Microbes and Infectious Diseases 2025; 6(1): 329-341

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Frequency of efflux pump-encoding genes *adeA* and *adeS* in *Acinetobacter baumannii* isolates from Ain Shams University Hospitals

Shimaa A. Abdel Salam[1*], Karim A. Montasser[2], Maha A. Anwar[3], Amira E. Abdel Hamid[1], Nagwa M Abo El Magd[1]

1- Medical Microbiology and Immunology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

2- Clinical Pathology Department, Faculty of Medicine, Helwan University, Cairo, Egypt

3- Clinical Pathology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt

ARTICLE INFO

Article history:
Received 27 October 2024
Received in revised form 3 December 2024
Accepted 8 December 2024

Keywords:
Acinetobacter baumannii
efflux pump
Antibiotic resistance

ABSTRACT

Background: *Acinetobacter baumannii* is one of the commonly encountered organisms in health care associated infections, posing a challenge in treatment due to multiple antibiotic resistance mechanisms. One of these mechanisms is the efflux pump. Three known Acinetobacter Drug Efflux pumps (Ade) belong to the Resistance Nodulation and Cell Division (RND) family are identified. The efflux pump *ade*ABC is detected in about 80 % of clinical isolates in some reports, leading to resistance to many antibiotics. **Aim:** This study aims to assess the frequency of efflux pump-encoding genes (*adeA* and *adeS*) in isolates of *Acinetobacter baumannii* detected among patients admitted to Ain Shams University Hospitals and to correlate their frequency with susceptibility to different classes of antibiotics. **Methods:** Eighty-four clinical isolates of *Acinetobacter baumannii* retrieved from Main Microbiology Laboratory, Ain Shams University Hospital, Cairo, Egypt, were included in the study. Identification was performed using conventional microbiological methods and antimicrobial sensitivity testing was performed. All isolates were subjected to molecular detection of *adeA* and *adeS* genes by conventional PCR. **Results**: The distribution of the *adeA* gene among clinical isolates was 78.5% and for *adeS* genes was 72.6% and both genes were present together in 72.6% of the tested isolates. There was a statistically significant association between the presence of *adeA* and *adeS* gene and resistance to imipenem, meropenem, ciprofloxacin, levofloxacin, and amikacin. **Conclusion**: The presence of *adeA* and *adeS* genes could have a pivotal involvement in resistance among *A. baumannii* isolates to several antibiotics.

Introduction

Acinetobacter baumannii (*A. baumannii*) is considered one of the commonest causes of healthcare associated infections (HAIs), especially in Intensive care units (ICU). Though it is an opportunistic pathogen, yet it can cause a variety of infections, including urinary tract infections,

DOI: 10.21608/MID.2024.331671.2320
* *Corresponding author:* Shimaa Ahmed Abdel Salam
E-mail address: *shimaa_ahmed@med.asu.edu.eg*

pneumonia, bacteraemia, and wound infections, with a mortality rate reaching 60% [1,2]

A. baumannii has different antibiotic resistance mechanisms, mainly through production of antimicrobial degrading enzymes, such as beta-lactamases and amino-glycosidase. Other mechanisms include changing the target sites, decreasing membrane permeability, biofilm formation, and efflux pumps expression, especially those with resistance nodulation cell division (RND) transporters (3). In this context, there are six major superfamilies of efflux pumps reported as a major cause of drug resistance. These efflux pumps are classified- based on their amino acid sequence and the source of energy used to export substrates- into: ATP-Binding Cassette (ABC) family, Multidrug and Toxic Compound Extrusion (MATE) family, Major Facilitator Superfamily (MFS) , Resistance Nodulation and Cell Division (RND) family , Small Multidrug Resistance (SMR) family and proteobacterial antimicrobial compound efflux (PACE) family [4,5].

RND family is highly distributed among Gram-negative bacteria, playing a pivotal role in the efflux of antibiotics. Three known Acinetobacter Drug Efflux pumps (Ade) belong to the RND family were identified and are widely expressed in *A. baumannii* isolates, AdeABC, AdeFGH, and AdeIJK efflux pumps. [6,7].

In about 80% of clinical isolates, the efflux pump adeABC was detected, with emergence of resistance to different types of antibiotics. [8]. It consists of three components: adeB, the transmembrane component, adeA, the inner membrane fusion protein and adeC which is the outer membrane protein. The gene encoding this type of efflux pump is regulated by two-component system called adeRS , adeS (sensor kinase) and adeR (response regulator), which together control the gene expression of the adeABC [9].

Due to the high prevalence of drug resistance among clinical *A. baumannii* in hospital settings, especially by AdeABC efflux pump, this study aims to investigate the pattern of resistance of *A. baumannii* clinical isolates collected from Ain Shams University hospitals and to investigate the frequency of efflux pump-encoding genes *adeA* and *adeS* among *A. baumannii* isolates.

Material and Methods

The current study was conducted on non-duplicate 84 clinical isolates. Sample size was calculated according to sample size calculator (PASS 15, version 15.0.10). The isolates were retrieved from Main Microbiology Laboratory, Ain Shams University Hospital, Cairo, Egypt. This study was approved by the Research Ethics Committee, Ain Shams University (FMASU R153/2024).

Identification of *A. baumannii* isolates

All isolates were identified by conventional microbiological methods by culturing on blood and MacConkey's agars (Bio-Rad, USA) at 37°C for 18-24h. Identification of non-lactose fermenting colonies on MacConkey's agar was performed by microscopic examination of Gram-stained film and biochemical reactions including triple sugar iron medium, urease agar, citrate agar, oxidase test, catalase test, motility testing, indole production test, and ornithine decarboxylase production test [10]. Identification to species level was performed by Vitek 2 (bioMérieux, Inc., Hazelwood, MO).

Antimicrobial Susceptibility Testing

It was done by disk diffusion method on Mueller Hinton agar plates (Bio-Rad, USA) was performed on all the isolates as shown in figure (1) and interpretation of results was done according to Clinical and Laboratory Standard Institute (CLSI) guidelines, 2024 [11].

Antibiotic discs tested were cefotaxime (30 µg), Ceftriaxone (30µg), ceftazidime (30 µg), piperacillin/tazobactam (100/10 µg), Ampicillin-Sulbactam (10/10µg), Meropenem (10µg), Imipenem (10µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), Gentamicin (10µg), Tobramycin (10µg), amikacin (30 µg), ciprofloxacin (5 µg) and levofloxacin (5 µg) (All antibiotic discs were supplied from Oxoid, USA). Isolates were defined as multi drug resistant (MDR) when the isolate was resistant to at least three classes of antimicrobial agents, including all penicillins (including penicillins-inhibitor combinations), cephalosporins, aminoglycosides and fluroquinolones. Extensive drug resistance (XDR) was defined as resistance to the classes of antimicrobials described among MDR in addition to resistance to carbapenems [12].

Molecular Detection of *adeA* and *adeS* genes

All *A. baumannii* clinical isolates were subjected to molecular detection of *adeA* and *adeS* genes by conventional PCR. DNA extraction was done by using Favor PrepTM Tissue Genomic DNA Extraction Mini Kit (Cat no. FATGK 001), Tiwan) . Following extraction, PCR was performed to

screen for *adeA* and *adeS* genes in thermal cycler (Applied Biosystems, USA) by using master mix (Cat no. K0171) supplied from Thermo Fisher scientific (USA). The amplification reaction with total volume 50 uL was prepared from PCR Master Mix (25 uL), forward primer (1uL), reverse primer (1uL), extracted DNA (10 uL) and nuclease free water (13 uL). The cycle parameters were as follow: initial hold for 2 min at 95 °C, 40 amplification cycles of (denaturation for 45 sec at 95°C, annealing for 45 sec at 55 °C, extension for 45 sec at 72 °C) and final extension for 5 min at 72 °C. The PCR products were analyzed through a 2% agarose gel containing ethidium bromide with using DNA ladder (100bp DNA Ladder) supplied from (Promega, USA). The product size of *adeA* gene was 74bp while for and *adeS* gene was 659bp as shown in Figure (2, 3).

Statistical analysis

Data were collected, tabulated, and statistically analyzed with SPSS statistical package version 23 (SPSS Inc. Released 2015. IBM SPSS statistics for windows, version 23.0, Armnok, NY: IBM Corp.)

Results

An eighty-four (84) non- repeated *A. baumannii* clinical isolates were included in this study. All the isolates were isolated from Ain Shams University Hospital. Isolates were collected from ICUs (61.9% (52/84 isolates), surgical wards (25% (21/84 isolates)) and burn units (13.05% (11/84 isolates)). Most of the bacterial isolates were obtained from blood samples (47.6 % (40/84 isolates)), followed by respiratory samples (Sputum, endotracheal aspirates & Bronchoalveolar lavage) (22.6 % (19/84 isolates)), pus from wounds (20.2 % (17/84 isolates)), and urine samples (9.5 % (8/84 isolates)) as in figure (4).

Antibiotic susceptibility results of the tested isolates were as follows: most of the isolates showed resistance to ampicillin-sulbactam (94%), piperacillin-tazobactam (92.8%), cefotaxime (90.5%), ceftriaxone (89%), ceftazidime (88%) while the least resistance was reported for imipenem (70%) and meropenem (72.6 %) as shown in table 2.

The isolates were classified according to the pattern of resistance into three groups: group I: isolates resistant to 1or 2 groups of antibiotics which represented 9.5% of the isolates (8/84). Group II: MDR isolates represented 15.5% of the isolates (13/84) and group III : XDR isolates represented 75% of the isolates (63/84).

The distribution of the *adeA* gene among clinical isolates was 78.5% (66/84) and for *adeS* genes was 72.6% (61/84) and both two genes were present together in 72.6% (61/84) of the tested isolates.

Table 3 illustrates the distribution of genes among antibiotic groups of *A. baumannii* isolates. A statistically significant difference between isolates that was resistant to 1 or 2 groups of antibiotics (group I) and MDR and XDR groups (group II, III) regarding *adeA* , *adeS* gene distribution was noted.

Analysis of resistance pattern of the included isolates and the presence of examined genes revealed that there is a statistically significant association between the presence of *adeA* gene and resistance to imipenem, meropenem, ciprofloxacin, levofloxacin, and amikacin. For *adeS* gene, a statistically significant association was observed with resistance to imipenem, meropenem, ciprofloxacin, levofloxacin, tobramycin, amikacin, and Trimethoprim-sulfamethoxazole as shown in table (4) and (5).

Table 1. The sequence of primers used in this study were as follows

Name of gene	Sequence	Product size	Ref.
adeA	**Forward 5′-TTG ATC GTG CTT CTA TTC CTCAAG -3′** **Reverse 5′-GGC TCG CCA CTG ATA TTA CGTT-3′**	**74 bp**	**13**
adeS	**Forward 5′- TGC CGC CAA ATT CTT TAT TC-3′** **Reverse 5′- TTA GTC ACG GCG ACC TCT CT-3′**	**659 bp**	**14**

Table 2. The Antibiotic resistance pattern of *A. baumannii clinical* isolates.

Antibiotic	**Sensitive No (%)**	**Resistant No (%)**
Ampicillin sulbactam (SAM)	(5/84) 6%	(79/84) 94%
Piperacillin-tazobactam (TZP)	(6/84) 7.2 %	(78/84) 92.8%
Cefotaxime (CTX)	(8/84) 9.5%	(76/84) 90.5%
Ceftriaxone (CRO)	(9/84) 11%	(75/84) 89 %
Ceftazidime (CAZ)	(10/84) 12%	(74/84) 88 %
Imipenem (IPM)	(25/84) 30%	(59/84) 70%
Meropenem (MEM)	(23/84) 27.4%	(61/84) 72.6%
Ciprofloxacin (CIP)	(16/84) 19%	(68/84) 81%
Levofloxacin (LEV)	(15/84) 18%	(69/80) 82%
Gentamycin (CN)	(13/84) 15.5%	(71/84) 84.5%
Tobramycin (TOB)	(14/84) 16.7%	(70/84)83.3%
Amikacin (AK)	(20/84) 24%	(64/84) 76%
Trimethoprim-sulfamethoxazole (SXT)	(17/84) 20.2%	(67/84) 79.8%

Table 3. Comparison between the different groups of *A. baumannii* isolates regarding the presence of *adeA* and *adeS* genes.

Genes	***A. baumannii* isolates**		**Test of significance**	**P value**
	Group I (isolates resistant to 1 or 2 groups of antibiotics) (No. 8)	**Group II (MDR) and III (XDR) (No.76)**		
Isolates positive for *adeA* gene. (No. 66)	**3**	**63**	**8.8589**	**0.002***
Isolates Negative for *adeA* gene. No (18)	**5**	**13**		
Isolates positive for *adeS* gene. (No. 61)	**2**	**59**	**10.0837**	**0.001***
Isolates Negative for *adeS* gene. No (23)	**6**	**17**		

Bold values are significant at $p<0.05$.

Table 4. Relation between presence of *adeA* gene and resistance to different antibiotics.

Antibiotic	Sensitive	Resistant	P value
Ampicillin sulbactam (SAM)	(5/84) 6%	(79/84) 94%	
***adeA* Positive (66)**	4	62	0.93
***adeA* Negative (18)**	1	17	
Piperacillin-tazobactam (TZP)	(6/84) 7.2 %	(78/84) 92.8%	0.76
***adeA* Positive (66)**	5	61	
***adeA* Negative (18)**	1	17	
Cefotaxime (CTX)	(8/84) 9.5%	(76/84) 90.5%	0.79
***adeA* Positive (66)**	6	60	
***adeA* Negative (18)**	2	16	
Ceftriaxone (CRO)	(9/84) 11%	(75/84) 89 %	0.42
***adeA* Positive (66)**	8	58	
***adeA* Negative (18)**	1	17	
Ceftazidime (CAZ)	(10/84) 12%	(74/84) 88 %	0.9
***adeA* Positive (66)**	8	58	
***adeA* Negative (18)**	2	16	
Imipenem (IPM)	(25/84) 30%	(59/84) 70%	**0.001***
***adeA* Positive (66)**	14	52	
***adeA* Negative (18)**	11	7	
Meropenem (MEM)	(23/84) 27.4%	(61/84) 72.6%	**0.01***
***adeA* Positive (66)**	14	52	
***adeA* Negative (18)**	9	9	
Ciprofloxacin (CIP)	(16/84) 19%	(68/84) 81%	**<0.0001***
***adeA* Positive (66)**	6	60	
***adeA* Negative (18)**	10	8	
Levofloxacin (LEV)	(15/84) 18%	(69/80) 82%	**0.008***
***adeA* Positive (66)**	8	58	
***adeA* Negative (18)**	7	11	
Gentamycin (CN)	(13/84) 15.5%	(71/84) 84.5%	0.4
***adeA* Positive (66)**	11	45	
***adeA* Negative (18)**	2	16	
Tobramycin (TOB)	(14/84) 16.7%	(70/84)83.3%	0.14
***adeA* Positive (66)**	7	49	
***adeA* Negative (18)**	7	21	
Amikacin (AK)	(20/84) 24%	(64/84) 76%	**0.003***
***adeA* Positive (66)**	11	55	
***adeA* Negative (18)**	9	9	
Trimethoprim-sulfamethoxazole (SXT)	(17/84) 20.2%	(67/84) 79.8%	0.12
***adeA* Positive (66)**	11	55	
***adeA* Negative (18)**	6	12	

Bold values are significant at $p<0.05$.

Table 5. Relation between *adeS* gene and resistance to different antibiotics.

Antibiotic	Sensitive	Resistant	P value
Ampicillin sulbactam (SAM)	(5/84) 6%	(79/84) 94%	
***adeS* Positive (61)**	4	57	0.7
***adeS* Negative (23)**	1	22	
Piperacillin-tazobactam (TZP)	(6/84) 7.2 %	(78/84) 92.8%	0.73
***adeS* Positive (61)**	4	57	
***adeS* Negative (23)**	2	21	
Cefotaxime (CTX)	(8/84) 9.5%	(76/84) 90.5%	0.49
***adeS* Positive (61)**	5	56	
***adeS* Negative (23)**	3	20	
Ceftriaxone (CRO)	(9/84) 11%	(75/84) 89 %	0.67
***adeS* Positive (61)**	6	55	
***adeS* Negative (23)**	3	20	
Ceftazidime (CAZ)	(10/84) 12%	(74/84) 88 %	0.84
***adeS* Positive (61)**	7	54	
***adeS* Negative (23)**	3	20	
Imipenem (IPM)	(25/84) 30%	(59/84) 70%	**0.0009***
***adeS* Positive (61)**	12	49	
***adeS* Negative (23)**	13	10	
Meropenem (MEM)	(23/84) 27.4%	(61/84) 72.6%	**0.001***
***adeS* Positive (61)**	11	50	
***adeS* Negative (23)**	12	11	
Ciprofloxacin (CIP)	(16/84) 19%	(68/84) 81%	**0.003***
***adeS* Positive (61)**	7	54	
***adeS* Negative (23)**	9	14	
Levofloxacin (LEV)	(15/84) 18%	(69/80) 82%	**0.001***
***adeS* Positive (61)**	6	55	
***adeS* Negative (23)**	9	14	
Gentamycin (CN)	(13/84) 15.5%	(71/84) 84.5%	0.29
***adeS* Positive (61)**	11	50	
***adeS* Negative (23)**	2	21	
Tobramycin (TOB)	(14/84) 16.7%	(70/84)83.3%	**0.0006***
***adeS* Positive (61)**	5	56	
***adeS* Negative (23)**	9	14	
Amikacin (AK)	(20/84) 24%	(64/84) 76%	**0.0001***
***adeS* Positive (61)**	8	53	
***adeS* Negative (23)**	12	11	
Trimethoprim-sulfamethoxazole (SXT)	(17/84) 20.2%	(67/84) 79.8%	**0.04***
***adeS* Positive (61)**			
***adeS* Negative (23)**	9	52	
	8	15	

Bold values are significant at $p<0.05$.

Figure 1. The antimicrobial susceptibility testing (AST) using Muller Hinton Agar streaked by *Acinetobacter baumannii* isolate revealed a multi-drug resistance (MDR) profile where isolates was resistant to all tested antibiotics.

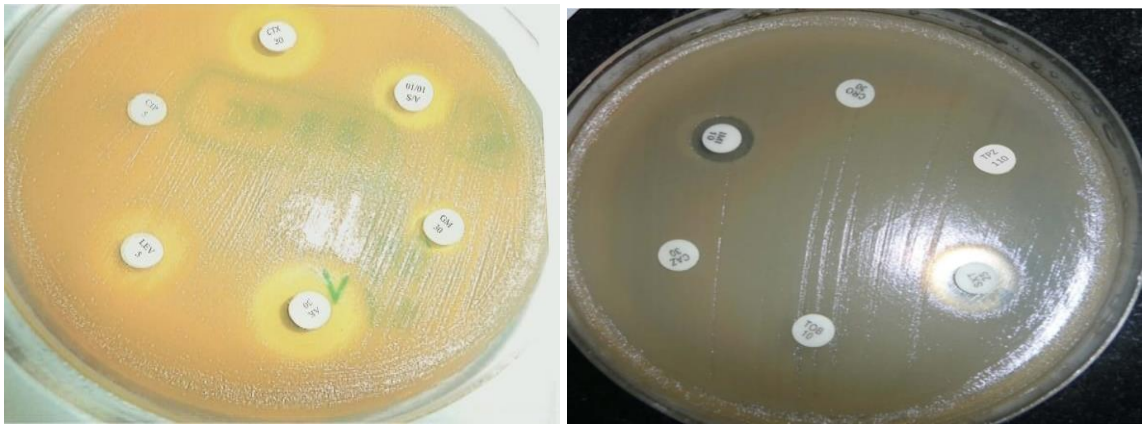


Figure 2. Agarose gel electrophoresis of PCR-assay for identification of *adeA* gene detected at 74bp.

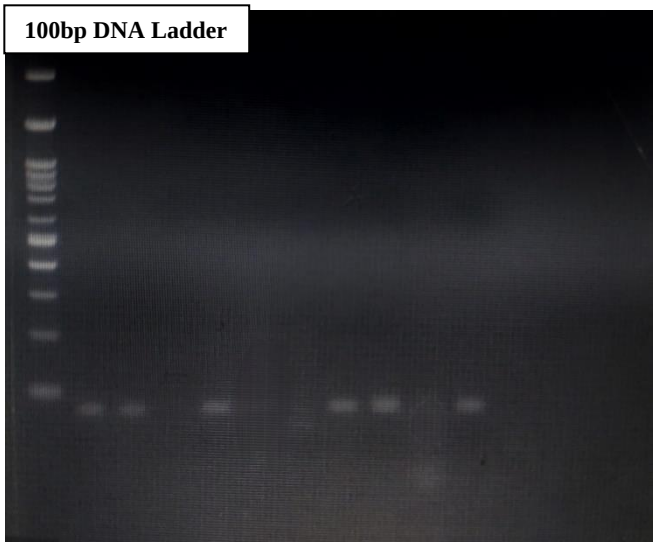


Figure 3. Agarose gel electrophoresis of PCR-assay for identification of *adeS* gene detected at 659bp.

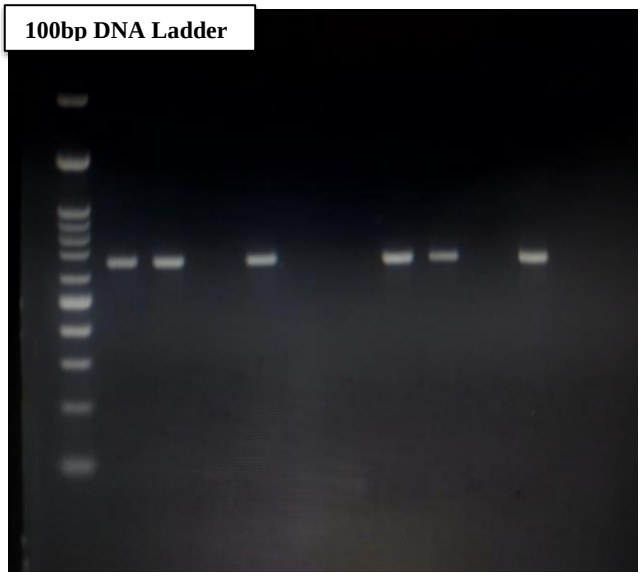
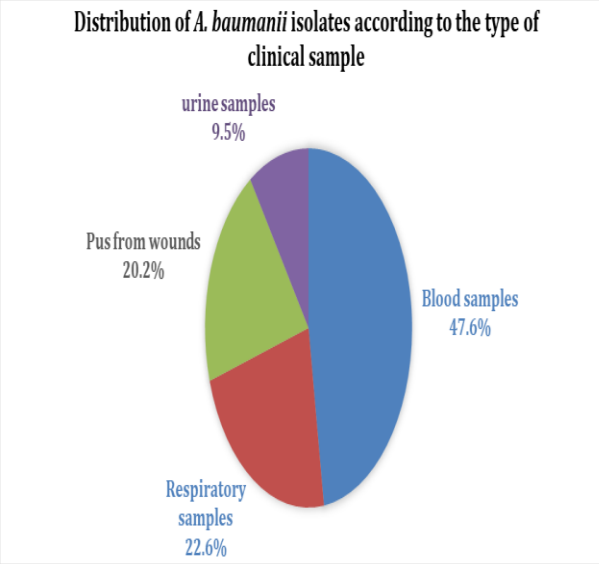


Figure 4. Distribution of *A. baumannii* isolates according to the type of clinical sample**.**



Discussion

A. baumannii is as one of the most important pathogens isolated in different hospital settings. It is characterized by high rate of antimicrobial resistance that imposes a challenge in treating infections caused by this pathogen. The mechanism of resistance of this pathogen is diverse, either by production of inactivating enzymes, biofilm formation and over expression of efflux pump genes **[15].**

One of the efflux pumps related to emergence of antimicrobial resistance among *A. baumannii* is *adeABC* system. Expression of these genes results in resistance to different antibiotics including beta-lactams, aminoglycoside, chloramphenicol, fluoroquinolones, and tetracycline, rendering it difficult to choose a proper drug for eradication **[15].**

In this study, we aimed to assess the frequency of presence of efflux pump-encoding genes, *adeA and adeS*, among *A. baumannii* Isolates retrieved from Ain Shams University Hospitals different settings.

This study was done on 84 non- repeated *A. baumannii* clinical isolates. Most of them were isolated from ICUs (61.9%) followed by surgical wards (25%) then burn units (13.05%). This come in accordance with a study performed by **Abdar et al. [16]** who concluded that most of the *A. baumannii* isolates were detected in ICUs and the least were from orthopedic ward. This could be attributed to the increased use of antibiotics in ICUs and the immune suppressed state of the patients that leads to infection with uncommon or opportunistic pathogens, in comparison to other hospital settings.

Most of the bacterial isolates were obtained from blood samples (47.6 %), followed by respiratory samples (sputum, endotracheal aspirates & BAL) (22.6 %), infected wounds (20.2 % isolates), and urine samples (9.5 %). A study performed in Ethiopia by **Araya and coworkers [17]** reported that *A. baumannii* was mostly isolated from blood samples (32.7%) followed by urine samples (19.4%) and least were from CSF (12.6%). In the same context, **Zheng et al.[18]** reported that *A. baumannii* was the commonest Gram-negative bacteria causing blood stream infection. An Egyptian study done by **Fahmy et al. [19]** reported that most of the isolates were retrieved from blood samples (42.86%), followed by wound swabs (23.81%) and sputum (15.47%).

On the other hand, a study performed by **El Edel et al. [20]** in Egypt, found that *A. baumannii* is commonly isolated from the respiratory samples. Also, **Bankan et al. [21]** stated that the majority of the isolates (39%) were from endotracheal tubes, followed by pus samples (24%). The discrepancy of results may be due to different sample size, different hospital settings either word or intensive care units.

In this study, the results of antibiotic susceptibility of tested isolates were as follows: most of the isolates were resistant to ampicillin sulbactam (94%), piperacillin-tazobactam (92.8%), cefotaxime (90.5%), ceftriaxone (89), ceftazidime (88%) while the least resistance was reported for imipenem (70%) and meropenem (72.6 %). Similar results were reported by **Abdar and his colleagues [16]** as the pattern of resistance of the tested isolates in their study was as follows: Meropenem, Gatifloxacin, levofloxacin, ceftazidime, piperacillin-tazobactam, cotrimoxazole and ticarcilin-clavualonic acid was 71%, 89%, 90%, 93%, 94%, 95% and 97%, respectively.

On the other hand, **Basatian-Tashkan et al. [22]** reported lower resistance rate to gentamicin and imipenem, which was 48.4% and 50% resistance, respectively, while the high resistance to piperacillin (100%), ceftazidime (98.4%), amikacin (96.6%) and tetracycline (91.6%) was observed. Also, a study done in Egypt by **El Edel et al. [20]** reported lower resistance to carbapenems

(imipenem and meropenem) as it was 52% and 53% respectively. Similarly, a study was done in Tiwan by **Yang et al. [23]** reported lower resistance to imipenem (65.67%), piperacillin (69.75%), ceftazidime (69.7%), ciprofloxacin (65.8%), gentamicin (60.8%), tigecycline (57.6%), and amikacin (56.17%) and higher resistance was reported to cefepime (96.2%) and sulfamethoxazole-trimethoprim (75.6%). A study performed by **AL-Kadmy et al. [24]** in Iraq reported higher resistance towards ciprofloxacin, levofloxacin and trimethoprim-sulfamethoxazole as all tested isolates were resistant to these antibiotics and more than 90% resistance was detected to tobramycin, tetracycline, cefepime, ceftriaxone, and β-lactams. Resistance to imipenem and meropenem was close to 86%.

In our study, MDR and XDR isolates represented (15.5%) and (75 %), respectively. On the contrary, results reported by **Araya el al. [17]** MDR isolates showed a percentage of 73.7% among the tested isolates, while **Fahmy et al. [19]** reported that all tested isolates were MDR. Another study done by **El Edel et al. [20]** reported that 28% were MDR and 58% were XDR of the tested isolates.

This difference in antibiotic resistance pattern may be due to the possession of different mechanisms of resistance in *A. baumannii* and difference in the triggering factors that initiate the stimulation of expression of these factors in different hospital settings worldwide.

In this study the distribution of the *adeA* gene among clinical isolates was (78.5%) and for *adeS* genes was (72.6%) and the two genes were detected together in (72.6%) of the examined isolates and there was a statistically significant difference between isolates that was resistant to 1 or 2 groups of antibiotics and MDR and XDR groups regarding *adeA , adesS* gene distribution. Similar results were reported by **Jassim et al. [25]** regarding *adeA* gene as they found that this gene is present in 77.4 % of their tested isolates.

Higher results were reported by **Basatian-Tashkan and coworkers [22]** as they found that 80% of the tested isolates had *adeA* gene and 81.66% had *adeS* gene. **Terkuran et al. [26]** reported the presence of ***adeS*** gene in 68% of their included isolates.

In another study performed in Egypt by **Ramadan et al. [27],** higher result regarding *adeA* gene was found, as they reported its presence in 82% of their tested isolates, while lower result was reported regarding *adeS* gene, as it was present in 64% of the isolates and both genes were found in 64% of isolates.

Mahmoudi el al. [15] examined *adeABC* efflux pump encoding genes among *A. baumannii*, and reported that the frequencies of *adeA*, *adeB*, and *adeC* genes were 86.7%, 90.7%, and 92%, respectively. **JaponiNejad et al. [28] and Khayat et al. [29]** reported the presence of *adeA* in 100% of the tested *A. baumannii* strains. **El Edel et al. [20]** reported that the expression of *adeS* genes among isolates was 88%. Also, **Atasoy et al. [30]** reported that 88% of all acinetobacter isolates carried *AdeS* genes, while **Noori et al. [31]** documented that 91% of the isolates carried that gene, which is higher than results in our study. On the contrary, **Lari et al. [32]** reported lower results, where 36% of the tested isolates carried *adeS* genes.

The variation among these results could be attributed to differences in sample sizes or different methods used for the detection of genes, qualitative or quantitative. However, the high prevalence of the tested genes indicates their possible pivotal role in antibiotic resistance in *A. baumannii.*

In this study, a statistically significant association between the presence of *adeA* gene and resistance to imipenem, meropenem, ciprofloxacin, levofloxacin and amikacin was observed, while for *adeS* gene, a statistically significant association was observed with resistance to imipenem, meropenem, ciprofloxacin, levofloxacin, tobramycin, amikacin, and trimethoprim-sulfamethoxazole. Similar results were found by **Basatian-Tashkan et al. [22]** who concluded that the presence of *adeA* and *adeS* genes are related to the resistance to ciprofloxacin, gentamicin, amikacin, and tetracycline. Also, **Ranjbar et al. [33]** concluded that the presence of *adeABC* genes can provoke the resistance to imipenem and trimethoprim in *A. baumannii* strains. **Jassim et al. [25]** concluded that *adeB* gene and its regulatory system have a role in multidrug and carbapenems resistance in clinical isolates of *A.baumannii.* These results could spotlight that these drugs are substrate for this efflux pump which plays a major role in resistance to them.

Understanding the mechanism of resistance of acinetobacter may provide more help in treatment options and may give a chance to introduce genetic diagnostic tests in laboratories and may help in developing new therapeutic approaches as efflux pump inhibitors.

Conclusion

Based on the findings in our study, there is a high rate of antimicrobial resistance among *A. baumannii, this is alarming and* warrants the rational use of antibiotics and need strict implementation of infection control guidelines to prevent the spread of MDR and XDR in hospital settings. The presence of *adeA* and *adeS* genes poses a pivotal role in resistance among *A. baumannii* isolates to several antibiotics. Further studies to investigate *adeA* and *adeS* genes expression would benefit confirmation of our results.

Limitation of the study

One of limitations of this study is the exclusion of colistin and tetracycline from susceptibility testing. Colistin needs tedious work by performing minimal inhibitory concentration technique. Also, Tetracyclines were excluded because of their broad protein synthesis inhibition which may interfere with the analysis of ade genes' role as efflux pumps. This exclusion may limit the understanding of the full spectrum of antibiotic resistance mechanisms and their correlation with efflux pump activity. Future research should include a broader range of antibiotics for a more comprehensive assessment.

Conflict of interest

All authors declare no conflict of interest in this work.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

References

1- Basatian-Tashkan B, Niakan M, Khaledi M, Afkhami H, Sameni F, Bakhti S, et al. Antibiotic resistance assessment of *Acinetobacter baumannii* isolates from Tehran hospitals due to the presence of efflux pumps encoding genes (adeA and adeS genes) by molecular method. BMC Res Notes 2020; 13(1):543. doi: 10.1186/s13104-020-05387-6.

2- Al-Shaabani M, Al-Ethawi A, Al-Mathkhury H. Eco-friendly synthesis of gold nanoparticles and study their effect with antibiotics against *Acinetobacter baumannii*. Iraqi J Agric Sci. 2020;51(4):1204–11. https://doi.org/10.36103/ijas.v51i4.1099.

3- Khaledi M, Shahini Shams Abadi M, Validi M, Zamanzad B, Vafapour R, Gholipour A. Phenotypic and genotypic detection of metallo-beta-lactamases in *A. baumanii* isolates obtained from clinical samples in Shahrekord, southwest Iran. BMC Res Notes 2019;12(1):597. https://doi.org/10.1186/s13104-019-4636-y.

4- Lucassen K, Muller C, Wille J, Xanthopoulou K, Hackel M, Seifert H, et al. Prevalence of RND efflux pump regulator variants associated with tigecycline resistance in carbapenem-resistant *Acinetobacter baumannii* from a worldwide survey. J Antimicrob Chemother. 2021;76(7):1724–30. https://doi.org/10.1093/jac/dkab079.

5- Gaurav A, Bakht P, Saini M, Pandey S, Pathania R. Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. Microbiology (Reading). 2023 May;169(5):001333. doi: 10.1099/mic.0.001333. PMID: 37224055; PMCID: PMC10268834.

6- Chin CY, Tipton KA, Farokhyfar M, Burd EM, Weiss DS, Rather PN. A high-frequency phenotypic switch links bacterial virulence and environmental survival in *Acinetobacter baumannii*. Nat Microbiol. 2018;3(5):563–9. https://doi.org/10.1038/s41564-018-0151-5 .

7- Abd El-Rahman OA, Rasslan F, Hassan SS, Ashour HM, Wasfi R. The RND Efflux Pump Gene Expression in the Biofilm Formation of *Acinetobacter baumannii*. Antibiotics. 2023; 12(2):419. https://doi.org/10.3390/antibiotics12020419

8- Ardehali SH, Azimi T, Fallah F, Owrang M, Aghamohammadi N, Azimi L. Role of efflux pumps in reduced susceptibility to tigecycline in *Acinetobacter baumannii*. New Microbes New Infect. 2019; 30:100547. PMC6541740]. https://doi.org/10.1016/j.nmni.2019.100547.

9- Zack KM, Sorenson T, Joshi SG. Types and Mechanisms of Efflux Pump Systems and the Potential of Efflux Pump Inhibitors in the Restoration of Antimicrobial Susceptibility, with a Special Reference to *Acinetobacter baumannii*. Pathogens. 2024; 13(3):197. https://doi.org/10.3390/pathogens13030197

10- Tille MP. Bailey and Scott. Diagnostic microbiology 15 ed. Chapter 19; Enterobacterales. 2022; 335-362.

11- Clinical and Laboratory Standards Institute (CLSI) Wayne PA. performance standards for antimicrobial susceptibility testing: 22nd informational supplement. CLSI 2024; document M100-S20.

12- Shi T and Xie L. Distribution and antimicrobial resistance analysis of gram-negative bacilli isolated from a tertiary hospital in Central China: a 10-year retrospective study from 2012 to 2021.Front. Microbiol. 2023 14:1297528. doi: 10.3389/fmicb.2023.1297528

13- Sun J-R, Perng C-L, Chan M-C, Morita Y, Lin J-C, Su C-M, et al. A truncated AdeS kinase protein generated by IS Aba1 insertion correlates with tigecycline resistance in *Acinetobacter baumannii*. PLoS ONE. 2012;7(11): e49534.

14- Lopes B, Amyes S. Insertion sequence disruption of adeR and ciprofloxacin resistance caused by efflux pumps and gyrA and parC mutations in *Acinetobacter baumannii.* Int J Antimicrob Agents. 2013;41(2):117–21.

15- Mahmoudi, H., Shokoohizadeh, L., Zare Fahim, N., Bardebari A.M., Moradkhani S., Alikhani M., Y. Detection of adeABC efflux pump encoding genes and antimicrobial effect of Mentha longifolia and Menthol on MICs of imipenem and ciprofloxacin in clinical isolates of *Acinetobacter baumannii*. BMC Complement Med Ther 20, 92 (2020). https://doi.org/10.1186/s12906-020-02887-7

16- Abdar MH, Taheri-Kalani M, Taheri K, Emadi B, Hasanzadeh A, Sedighi A, et al. Prevalence of extended-spectrum beta-lactamase genes in Acinetobacter spp. strains isolated from nosocomial infections in Tehran, Iran. GMS Hygiene and Infection Control 2019; 14.

17- Araya S, Gebreyohannes Z, Tadlo G, Gessew GT, Negesso AE. Epidemiology and Multidrug Resistance of Pseudomonas aeruginosa and Acinetobacter baumanni Isolated from Clinical Samples in Ethiopia. Infection and Drug Resistance 2023; 16: 2765–2773. https://doi.org/10.2147/IDR.S402894

18- Zheng C, Cai J, Liu H, Zhang S, Zhong L, Xuan N, et al. Clinical characteristics and risk factors in mixed-enterococcal bloodstream infections. Infect Drug Resist 2019; 12:3397-3407

19- Fahmy G, Abdel-Rahman S, Khalifa R, & Mohamed R . The effect of tigecycline-usnic acid combination on tigecycline-non-susceptible *Acinetobacter baumannii* clinical isolates and the role of usnic acid as an adjuvant efflux pump inhibitor. Microbes and Infectious Diseases, 2024; 5(4), 1471-1485. doi: 10.21608/mid.2024.279500.1866

20- El Edel RH, El-Halim A, Fahim E, Diab SM, ElKholy RM. Genetic expression of AdeR and AdeS genes in multidrug resistant Acinetobacter spp., isolated from patients in Menoufia University Hospitals. Microbes and Infectious Diseases 2021 May 1;2(2):352-60.

21- Bankan N, Koka F, Vijayaraghavan R, Basireddy SR, Jayaraman S. Overexpression of the adeB Efflux Pump Gene in Tigecycline-Resistant *Acinetobacter baumannii* Clinical Isolates and Its Inhibition by (+) Usnic Acid as an Adjuvant. Antibiotics. 2021; 10(9):1037. https://doi.org/10.3390/antibiotics10091037.

22- Basatian-Tashkan B, Niakan M, Khaledi M, Afkhami H, Sameni F, Bakhti S, Mirnejad R. Antibiotic resistance assessment of *Acinetobacter baumannii* isolates from Tehran hospitals due to the presence of efflux pumps encoding genes (adeA and adeS genes) by molecular method. BMC Research Notes. 2020 Dec; 13: 1-6.

23- Yang CH, Su PW, Moi SH, Chuang LY. Biofilm formation in Acinetobacter spp.: genotype-phenotype correlation. Molecules 2019; 24(10):1849.

24- AL-Kadmy IM, Ali AN, Salman IM, Khazaal SS. Molecular characterization of *Acinetobacter baumannii* isolated from Iraqi hospital environment.New Microbes New Infect. 2018; 21:51-57

25- Jassim KA, Ghaima KK, Saadedin SM. AdeABC efflux pump genes in multidrug resistant *Acinetobacter baumannii* isolates. Avicenna Journal of Clinical Microbiology and Infection. 2016 Oct 24;3(4):40898-.

26- Terkuran M, Erginkaya Z, Konuray G, Meral M, Ünal N, Sertdemir Y,et al.,Evaluation of Antibiotic Resistance and adeABC, adeR, adeS Efflux Pump Genes among Foodborne and Clinical Acinetobacter spp. in Türkiye. Iran J Public Health.2022;51(12):2753-2763

27- Ramadan AE, Elgazar AS, Amin NA, Allam AH, Elmahdy MA, Eldeen NA et al. Efflux pumps encoding genes (adeA and adeS) in relation to antibiotic resistance pattern in *Acinetobacter baumannii* strains isolated from Benha university hospital. Egypt J Chest Dis Tuberc 2024 Jul 1;73(3):241-7.

28- JaponiNejad AR, Sofian M, Ghaznavi-Rad E. Molecular detection of AdeABC efflux pump genes in clinical isolates of *Acinetobacter baumannii* and their contribution in imipenem resistance. Iran South Med J. 2014;17(5):815–23.

29- Khayat H, Sadeghifard N, Pakzad I, Azimi L, Delfani S, Sayehmiri K, et al. Determination of different fluoroquinolone mechanisms among clinical isolates of *Acinetobacter baumannii* in Tehran. Iran Iranian Red Crescent Medical Journal. 2017; 19(9): e58798.

30- Atasoy AR, Ciftci IH, Petek M, Terzi HA. Investigation of mutations in adeR and adeS

31- gene regions in gentamicine resistant Acinetobacter spp. isolates. Biotechnology & Biotechnological Equipment 2016;30(2):360-367.

32- Noori M, Mohsenzadeh B, Bahramian A, Shahi F, Mirzaei H, Khoshnood S. Characterization, and frequency of antibiotic resistance related to membrane porin and efflux pump genes among *Acinetobacter baumannii* strains obtained from burn patients in Tehran, Iran. Journal of Acute Disease. 2019 Mar 1;8(2):63-6.

33- Lari AR, Ardebili A, Hashemi A. AdeR-AdeS mutations & overexpression of the AdeABC efflux system in ciprofloxacin-

resistant Acinetobacter spp. clinical isolates. The Indian journal of medical research 2018;147(4): 413.

34- Ranjbar R, Zayeri S, Afshar D. High Frequency of AdeA, AdeB and AdeC Genes among *Acinetobacter baumannii* Isolates. Iran J Public Health. 2020;49(8):1539-1545. doi: 10.18502/ijph.v49i8.3898. PMID: 33083331; PMCID: PMC7554404.

Abdel Salam S, Montasser K, Anwar M, Abdelhamid A, Abo El Magd N. Frequency of efflux pump-encoding genes *ade*A and *ade*S in *Acinetobacter baumannii* isolates from Ain Shams University Hospitals. Microbes Infect Dis 2025; 6(1): 329-341.