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ORIGINAL ARTICLE

Identification of Efflux Pumps Genes (adeB, adeJ, and adeG) in Acinetobacter Baumannii Resistant to Tigecycline

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ABSTRACT

Background: Acinetobacter baumannii is an opportunistic bacterium commonly associated with multidrug-resistant infections. One of the major therapeutic challenges is tigecycline resistance in clinical isolates. This study aimed to identify the presence of efflux pump genes) adeB, adeJ, and adeG) in tigecycline-resistant A. baumannii, to support efforts in controlling antibiotic resistance.

Methods: A cross-sectional study was conducted on 44 patients from Intensive Care Unit and Surgery Departments of Zagazig University Hospitals at the Clinical Pathology Department, Zagazig University Hospitals. The presence of adeB, adeJ, and adeG efflux pump genes was assessed, and antimicrobial susceptibility testing was performed.

Results: Among the 44 patients, the mean age was 55.3±16.86 years, with 68.2% males. ICU patients represented 68.2%, and median hospital stay was 16 days. Tigecycline resistance was observed in 54.5% of isolates, with resistance also noted for tetracycline (56.8%), ticarcillin/clavulanate (93.2%), piperacillin (95.5%), levofloxacin (93.2%), ceftriaxone (95.5%), cefepime (95.5%), and meropenem (93.2%). Significant risk factors for tigecycline resistance included prior antibiotic use, chronic lung disease, mechanical ventilation, ICU admission, prolonged hospitalization, and the presence of adeB and combined (adeB+adeJ) efflux pump genes ($p<0.05$). Tigecycline resistance was significantly associated with decreased susceptibility to tetracycline and minocycline. Multivariate analysis identified adeB positivity, combined (adeB+adeJ), prior antibiotic use, ICU admission, mechanical ventilation, and prolonged hospitalization as independent predictors of resistance.

Conclusion: Tigecycline-resistant A. baumannii is prevalent among ICU patients, particularly those with prior antibiotic exposure and invasive procedures. Efflux pump genes, especially adeB and ombined (adeB+ade), play a central role in mediating resistance. Continuous surveillance, antimicrobial stewardship, and infection control interventions are essential to limit the spread of resistant strains and optimize clinical outcomes.

Keywords: Acinetobacter baumannii; Tigecycline resistance; Efflux pumps; AdeB

INTRODUCTION

Gram-negative, aerobic, non-motile, non-fermentative, oxidase-negative, and catalase-positive, Acinetobacter baumannii (A. baumannii) is a coccobacillus [1]. It is an opportunistic pathogen that causes a range of hospital- and community-acquired illnesses, accounting for 2–10% of all Gram-negative hospital infections [2]. Acinetobacter infections are caused by several risk factors, including recent surgery, prolonged hospitalization, admission to the critical care unit, mechanical ventilation, and previous exposure to antibiotics.

In recent years, tigecycline resistance in A. baumannii has been reported worldwide with prevalence ranging up to 53.0 % in certain clinical reports. A. baumannii is implicated in serious nosocomial infections and is becoming increasingly multidrug resistant

(MDR). According to international guidelines research, multidrug-resistant Acinetobacter baumannii was defined as an isolate resistant to at least three different classes of antimicrobial agents, including carbapenems, cephalosporins, fluoroquinolones, and aminoglycosides. One of the main reasons hospital-acquired illnesses do not respond to therapy is multidrug resistance. Numerous antibiotic-resistant genes are intrinsically present in the bacteria [3]. Biofilm development, enzymatic antibiotic inactivation, reduced outer membrane permeability, and efflux pump overexpression are among its primary resistance mechanisms [4]. Because of their wide substrate specificity and cooperative relationships with other resistance mechanisms, multi-drug efflux pumps are among those that contribute significantly to the establishment of MDR strains [5].

One of the few available treatments for MDR A. baumannii infections is tigecycline, the first drug of the glycylcycline class [6]. Nevertheless, there have been reports of growing tigecycline resistance, which has been attributed to efflux pumps like adeB, adeG, and adeJ [7]. Tigecycline resistance in A. baumannii has been associated with overexpression of these resistance-nodulation-division (RND) efflux pumps [8]. Membrane-associated protein complexes called efflux pumps eliminate hazardous chemicals, including antibiotics, and metabolic waste products, lowering intracellular medication concentrations [9].

A periplasmic membrane fusion protein (MFP), an outer membrane pore protein (OMP), and a cytoplasmic membrane transporter work together to enable the passage of medications over the bacterial membrane, making the RND-type efflux system clinically significant. The creation and application of efflux pump inhibitors, which increase bacterial sensitivity to antibiotics, is essential for managing resistance considering the rising incidence of MDR A. baumannii [10]. This study aimed to identify the presence of efflux pump genes) adeB, adeJ, and adeG) in tigecycline-resistant A. baumannii, to support efforts in controlling antibiotic resistance.

METHODS

This cross-sectional study was carried out between April 2024 and April 2025 at the Zagazig University Hospitals' Clinical Pathology, Intensive Care Unit (ICU), and Surgery Departments. The Zagazig Medical Research Ethical Committee accepted the study protocol (IRB No. ZU-IRB #86/11-2-2024), and each patient or their first-degree relatives provided written informed permission. Every procedure was carried out in compliance with the ethical guidelines for research with human beings provided in the Declaration of Helsinki by the World Medical Association.

From a variety of clinical materials, such as sputum, bronchoalveolar lavage (BAL), urine, pus, blood, and peritoneal fluid, 44 non-duplicate clinical isolates of Acinetobacter baumannii were found. Twenty (45%) of these isolates were tigecycline-sensitive, and twenty (55%) were tigecycline-resistant. Age, sex, history of antibiotic exposure, underlying diseases such as diabetes mellitus, hypertension, chronic lung disease, and mechanical ventilation use, as well as the hospital unit of admission and surgical drainage history, were among the clinical and demographic information gathered about the patients.

Adult patients between the ages of 20 and 80 who gave written informed consent and whose culture samples were positive for Acinetobacter baumannii met the inclusion criteria. Patients undergoing antibiotic therapy at the time of sample collection, those whose culture samples tested positive for bacteria other than A. baumannii, and those who declined to take part in the study were all excluded.

Identification of Bacterial Isolates

An automated system and standard microbiological methods were used to identify every clinical isolate of Acinetobacter baumannii. The isolates were first cultivated on Blood agar and MacConkey agar and analyzed under a microscope using Gram staining. The VITEK® 2 COMPACT SYSTEM with GN ID cards (BioMérieux, Marcy L'Etoile, France) was then used to validate the identity and only isolates showing

green (fully consistent) results in the system were confirmed as A. baumannii.

Antimicrobial Susceptibility Testing (AST)

The VITEK® 2 COMPACT SYSTEM was used for antimicrobial susceptibility testing. After being suspended in 3 milliliters of 0.45% saline, fresh colonies were further diluted for loading into AST GN-XN05 cards and adjusted to 0.5 McFarland using DensiCHEKTM plus. Tigecycline, Tetracycline, Colistin, Minocycline, Ticarcillin/clavulanate, Piperacillin, Levofloxacin, Ceftriaxone, Cefepime, and Meropenem were among the antibiotics on the panel. AST performance was validated by using quality control strain **(**Pseudomonas aeruginosa ATCC 27853**)**.

Cascades were loaded into the loading and filling stations, and patient and specimen data were input into the device. The VITEK® 2 Advanced Expert SystemTM (AES), which employs color-coded indicators to reflect confidence levels—green is entirely consistent, yellow is consistent with correction, red is unknown phenotype, check results, and purple is phenotypic not in database—was used to interpret susceptibility results.

Molecular Detection of Efflux Pump Genes by Multiplex PCR

The efflux pump genes (adeB, adeJ, and adeG) were molecularly detected using multiplex PCR, a commonly used technique for determining the genes responsible for bacterial virulence and resistance. In accordance with the manufacturer's instructions, genomic DNA was extracted from overnight cultures of A. baumannii isolates using the i-genomic BYF DNA Extraction Mini Kit (Lot No. 13010348). This kit makes it possible to quickly purify high-quality DNA while eliminating all impurities and inhibitors. RNase A, Proteinase K, lysozyme, spin columns, collection tubes, pre-lysis buffer (MP), lysis buffer (MG), binding buffer (MB), washing buffer (MW), and elution buffer (ME) were among the kit's ingredients. Ethanol (96–100%), Tris-EDTA buffer (1x), pipette tips with aerosol barrier, a microcentrifuge with rotor for 2 mL tubes, a vortex mixer, water bath or heating block at 56°C, and phosphate-buffered saline (PBS) when needed were additional reagents and equipment needed.

DNA extraction works based on bacterial cell lysis to liberate genomic DNA, protein removal with protease treatment, purification with ethanol in spin columns, and elution in an appropriate buffer for PCR applications later [11]. To summarize, 7–10 medium-sized bacterial colonies were placed in 2 mL tubes filled with Tris-EDTA buffer, centrifuged for 1 minute at 13,000 rpm to pellet them, and then resuspended in 200 µL of leftover supernatant. After adding 300 µL of buffer MG, 20 µL of proteinase K, and 5 µL of RNase A, lysis was accomplished by incubating for 15 minutes at 56°C. After that, 250 µL of buffer MB and 250 µL of 80% ethanol were added to the lysate, and the mixture was sent to spin columns for centrifugation. After being cleaned with 700 µL of buffer MW, the columns were further centrifuged to dry them. Finally, 50–100 µL of buffer ME was used to elute the genomic DNA The purity and concentration of the extracted DNA were assessed using a spectrophotometer (NanoDrop, Thermo Scientific, USA) at 260/280 nm ratio before PCR amplification to ensure DNA quality and integrity., which was then kept at -20°C until PCR analysis.

DNA Amplification:

Biokit (Willowfort Co., UK) provided lyophilized primers for the universal bacterial 16S rRNA gene and the efflux pump genes (adeB, adeJ, and adeG). To create stock solutions, they were reconstituted using nuclease-free water in accordance with the manufacturer's recommendations. These were then aliquoted to prevent frequent thawing and freezing, and they were kept at -20°C until they were needed.

Each primer tube received the following amounts of distilled water for reconstitution:

adeB: forward 318 µL, reverse 260 µL

adeJ: forward 331 µL, reverse 238 µL

adeG: forward 310 µL, reverse 270 µL

16S rRNA: forward 382 µL, reverse 233 µL

Working solutions were prepared by performing a 1:15 dilution of each primer (15 µL primer + 85 µL nuclease-free water) before use in PCR reactions Table 1. The final

working concentration of each primer in the reaction mixture was 0.2 µM.

A 30µL reaction mixture including 7µL of genomic DNA, 2µL of each forward and reverse primer solution, 15µL of 2X PCR master mix (TOPsimple™ DyeMIX-nTaq), and 4µL of distilled water was used for multiplex PCR amplification of the adeB, adeJ, and adeG efflux pump genes. To guarantee the quality of amplification, 16S rRNA, a universal bacterial gene, was used as an internal check in every PCR experiment.

A thermal cycler (Applied Biosystems) (Thermo Fisher Scientific, USA) was used to perform PCR amplification. The conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing at 50°C for 35 seconds, and extension at 72°C for 35 seconds, with a final extension step at 72°C for 10 minutes.

Using agarose gel electrophoresis, both isolated genomic DNA and PCR-amplified products were detected. Each 6 µL DNA sample was treated with 3 µL of loading dye before the PCR products were separated on a 2% agarose gel. As a molecular weight marker, SizerTM TM-100 Plus DNA Marker Solution (iNtRON, Cat No: 24073) was injected into a specific well for size reference. A trans-illuminator was used to view the amplified DNA bands to verify that the target efflux pump genes were present.

Statistical Analysis

Statistical Package for Social Sciences (SPSS) version 27 was used to analyze the data. The chi square test and, when applicable, the Fisher exact test was used to compare categorical variables, which were presented using their absolute frequencies. The chi square trend test was used to examine ordinal data between two groups. Assumptions for use in parametric testing were validated using the Shapiro-Wilk test. Depending on the type of data, the means, standard deviations (SD), median, and interquartile range were used to characterize quantitative variables. The independent sample t test (for regularly distributed data) and the Mann Whitney test (for non-normally distributed data) were employed to compare quantitative data between two groups. To find independent risk factors linked to specific health issues, binary logistic regression was employed. $P<0.05$ was chosen as the threshold for statistical significance. There was a highly significant difference if $p\leq0.001$.

RESULTS

The study comprised 44 patients, whose mean age was 55.3±16.86 years, with ages ranging from 19 to 76. While women made up 31.8% of the patients, men made up most of the study population (68.2%). Sputum samples were the most collected clinical specimen type (47.7%), followed by bronchoalveolar lavage (BAL) samples (27.3%). Samples of urine, pus, blood, and peritoneal fluid made up 11.4%, 6.8%, 4.5%, and 2.3% of the total. The intensive care unit (ICU) accounted for 68.2% of all patient admissions, followed by medical wards (20.4%) and surgical wards (11.4%). The median length of hospital stay was 16 days, with a range of 3 to 75 days; 86.4% of patients stayed for 7 days or more, while 13.6% of patients stayed for fewer than 7 days. Prior use of antibiotics was the most common risk factor for infection (65.9%), followed by diabetes mellitus (47.7%), chronic lung illness (52.3%), hypertension (54.5%), mechanical ventilation (40.9%), and surgical drainage (27.3%) (Table 2).

This Table 3 showed that specifically, 54.5% of isolates were resistant to tigecycline, while 45.5% were sensitive. Tetracycline resistance was observed in 56.8% of isolates, with 36.4% sensitive and 6.8% intermediate. Colistin remained the most effective agent, with 93.2% of isolates susceptible. Minocycline demonstrated 75% sensitivity, 11.4% intermediate resistance, and 13.6% resistance. High resistance rates were observed for ticarcillin/clavulanate (93.2%), piperacillin (95.5%), levofloxacin (93.2%), ceftriaxone (95.5%), cefepime (95.5%), and meropenem (93.2%).

This Table 4 showed that thirty-two of total isolates (72.7%) carried adeB (Figure 1) and 65.9% of total isolates carried adeJ (Figure 2) and all had no adeG. Combined (adeB+adeJ) occurred in 43.2% of total A. baumannii isolates.

Table 5 showed that there was statistically significant relation between presence of Tigecycline-resistant strains and adeB (91.7%

of isolates carried adeB versus 50% of Tigecycline-sensitive strains). There was statistically non-significant relation between presence of Tigecycline-resistant strains and presence of adeJ gene. There was statistically significant relation between presence of Tigecycline-resistant strains and combined adeB and adeJ (62.5% of Tigecycline-resistant strains had combined (adeB+adeJ) versus 20% of Tigecycline-sensitive strains).

Table 6 showed that there was statistically significant relation between susceptibility to Tigecycline and tetracycline, and minocycline. There was statistically non-significant relation between susceptibility to Tigecycline and colistin, minocycline, Ticarcillin/clavulanate**,** Piperacillin, Levofloxacin, ceftriaxone, Cefepime and Meronym.

Positive adeB, combined (adeB+adeJ), prior use of antibiotics, Mechanical ventilation, ICU admission, high Length of hospital stay increased risk of infection with Tigecycline resistant strains by 1.541, 7.861, 18.187, 1.61, 8.805, and 1.098 folds respectively as shown in Table 7.

Table (1): Primers used in Multiplex polymerase chain reaction (PCR) in this study [12].

Target genes	Primer sequence (5′–3′)	Product Size (bp)
adeB	**F: ATTTGGATTGCTGAGCATTC** **R: GTAAACCTTGCTGACGTACA**	**340 bp**
adeJ	**F: GGTCATTAATATCTTTGGC** **R: GGTACGAATACCGCTGTCA**	**221 bp**
adeG	**F: TTCATCTAGCCAAGCAGAAG** **R: GTGTAGTGCCACTGGTTACT**	**468 bp**
16S rRNA	**F: GACGTACTCGCAGAATAAGC** **R: TTAGTCTTGCGACCGTACTC**	**426 bp**

Table 2: Demographic characteristics, specimen types, admission site, risk factors (N=44)

	N=44	%
Gender		
Female	**14**	**31.8%**
Male	**30**	**68.2%**
	Mean ± SD	**Range**
Age (year)	**55.3 ± 16.86**	**19 – 76**
Specimen		
Sputum	**21**	**47.7%**
BAL	**12**	**27.3%**
Urine	**5**	**11.4%**
Pus	**3**	**6.8%**
Blood	**2**	**4.5%**
Peritoneal fluid	**1**	**2.3%**
Admission site		
ICU	**30**	**68.2%**
Medical ward	**9**	**20.4%**
Surgical ward	**5**	**11.4%**
	Median (IQR)	**Range**
Length of hospital stay (day)	**16(8 – 35)**	**3 – 75**
<7 days	**6**	**13.6%**
≥7 days	**38**	**86.4%**
Risk factors		
Prior use of antibiotics	**29**	**65.9%**
Hypertension	**24**	**54.5%**

	N=44	%
Chronic lung disease	**23**	**52.3%**
Diabetes mellitus	**21**	**47.7%**
Mechanical ventilation	**18**	**40.9%**
Surgical drainage	**12**	**27.3%**

Table (3): Antimicrobials susceptibility pattern of Acinetobacter baumannii isolates used in the study

	Sensitive		Intermediate		Resistant	
	n	%	n	%	n	%
Tigecycline	**20**	**45.5%**	**0**	**0%**	**24**	**54.5%**
Tetracycline	**16**	**36.4%**	**3**	**6.8%**	**25**	**56.8%**
Colistin	**41**	**93.2%**	**0**	**0%**	**3**	**6.8%**
Minocycline	**33**	**75%**	**5**	**11.4%**	**6**	**13.6%**
Ticarcillin/clavulanate	**1**	**2.3%**	**2**	**4.5%**	**41**	**93.2%**
Piperacillin	**1**	**2.3%**	**1**	**2.3%**	**42**	**95.5%**
Levofloxacillin	**2**	**4.5%**	**1**	**2.3%**	**41**	**93.2%**
Ceftriaxone	**0**	**0%**	**2**	**4.5%**	**42**	**95.5%**
Cefepime	**2**	**4.5%**	**0**	**0%**	**42**	**95.5%**
Meropenem	**2**	**4.55**	**1**	**2.3%**	**41**	**93.2%**

Table (4): Distribution of efflux pumps genes adeB, adeJ, adeG and Combined (adeB + adeJ) among total Acinetobacter baumannii isolates

	N=44	%
adeB		
Negative	**12**	**27.3%**
Positive	**32**	**72.7%**
adeJ		
Negative	**15**	**34.1%**
Positive	**29**	**65.9%**
adeG		
Negative	**44**	**100%**
Positive	**0**	**0%**
Combined (adeB + adeJ)	**19**	**43.2%**

Table (5): Distribution of efflux pump genes, combined, adeB, adeJ among Tigecycline sensitive and Tigecycline resistant Acinetobacter baumannii isolates

	Tigecycline-sensitive n=20 (45%)	Tigecycline-resistant n=24 (55%)	χ^2	p
adeB				
Negative	**10 (50%)**	**2 (8.3%)**	**9.549**	**0.002***
Positive	**10 (50%)**	**22 (91.7%)**		
adeJ				
Negative	**8 (40%)**	**7 (29.2%)**	**0.57**	**0.45**
Positive	**12 (60%)**	**17 (70.8%)**		
Combined (adeB + adeJ)				
Negative	**16 (80%)**	**9 (37.5%)**	**8.031**	**0.005***
Positive	**4 (20%)**	**15 (62.5%)**		

*p<0.05 is statistically significant, χ^2: Chi square test, **p≤0.001 is statistically highly significant.

Table (6): Relation between Susceptibility to Tigecycline and different antimicrobials susceptibility

	Tigecycline-sensitive n=20 (%)	Tigecycline-resistant n=24 (%)	χ^2	p
Tetracycline				
Sensitive	**16 (80%)**	**0 (0%)**	**36.686¥**	**<0.001****
Intermediate	**3 (15%)**	**0 (0%)**		
Resistant	**1 (5%)**	**24 (100%)**		
Colistin				
Sensitive	**20 (100%)**	**21 (87.5%)**	**Fisher**	**0.239**
Resistant	**0 (0%)**	**3 (12.5%)**		
Minocycline				
Sensitive	**20 (100%)**	**13 (54.2%)**	**10.492¥**	**0.001****
Intermediate	**0 (0%)**	**5 (20.8%)**		
Resistant	**0 (0%)**	**6 (25%)**		
Ticarcillin/clavulanate				
Sensitive	**1 (5%)**	**0 (0%)**	**3.329¥**	**0.068**
Intermediate	**2 (10%)**	**0 (0%)**		
Resistant	**17 (85%)**	**24 (100%)**		
Piperacillin				
Sensitive	**0 (0%)**	**1 (4.2%)**	**0.333¥**	**0.564**
Intermediate	**1 (5%)**	**0 (0%)**		
Resistant	**19 (95%)**	**23 (95.8%)**		
Levofloxacin				
Sensitive	**1 (5%)**	**1 (4.2%)**	**0.035¥**	**0.852**
Intermediate	**0 (0%)**	**1 (4.2%)**		
Resistant	**19 (95%)**	**22 (91.6%)**		
Ceftriaxone				
Intermediate	**1 (5%)**	**1 (4.2%)**	**Fisher**	**>0.999**
Resistant	**19 (95%)**	**23 (95.8%)**		
Cefepime				
Sensitive	**1 (5%)**	**1 (4.2%)**	**Fisher**	**>0.999**
Resistant	**19 (95%)**	**23 (95.8%)**		
Meropenem				
Sensitive	**1 (5%)**	**1 (4.2%)**	**0.035¥**	**0.852**
Intermediate	**0 (0%)**	**1 (4.2%)**		
Resistant	**19 (95%)**	**22 (91.7%)**		

¥Chi square for trend test, χ^2: Chi square test, *p<0.05 is statistically significant, χ^2: Chi square test **p≤0.001 is statistically highly significant.

Table (7): Multivariate regression analysis of factors associated with Tigecycline resistant strains

	β	p	AOR	95% CI	
				Lower	Upper
ade B positive	**-0.112**	**0.045***	**0.894**	**0.0367**	**21.789**
Combined (adeB + adeJ)	**2.452**	**0.055**	**11.613**	**0.954**	**141.43**
Prior use of antibiotics	**2.901**	**0.012***	**18.187**	**1.887**	**175.278**
ICU	**2.185**	**0.188**	**8.892**	**0.344**	**230**
Mechanical ventilation	**0.744**	**0.495**	**2.105**	**0.248**	**17.886**
Length of hospital stay	**0.084**	**0.023***	**1.088**	**1.012**	**1.17**
Chronic lung disease	**-0.462**	**0.67**	**0.63**	**0.075**	**5.275**
Prior use of antibiotics	**2.913**	**0.045***	**18.412**	**1.064**	**318.53**

AOR: adjusted odds ratio, CI: Confidence interval, *p<0.05 is statistically significant.

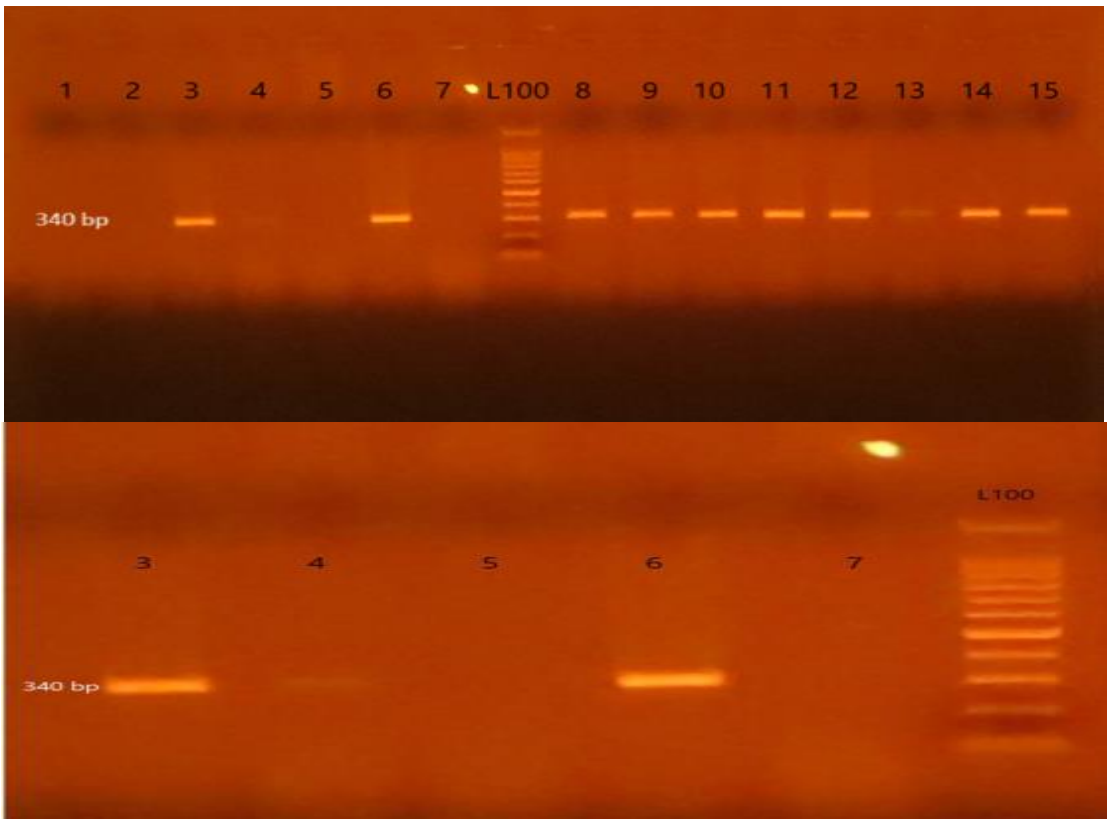

Figure (1): Gel electrophoresis for detection of PCR multiplex with adeB genes primers. DNA molecular size marker (100 bp ladder), Lanes (3-4-6) and (from 8 to 15) show positive results at 340 bp, while Lane (1-2-5-7) show negative results.

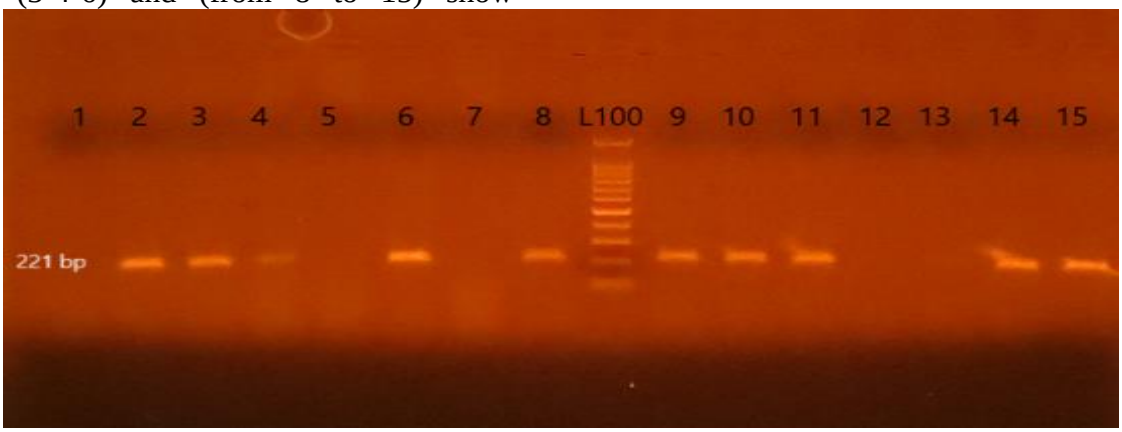

Figure (2): Gel electrophoresis for detection of PCR multiplex with adeJ genes primers. DNA molecular size marker (100 bp ladder), Lanes (2-3-4-6-8) and (9-10-11-14-15) show positive results at 221 bp, while Lanes (1-5-7-12-13) show negative results.

DISCUSSION

This cross-sectional study was conducted at clinical pathology Department, Faculty of Medicine, Zagazig University Hospitals on 44 patients (mean age: 55.3 years; range: 19-76), with 68.2% males and 31.8% females**.**

This study provides a unique contribution by exploring the association between adeB and adeJ efflux pump genes and tigecycline resistance in A. baumannii clinical isolates, the results provide clinically relevant insights that can support infection control and guide antibiotic stewardship programs in hospitals.

To our knowledge, this is one of the few studies from Egypt addressing this mechanism. Our study can be supported by a retrospective study by Shin et al. [13] involving 27 patients treated with tigecycline for multidrug-resistant A. baumannii

infections reported a mean age of 66.2 years and 74.1% male patients.

Our study analyzed various specimen types, with sputum (47.7%) and BAL (27.3%) being most common, followed by urine (11.4%), pus (6.8%), blood (4.5%), and peritoneal fluid (2.3%).

Hospital admission data showed that 68.2% of patients were admitted to ICUs, 20.4% to medical wards, and 11.4% to surgical wards, with a median hospital stay of 16 days (range 3–75). The significant association between tigecycline resistance, ICU admission, and prolonged hospitalization ($p<0.05$) supports findings by Spiliopoulou et al. [14] and Park et al. [15], indicating that ICU stay and longer hospital exposure are critical risk factors for resistant A. baumannii.

Patient risk factors included prior antibiotic use (65.9%), hypertension (54.5%), chronic lung disease (52.3%), diabetes mellitus (47.7%), mechanical ventilation (40.9%), and surgical drainage (27.3%). This is consistent with Barbier et al. [16], who identified prior broad-spectrum antibiotic exposure as a risk factor for multidrug-resistant A. baumannii.

Antimicrobial susceptibility analysis revealed high resistance rates: tigecycline (54.5%), tetracycline (56.8%), and very high resistance to ticarcillin/clavulanate (93.2%), piperacillin (95.5%), levofloxacin (93.2%), ceftriaxone (95.5%), cefepime (95.5%), and meropenem (93.2%). These findings are in line with Maraki et al. [17] and Jin et al. [18], who reported increasing resistance trends over time in A. baumannii isolates.

Efflux pump gene analysis revealed that 72.7% of isolates carried adeB, 65.9% carried adeJ, none carried adeG, and 43.2% carried both adeB and adeJ Tigecycline resistance was significantly associated with adeB positivity (91.7% in resistant vs. 50% in sensitive strains) and combined (adeB+adeJ) (62.5% vs 20%). adeJ alone showed no significant association (70.8% tigecycline resistance vs 60% tigecycline sensitive). These results correspond to ALTabbakh et al. [12], who reported similar patterns, emphasizing the central role of the adeABC efflux pump in mediating tigecycline resistance. Mechanistically, adeB encodes the main efflux transporter that expels tigecycline from the bacterial cell, while adeJ may potentiate this action when co-expressed, leading to higher resistance levels [19-21].

Co-susceptibility analysis showed significant correlations between tigecycline susceptibility and tetracycline and minocycline ($p<0.05$), whereas no significant associations were observed with colistin, ticarcillin/clavulanate, piperacillin, levofloxacin, ceftriaxone, cefepime, or meropenem ($p>0.05$). These results reflect the shared mechanism of action of tetracycline-class antibiotics and cross-resistance via efflux pumps [22,23].

Multivariate regression analysis Positive adeB, combined (adeB+adeJ), prior use of antibiotics, and prolonged hospital stay, identified multiple independent predictors for tigecycline resistance: adeB positivity (1.541-fold), combined (adeB+adeJ) (7.861-fold), prior antibiotic use (18.187-fold), mechanical ventilation (1.61-fold), ICU admission (8.805-fold), and prolonged hospital stay (1.098-fold). These findings confirm the combined effect of efflux pump activity, prior antibiotic exposure, critical care environment, and prolonged hospitalization as major determinants of tigecycline resistance [24,25,26]. However, certain factors, such as ICU admission, Chronic lung disease and mechanical ventilation, are not statistical significance in the multivariate regression model. This may be due to multicollinearity with more dominant predictors, including prior antibiotic exposure and prolonged hospitalization. Consequently, their association with tigecycline resistance is likely indirect, reflecting the severity of patients' conditions and extended healthcare exposure rather than serving as independent determinants of resistance.

Study Limitations

This study has several limitations even if it offers insightful information. First, the results may not be as applicable to larger patient groups due to the single-center design and the very small sample size (n=44). Second, the study limited a better

understanding of the underlying resistance mechanisms by not quantifying efflux pump expression levels or molecularly characterizing regulatory mechanisms, such as adeRS mutations. Third, the absence of clinical outcome data, including mortality rates, treatment response, or length of ICU stay in relation to resistant infections, prevents the establishment of correlations between genetic resistance markers and patient prognosis. Additionally, potential biases in sample collection and antibiotic susceptibility testing methods may have influenced the results. Future multicenter studies with larger cohorts and more comprehensive genetic analyses are warranted to validate these findings and provide more robust evidence.

CONCLUSION

This study demonstrates a high prevalence of tigecycline-resistant Acinetobacter baumannii, especially among ICU patients. Resistance was mainly linked to prior antibiotic use, prolonged hospitalization, and the presence of adeB and combined adeB+adeJ efflux pump genes. These results highlight the key role of efflux mechanisms in tigecycline resistance and the importance of antibiotic stewardship and strict infection control to prevent its spread. Future studies must include quantitative Real-Time PCR (qRT-PCR) and clinical outcome evaluations to create focused management, and mitigation plans for multidrug-resistant A. baumannii infections.

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