Efflux pumps encoding genes (*adeA* and *adeS*) in relation to antibiotic resistance pattern in Acinetobacter *baumannii* strains isolated from Benha university hospital

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Background
Acinetobacter baumannii become a growing problem in hospitals as a predominant multidrug-resistant (MDR). This resistance capacity is generated by various mechanisms including efflux pumps that can direct antibiotics outwards and prevent antibiotics from affecting the bacteria.
Methods
Clinical samples were cultured on blood agar and MacConkey agar medium, identified by Gram stain and biochemical reactions, and then identified to the species level by Vitek2 automated system. The *adeA* and *adeS* genes among isolated strains were detected by conventional PCR. This study included 50 A. baumannii strains to assess the antibiotic resistance pattern of A. baumannii.
Results
A. baumannii strains were fully resistant to Piperacillin (100%) and lowest resistance to Imipenem (54%) was observed. Out of 50 isolates, 41 (82%) of A. baumanii isolates had *adeA* gene and 32 (64%) had *adeS* gene. The clinical strains that had *adeA* gene and *adeS* gene showed significantly higher resistance to Ciprofloxacin (92.7%) and (100%), respectively. Additionally, borderline significant differences were reported regarding Gentamycin (P = 0.065), Ceftazidime (P = 0.08), and Meropenem (P = 0.08), with the resistance being higher in the *adeA* positive group.
Conclusion
Efflux pumps genes (*adeA*, *adeS*) played a key role in antibiotic resistance of A. baumanii.

Keywords:
Acinetobacter, efflux pump genes, multidrug-resistant

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Introduction

Acinetobacter baumannii is gram-negative bacteria from the class of Gammaproteobacteria [1]. This bacterium become a growing problem in hospitals as a predominant multidrug-resistant (MDR) bacterium in the intensive care and burn units. Horizontal acquisition of resistance genes is the main factors involved in the emergence of MDR [2].

This resistance capacity is generated by various mechanisms and contributes to resistance against a wide variety of antimicrobials, such as ß-lactams, macrolides, fluoroquinolones, aminoglycosides [3].

Hospital-associated *Acinetobacter* infections are often device-associated, including ventilator-associated pneumonias, and catheter-associated urinary tract infections. Risk factors for colonization or infection with *A. baumannii* include prolonged hospital stay, mechanical ventilation, intravascular devices, advanced age, immunosuppression, admission to an intensive

care unit (ICU), recent surgery or invasive procedures, and severe burns [4].

The main mechanisms to confer resistance to a different classes of antibiotics in *A. baumannii* include drug hydrolyzing enzymes, modifying enzymes, permeability defects, alteration of target sites and multidrug efflux pumps, these efflux pumps are important source of MDR, which export antibiotics from the cell, increasing their antibiotic resistance [5].

Based upon the similarity in amino acid composition and energy source, efflux pumps have been classified into five major families, namely, resistance nodulation division (RND) family, ATP binding cassette (ABC) transporter family, multidrug and toxin extrusion (MATE) family, small multidrug resistance (SMR) family, and major facilitator superfamily (MFS). Recently, a new family of efflux pumps have been identified in *A. baumannii* known as Proteobacterial Antimicrobial Compound Efflux. Among those families of pumps, the RND systems are the most prevalent in MDR Acinetobacter. The major clinically relevant in the RND efflux system is AdeABC efflux pump [6]. Patients with infection due to resistant strains seem to have higher mortality than patients with infection due to susceptible strains [7]. Bacterial resistance to antimicrobial agents is a threat to public health in Egypt [8].

Aim of the study: This investigation aims to evaluate A. baumannii's pattern of antibiotic resistance.

Patients and methods

This work was carried out in Microbiology and Immunology Department, Benha Faculty of Medicine in the period between January 2023 and March 2023. The patients included in the study were 37 females and 13 males, ranging in age from 20 to 50. The study was approved by the Benha University ethical committee, and informed consent was obtained from all patients. It included 50 strains of A. baumannii that were isolated from sputum and bronchoalveolar lavage (BAL).

The samples were obtained from patients by using standard microbiological sample collection methods. They were cultured on blood agar and MacConkey agar (Bio-Rad, USA) at 37°C for 24 h. Each nonlactose fermenting colony on MacConkey agar media was picked up, further identified by microscopic examination using Gram stain, culture characteristics, and standard biochemical reactions.

Vitek 2 compact system, Biomerieux, France performed the antimicrobial susceptibility test in accordance with the manufacturer's instructions by preparing a bacterial suspension in 3.0 ml of sterile saline (0.45%) and adjusting the turbidity accordingly (0.50–0.63) before using the solution to rehydrate the antimicrobial medium inside the card. After filling it out, sealing it, and inserting it into the instrument incubator/reader VITEK 2 system. Over a predetermined amount of time (up to 18 h for bacteria), the equipment tracked the development of each well in the card. Each antibiotic listed on the card had its MIC values (or test results, if appropriate) determined at the end of the incubation cycle (AST-N 233).

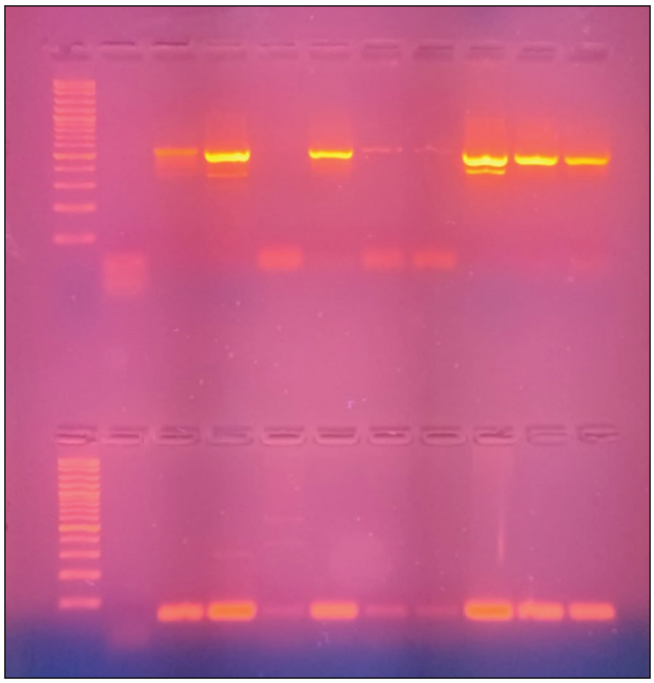
PCR: Following the manufacturer's recommendations, DNA was extracted using a Quick-DNATM Miniprep Plus Kit from Zymo Research in the United

Table 1 Primers that were utilized in this investigation in order

ade A-F	5′-TTG ATC GTG CTT CTA TTC CTCAAG -3′
ade A-R	5′-GGC TCG CCA CTG ATA TTA CGTT - 3′
Ade S – F	5′- TGC CGC CAA ATT CTT TAT TC -3′
Ade S – R	5′- TTA GTC ACG GCG ACC TCT CT -3′

Two PCR reactions were made for each sample. Each reaction contained either ade- A primer or ade-s primer in a final reaction volume of 50 µL contained: Green PCR Master Mix: 25 µl, forward Primer: 2 µl Reverse Primer: 2 µl, Template DNA: 5 µl.nuclease-free Water: 16 µl, Amplification was performed in 40 cycles: 3 min of initial denaturation at 94°C, primer connection for *adeA* at 55.5°C and For *adeS* at 54.5°C for 30 s, 90 s at 72°C, and 5 min as a final extension at 72°C. The PCR products were electrophoresed by a gel agarose (Hopkins and Williams, England)) and visualized by a UV transilluminator (Biometra,Germany).
Results: A. baumanii infection was higher in females (56%) than males (44%).

Figure 1



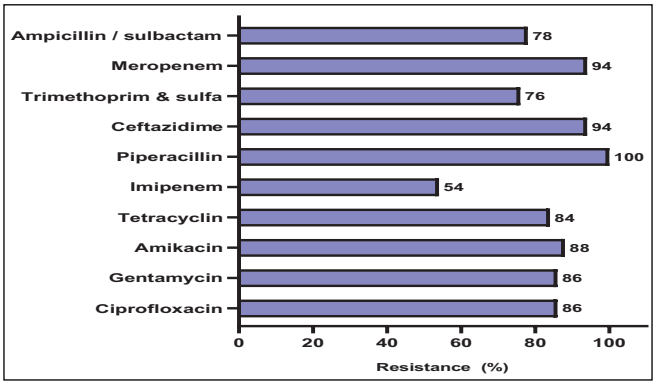
The PCR by gel electrophoresis results. Lane 1: DNA Ladder 100 bp.

States. A conventional PCR (Master mix Dream Taq. fermentas, life science, Thermo Fisher Scientific) was then used to detect the *adeA-adeS* genes listed in, Table 1, Fig. 1.

Figure 2 Demonstrates that imipenem had the lowest amount of resistance (54%) and piperacillin had the highest level of resistance (100%) in A. baumannii.

Table 2 shows that the study included 50 patients with a mean age of 53 ± 15 years. More than half were females (56%). The specimens collected included bronchoalveolar lavage (BAL) (32%), pus (18%), sputum (34%), and urine (16%). Most patients (74%) had a hospital stay over seven days. About one-third had hypertension (36%), and more than half (56%) had diabetes mellitus. The majority of patients were on mechanical ventilation (70%). Ade-A and Ade-S were positive in 82% and 64% of patients, respectively. About two-thirds (64%) had a double positive result.

Figure 2



Antimicrobial susceptibility pattern of Acinetobacter baumannii isolates.

Table 2 General characteristics of the studied patients

General characteristics	
Age (years)	53 ± 15
Sex	
Males	22 (44)
Females	28 (56)
Specimens	
Bronchoalveolar lavage	16 (32)
Pus	9 (18)
Sputum	17 (34)
Urine	8 (16)
Length of hospital stay	
≤7 days	13 (26)
>7 days	37 (74)
Hypertension	18 (36)
Diabetes mellitus	28 (56)
Mechanical ventilation	35 (70)
Positive Ade-A	41 (82)
Positive Ade-S	32 (64)
Double positive genes	32 (64)

Data are presented as mean±SD or number (percentage).

Table 3 shows that patients were classified according to *adeA* results. Of the studied patients, 41 tested positive, while 9 were negative. The positive group demonstrated a significantly higher percentage of patients with a hospital stay of more than 7 days (80.5% vs. 44.4%, $P = 0.026$).

Regarding antimicrobial resistance, the positive groups showed significantly higher resistance to Ciprofloxacin (92.7% vs. 55.6%, $P = 0.004$), Amikacin (97.6% vs. 44.4%, $P < 0.001$), and Tetracyclin (90.2% vs. 55.6%, $P = 0.01$).

Additionally, borderline significant differences were reported regarding Gentamycin ($P = 0.065$), Ceftazidime ($P = 0.08$), and Meropenem ($P = 0.08$), with the resistance being higher in the *adeA* positive group.

No significant differences were observed regarding age, sex, hypertension, diabetes mellitus, mechanical ventilation, imipenem, piperacillin, Trimethoprim and sulfa, and Ampicillin/sulbactam.

Table 4 shows that patients were classified according to *adeS* results. Of the studied patients, 32 tested positive, while 18 were negative.

Regarding antimicrobial resistance, the positive groups showed significantly higher resistance to Ciprofloxacin (100.0% vs. 61.1%, $P < 0.001$), Gentamycin (96.9% vs. 66.7%, $P = 0.006$), and Amikacin (96.9% vs. 72.2%, $P = 0.018$).

A borderline significant difference was observed regarding tetracycline ($P = 0.088$), with the resistance being higher in the *adeS* positive group.

No significant differences were observed regarding age, sex, length of hospital stay, hypertension, diabetes, mechanical ventilation, imipenem, piperacillin, Ceftazidime, Trimethoprim and sulfa, Meropenem, and Ampicillin/sulbactam.

Discussion

A. baumannii has now emerged as a leading cause of nosocomial and community-acquired infections. MDR A. baumannii has been increasingly reported worldwide.

In the present study, A. baumanii infection was higher in females (56%) than males (44%). This finding agrees

Table 3 Patients' characteristics and antimicrobial resistance according to Ade-A status

	Ade-A		*P*-value
	Positive (*n*=41)	Negative (*n*=9)	
Age (years)	54 ± 15	50 ± 13	0.428
Sex			
Males	17 (41.5)	5 (55.6)	0.441
Females	24 (58.5)	4 (44.4)	
Length of hospital stay			
≤7 days	8 (19.5)	5 (55.6)	**0.026**
>7 days	33 (80.5)	4 (44.4)	
Hypertension	16 (39)	2 (22.2)	0.342
Diabetes mellitus	24 (58.5)	4 (44.4)	0.441
Mechanical ventilation	30 (73.2)	5 (55.6)	0.296
Ciprofloxacin			
Resistant	38 (92.7)	5 (55.6)	**0.004**
Sensitive	3 (7.3)	4 (44.4)	
Gentamycin			
Resistant	37 (90.2)	6 (66.7)	0.065
Sensitive	4 (9.8)	3 (33.3)	
Amikacin			
Resistant	40 (97.6)	4 (44.4)	**<0.001**
Sensitive	1 (2.4)	5 (55.6)	
Tetracyclin			
Resistant	37 (90.2)	5 (55.6)	**0.01**
Sensitive	4 (9.8)	4 (44.4)	
Imipenem			
Resistant	24 (58.5)	3 (33.3)	0.270
Sensitive	17 (41.5)	6 (66.7)	
Piperacillin			
Resistant	41 (100)	9 (100)	-
Sensitive	0	0	
Ceftazidime			
Resistant	40 (97.6)	7 (77.8)	0.08
Sensitive	1 (2.4)	2 (22.2)	
Trimethoprim and sulfa			
Resistant	33 (80.5)	5 (55.6)	0.113
Sensitive	8 (19.5)	4 (44.4)	
Meropenem			
Resistant	40 (97.6)	7 (77.8)	0.08
Sensitive	1 (2.4)	2 (22.2)	
Ampicillin/sulbactam			
Resistant	33 (80.5)	6 (66.7)	0.365
Sensitive	8 (19.5)	3 (33.3)	

Data are presented as mean±SD or number (percentage); Significant *P* values are marked in bold.

with that of Ranjbar and colleagues [9].who reported that A. baumannii infection was higher in females (56%) than males (44%). In fact, in terms of sex, the prevalence of A. baumannii strains was almost the same in females as well as males Ranjbar and colleagues [9].

The current study founded that A. baumannii recovered more from sputum, followed by endotracheal aspirates, pus and urine, which agrees with Tolba and colleagues [10], who found that sputum samples was the major site for A. baumannii isolation followed by wound swabs, urine and BAL. A. baumannii is a leading cause of pneumonia in ICU patients and it is also a main cause of urinary, blood stream and wound infections, which makes it a serious threat Tantawy and colleagues [11].

In the present study, the prolonged stay in hospital was associated with A. baumannii infection as 74% of patients infected with A. baumannii were staying in hospital more than 7 days which coincides with Ren and colleagues [12], who reported that prolonged hospitalization was a significant risk of infection.

Patients on mechanical ventilation had higher incidence of A. baumannii infection (70%).Other

Table 4 Patients' characteristics and antimicrobial resistance according to Ade-S status

	Ade-S		*P*-value
	Positive (*N*=32)	Negative (18)	
Age (years)	55 ± 15	51 ± 14	0.312
Sex			
Males	15 (46.9)	7 (38.9)	0.585
Females	17 (53.1)	11 (61.1)	
Length of hospital stay			
≤7 days	6 (18.8)	7 (38.9)	0.119
> 7 days	26 (81.3)	11 (61.1)	
Hypertension	13 (40.6)	5 (27.8)	0.364
Diabetes mellitus	18 (56.3)	10 (55.6)	0.962
Mechanical ventilation	24 (75)	11 (61.1)	0.304
Ciprofloxacin			
Resistant	32 (100)	11 (61.1)	**<0.001**
Sensitive	0	7 (38.9)	
Gentamycin			
Resistant	31 (96.9)	12 (66.7)	**0.006**
Sensitive	1 (3.1)	6 (33.3)	
Amikacin			
Resistant	31 (96.9)	13 (72.2)	**0.018**
Sensitive	1 (3.1)	5 (27.8)	
Tetracycline			
Resistant	29 (90.6)	13 (72.2)	0.088
Sensitive	3 (9.4)	5 (27.8)	
Imipenem			
Resistant	19 (59.4)	8 (44.4)	0.309
Sensitive	13 (40.6)	10 (55.6)	
Piperacillin			
Resistant	32 (100)	18 (100)	-
Sensitive	0	0	
Ceftazidime			
Resistant	31 (96.9)	16 (88.9)	0.291
Sensitive	1 (3.1)	2 (11.1)	
Trimethoprim and sulfa			
Resistant	26 (81.3)	12 (66.7)	0.246
Sensitive	6 (18.8)	6 (33.3)	
Meropenem			
Resistant	31 (96.9)	16 (88.9)	0.291
Sensitive	1 (3.1)	2 (11.1)	
Ampicillin/sulbactam			
Resistant	26 (81.3)	13 (72.2)	0.459
Sensitive	6 (18.8)	5 (27.8)	

Data are presented as mean±SD or number (percentage); Significant *P* values are marked in bold.

researcher found that 80% of their studied patients with A. baumannii infection were on mechanical ventilation Arafa and colleagues [13].

A. baumannii is an opportunistic hospital pathogen that causes a wide range of nosocomial infections. Due to the indiscriminate use of broad-spectrum antibiotics, There is high antibiotic resistance caused by this bacterium. One of the unique characteristic of A. baumannii is resistance to antibiotics and acquiring resistance genes to antibiotics, which exacerbate the treatment in patients with immune deficiency system.

Recently, A. baumannii strains with the resistant ability to all common antibiotic classes are undergone prevalence in healthcare systems Rafiei and colleagues [14].

Various studies have shown that A. baumannii is resistant to most ß -lactam antibiotics and Quinolones, and its resistance to Aminoglycosides is increasing. In this study, the most effective antibiotic used against A. baumannii was Imipenem, which had 54% resistance. This is in agreement with Basatian-Tashkan *et al*., [15] who reported that the antibiotic resistance to Imipenem was 50%.

They were more resistant to Amikacin (88%), Ciprofloxacin (86%), Gentamycin (86%), Tetracyclin (84%),Ampicillin/sulbactam (78%) and Trimethoprim-

sulfamethoxazole (76%). On the other hand the antibiotic resistance to Amikacin, Gentamycin, Tetracyclin, Ampicillin/sulbactam was reported by Basatian-Tashkan *et al*., [15] to be 96.6, 48.4.1, 91.6% and 65 and 76.1%, respectively.

Our clinical A. baumannii strains were fully resistant to Piperacillin (100%) and nearly fully resistant (95%) to Ceftazidime and Meropenem. Noori and colleagues [16] and Basatian-Tashkan *et al.* [15] reported the same result for piperacillin, Ghasemian and colleagues [17] and Tantawy and colleagues [11] reported the same result for Meropenem and Ceftazidime, respectively.

In Abdar and colleagues [18], study, the resistance to Meropenem and Ceftazidime was reported to be 71 and 93%, respectively.

In a study by Jia and colleagues [19] in China, resistance to Piperacillin and Ceftazidime was reported to be 92.2% that was less resistance than this study. Variability of antibiotic resistance patterns of A. baumannii could be attributed to the use of different antimicrobial panels, antibiotic abuse among countries Tohamy and colleagues [20].

A. baumannii exhibits different mechanisms of resistance to many classes of antibiotics. One of the resistance mechanisms in the A. baumannii is the presence of efflux pumps. These pumps cause the leakage of antibiotics and a wide range of substances out of the bacteria, decreasing drug levels in the bacterial cells Cortez-Cordova and Kumar [21].

Although high levels of resistance do not occur only as a result of multi-drug efflux pumps, the expression of their genes among isolates with high antibiotic resistance cannot be neglected. Therefore, it is necessary to identify resistance mechanisms, including efflux pumps Mohamed and colleagues [5].

In our study, *adeA* gene is shown to be the most prevalent in our clinical isolates, 82% of A. baumannii isolates had *adeA* gene. The *adeA* gene encodes for one of the proteins that make up the tripartite system of efflux pump adeABC Kor and colleagues [22].

Out of 50 isolates, 32(64%) had *adeS* gene and 64% of isolates had both genes. Nemec and colleagues [23] in France reported the same result for *adeA* gene (81.9%) and Terkuran and colleagues [24] in Turkey reported the same result for *adeS* gene (68%).

On the other hand, studies conducted in Iran, Beheshti and colleagues [25] founded that (100%) of isolates had *adeA* gene and Ardabili and colleagues [26] founded (100%) of isolates had *adeS* gene.

Regarding antimicrobial resistance in our study, the clinical strains that had *adeA* gene and *adeS* gene showed significantly higher resistance to Ciprofloxacin (92.7%, $P = 0.004$) and Ciprofloxacin (100.0%, $P < 0.001$), respectively.

Ardabili and colleagues [26] suggest that drug efflux pumps are involve in resistance to fluoroquinolone in clinical isolates of *A. baumannii*. AdeABC is an efflux pump described in *A. baumannii,* overexpression of which confers resistance to fluoroquinolones and other antimicrobial agents.

Out of 50 isolates, 37 (90.2%) strains showing resistance to Tetracyclin had *adeA* gene and 29 (90.6%) strains showing resistance to Tetracycline had *adeS* gene.

Beheshti and colleagues [25] have reported the role of efflux pumps in the resistance of A. baumannii isolates to Tetracycline antibiotics.

The reason for the differences in different studies could be due to differences in the patterns of antibiotic use, the type of clinical sample, the number of samples studied, sampling method, environmental factors and the different geographical distribution of these genes.

Antibiotic-sensitive Acinetobacter spp. could be carry *adeA* and *adeS* genes, but some of them implied that only resistant strains carried those genes Ranjbar and colleagues [9].

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