg Ciprofloxacin Cip 30 µg Ceftriaxone CTR 5 µg, Ceftoexam CTX 5 µg Amikacine (AK) 30 µg.

The molecular study employed PCR technology to boost gene amplification of AdeE and AdeY through a sequence of experiments on 22 different Acinetobacter baumannii isolates. The examined figures (3 and 4) demonstrate that isolates (6, 11, 12, 22) displayed successful AdeE and AdeY gene amplification resulting in the production of specific amplicons measuring 370 bp and 587 bp. A different observation revealed that isolates numbered (2, 6, 9, 11, 12, 15, 22) contained only the AdeE gene along with no detectable presence of AdeY. The AdeY gene was solely expressed by isolates numbered (3, 6, 8, 11, 12, 14, 18, 19, 22) among the microbial isolates. Evaluation showed that AdeE and AdeY gene amplification remained absent in all remaining bacteria strains which implied some bacteria lacking these critical genes entirely. (Zack et al., 2024)

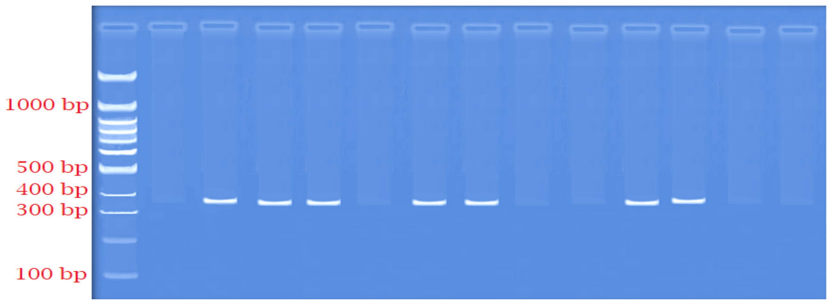

Figure 3. Agarose gel electrophoresis for detection of AdeE gene in Acinetobacter baumannii by PCR (amplicon size 370 bp; electrophoresis carried out at 60V for 80 minutes).

Line 1: Ladder DNA (100bp)

Line 3,4,5,7,8,11,12: isolates No. 2,6,9,11,12,15,22 of Acinetobacter baumannii with positive result respectively.

Line 2,6,9,10,13,14: negative results of amplification

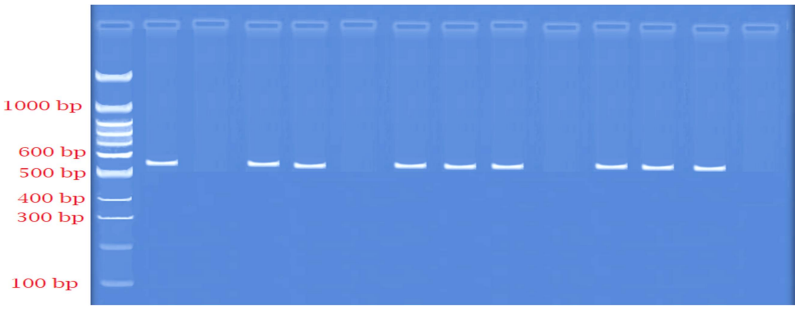

Figure 4. Agarose gel electrophoresis for detection of AdeY gene in A. baumannii by PCR (amplicon size 587 bp; electrophoresis carried out at 60V for 80 minutes).

Line 1: Ladder DNA (100bp)

Line 2,4,5,7,8,9,11,12,13: isolates No. 3,6,8,11,12,14,18,19,22 of Acinetobacter baumannii with positive result

Line 3,6, 10,14: negative results of amplification

An analytical and detailed examination comparing traditional and conventional methods with advanced molecular techniques (as illustrated in the corresponding table 2) indicated that isolates exhibiting notable resistance to several antibiotics such as Ti, Cip, Gen, CTR, AK, LE, GAT, RA, and CTX contained either the AdeY or AdeE gene. In contrast, the isolates that demonstrated resistance specifically to antibiotics such as RA, Cip, and Ti possessed only the AdeE gene. Furthermore, the presence of the AdeY gene was identified and confirmed in isolates that were resistant to RA, Cip, and CTX, highlighting the complex

genetic relationships underlying antibiotic resistance mechanisms. This distinction in genetic markers between various resistance profiles emphasizes the necessity for precise molecular diagnostics to accurately identify and characterize these resistant strains. (Zack et al., 2024) The findings of the detailed statistical analysis indicate that there is no noteworthy difference or significant correlation between the levels of bacterial resistance to the specified antibiotics in the present study and the existence of Ade Y and E in the bacterial samples observed. (Esmaeili et al., 2025)

Table 2. Compression between conventional and molecular technique.

	Ak	CTX	CTR	Cip	Gen	LE	RA	Ti	AdeE	AdeY
1	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**		
2	**R**	**R**	**R**	**R**	**S**	**S**	**R**	**R**	+	
3	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**		+
4	**R**	**S**	**R**	**R**	**S**	**S**	**R**	**R**		
5	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**S**		
6	**R**	**R**	**R**	**S**	**S**	**S**	**R**	**R**	+	+
7	**S**	**R**	**S**	**S**	**R**	**S**	**R**	**R**		
8	**S**	**R**	**R**	**R**	**R**	**S**	**R**	**R**		+
9	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	+	
10	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**		
11	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	+	+
12	**R**	**S**	**R**	**R**	**R**	**S**	**R**	**R**	+	+
13	**R**	**R**	**R**	**R**	**R**	**S**	**R**	**S**		
14	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**		+
15	**S**	**S**	**S**	**S**	**S**	**S**	**R**	**R**	+	
16	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**		
17	**S**	**R**	**R**	**S**	**S**	**S**	**S**	**R**		
18	**S**	**R**	**R**	**S**	**S**	**S**	**R**	**R**		+
19	**R**	**R**	**R**	**S**	**R**	**S**	**R**	**R**		+
20	**S**	**R**	**R**	**R**	**R**	**S**	**R**	**R**		
21	**S**	**S**	**S**	**R**	**S**	**S**	**R**	**R**		
22	**R**	**S**	**R**	**R**	**R**	**S**	**R**	**R**	+	+

Resistant microbial populations employ four main resistance mechanisms including active enzyme production to destroy broad-range antimicrobials and modification of their target sites and decreased membrane permeability as well as biofilm formation and increased membrane-active efflux system activity [Gordon, 2010]. Several crucial enzymes identify antimicrobials for destruction which leads to medication degradation and multiple strains develop resistance to different drugs. The specified substrates processed by inactive enzymes

display high target affinity which indicates their anti-efficiency relies on matching targets. The enzyme -lactamases activity leads to -lactam antibiotic inactivation while other specific resistance enzymes deactivate aminoglycosides [Lee et al., 2017]. Active efflux pumps exhibit broad-reaching distribution in microbial populations since they possess an extensive substrate range which leads to widespread drug resistance diversity [Peleg et al., 2008]. The research by He et al. [2015] demonstrates that A. baumannii biofilm formation possibly involves genes which control efflux pump activity. The existence of multi-drug resistant Acinetobacter baumannii remains evident even though minocycline and tigecycline target these strains effectively since A. baumannii avoids treatment response. The mutations causing efflux pump overexpression serve as the main reason behind antibiotic resistance in microorganisms. (Huang et al.2022)

Table 3. Chi-square/Fisher test results for each antibiotic based on the presence of adeE and adeY.

Antibiotic	AdeE p-value	AdeY p-value
Ak	0.16	0.20
CTX	0.33	1
CTR	1	0.11
Cip	0.64	1
Gen	0.37	0.20
LE	1	1
RA	1	1
Ti	1	0.49

t-test results comparing the average number of antibiotic resistances are:

- **AdeE:** No significant difference (p = 0.733)
- **AdeY:** No significant difference (p = 0.152)

The statistical analysis was designed to explore whether the adeE and adeY genes are associated with antibiotic resistance in *Acinetobacter baumannii* isolates. For the Chi-square / Fisher's Exact Test Results These tests evaluated the relationship between gene presence and resistance to individual antibiotics. Across all 8 antibiotics, the p-values for both adeE and adeY were above 0.05, indicating no statistically significant associations between gene presence and resistance to any specific antibiotic. This suggests that the presence or absence of either gene does not independently predict resistance to any particular drug tested in this study. The t-test assessed whether the average number of

resistances (out of 8 antibiotics) differed significantly between gene-positive and gene-negative groups. The AdeE difference in resistance count was negligible ($p = 0.733$), showing no evidence of increased resistance in strains carrying adeE.AdeY: There was a trend toward higher resistance in adeY-positive isolates, but it was not statistically significant ($p = 0.152$).

4. CONCLUSION

This study demonstrates that while Acinetobacter baumannii isolates exhibited high levels of multidrug resistance, no significant correlation was found between the presence of the adeE and adeY efflux pump genes and the observed resistance profiles. These findings indicate that other mechanisms, such as alternative efflux systems or enzymatic degradation, may play a more substantial role in antibiotic resistance in these clinical isolates. Further research is needed to identify these mechanisms and to determine their clinical impact.

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