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Efflux pump gene evaluation in multidrug resistant *Acinetobacter baumannii*

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Abstract
Acinetobacter baumannii is a Gram-negative bacterium that is responsible for causing infection at different site the bacteria have been reported to induce infection in the urinary tracts, wound and burns areas specially in hospitalized cases. The current study embraced collecting specimen from 240 clinical cases to identify *A. baumanni* causes of infection through the period between February 2025 until June 2025 at Tikrit Teaching Hospital. All specimens underwent routine culture and biochemical identification in addition to confirmation via DL microbiology identification kit, Efflux activity was measured via Ethidium bromide method in presence and absence of Efflux inhibitor, in addition, DNA extraction was performed on all isolates to detect the presence of adeA gene. Our results revealed that males to female's ratio was 40:60 while the mean age of them were 47 and 35 years respectively. *A. baumanni* was positive in
13.33the activity of Efflux pump was detected in 78.13% of all 32 *A. baumanni* isolates. The gene responsible for Efflux pump production adeA was found in 84.38% of all isolates. In conclusion, *A. baumanni* Efflux pump was closely matched phenotypically and genotypically in MDR and XDR isolated which greatly support the virulence capability of the bacteria in clinical infections.

Keywords: *A. baumanni*, Efflux pump, adeA gene

Introduction
Acinetobacter baumannii's exceptional capacity to endure harsh environmental conditions and develop resistance to a wide range of antimicrobial agents has made it a significant nosocomial pathogen and a serious threat in healthcare settings, especially in intensive care units (ICUs). In patients who are immunocompromised or critically ill, this Gram-negative, non-fermentative, aerobic coccobacillus is linked to a number of serious infections, such as ventilator-associated pneumonia, bloodstream infections, urinary tract infections, wound infections, and meningitis [1, 2]. Membrane-associated protein complexes called efflux pumps actively extrude antibiotics and other toxic substances from bacterial cells, minimizing intracellular drug
accumulation and efficacy. This is one of the main ways that the bacteria develop and maintains multidrug resistance (MDR). Because the AdeABC, AdeIJK, and AdeFGH systems are known to confer resistance to aminoglycosides, fluoroquinolones, β-lactams, chloramphenicol, tetracyclines, and other substances, the resistance-nodulation-cell division (RND) family of efflux pumps is the most studied among the various families [3, 4]. Overexpression of efflux pump genes is highly associated with an MDR phenotype and treatment failure, frequently as a result of mutations in their regulatory elements, such as adeR and adeS [5]. Interestingly, it has been determined that the AdeABC efflux pump plays a major role in high-level resistance, and clinical isolates with extensive drug resistance (XDR) profiles often exhibit its activity.
Moreover, efflux pumps contribute to pathogenicity and biofilm formation in addition to facilitating resistance, making treatment and eradication tactics even more challenging [6]. The increasing incidence of efflux pump-mediated resistance emphasizes how urgently efflux pump inhibitors (EPIs) as supplemental treatments to regain antibiotic sensitivity, as well as efficient surveillance and diagnostic techniques, are needed. Additionally, horizontal gene transfer events and plasmid-mediated dissemination of efflux pump genes have been discovered in recent genomic investigations, highlighting their role in the swift global development of resistance [7]. Understanding the molecular mechanisms behind efflux pump production and regulation is crucial for developing innovative therapeutic approaches and guiding infection control procedures, especially in light of the global burden of MDR *A. baumanni*. This study was designated to the study the Efflux pump effect antibiotic resistance both phenotypically and genotypically in MDR and XDR resistance to antibiotics *A. baumanni* isolates.

Materials and Methods
The current study was conducted at Tikrit Teaching Hospital and included 240 cases with various clinical condition, specimens involved urine, vaginal swabs as well as wound and burn swabs were obtained from all participants after acquiring written consent form and a questionnaire for patient medical record. The study spans the period between February 2025 until June 2025. Clinical samples submitted to routine culture and identification via biochemical test and further confirmed with DL microbiology diagnostic system (DL biotech. China).

Efflux pump
The activity of efflux pump was assessed in all *A. baumanni* isolates according to Ethidium bromide method in the presence of Efflux pump inhibitor. Bacterial broth was incubated in sterile 96 flat bottom wells plate with ethidium bromide in the present and absent of Efflux inhibitor. The data were measured via ELISA reader at 620nm over 6-hour period.

DNA extraction
DNA extraction was performed on all the isolates following the manufacturer procedure (Genomic DNA extraction,

Promega, USA) in order to identify the gene responsible for Efflux pump.

PCR protocol

The master mix prepared in the following ingredients, 13.5µl G2 green master mix (Promega, USA) with 1µl of 10 pmol for forward and reverse primers (5'-GGGCTGATATTACAGGGGTG- 3' and 5'-CGCCCAGAAATAGGCGCTCG- 3') in addition to 5µl of DNA temples and the volume was calibrated to 25µl with nuclease free water. The PCR mix was transferred into PCR machine (G storm gradient PCR, USA). The amplification protocol was set as initial denaturation at 95° C for 4 min, denaturation at 95° C for15 seconds, annealing 55° C for 30 seconds and extension at 72° C for 1 min for 35 cycles for the last three steps. The PCR products were run on 1.5% agarose gel (Promega, USA) for 1h at 80 volts. The Gel was viewed with UV transilluminator (ClearView, UK) to detect the presence of the 658bp migrated bands.

Ethical approval

The study was gained ethical approval from Ethical Approval Committee at College of Medicine/ University of Tikrit (issue number 3/7/570 at 22/12/2024).

Statistical analysis

The data were analyzed and categorized and analyzed as descriptive tables and comparison were made with Qi square and tTest wherever required via Graph Pad Prisim version 10.1.

Result and Discussion

According to age distribution of *A. baumanni*i-infected male and female as revealed in Table (4.1). The mean age of male 45.92 years (±14.58), was significantly older than females, whose mean age was 37.38 years (±13.22). This age gap implies that older men may be more likely than women to have *A. baumanni*i infections, potentially as a result of immunosenescence, underlying comorbidities, and increased exposure to healthcare environments. The higher mean age of males is consistent with a number of other studies that found that older male patients, especially those in intensive care units (ICUs) and those in need of invasive procedures or mechanical ventilation, had a greater prevalence of the bacterial infections. Distinct immunological responses, occupational exposures, or the greater probability of males undertaking invasive or surgical operations at later ages could all account for this age- and gender-based susceptibility [8, 9]. Additionally, due to the use of broad-spectrum antibiotics, extended hospital stays, and age-related immune function decrease, age has been found to be an independent risk factor for both colonization and infection with multidrug-resistant *A. baumanni*i [10]. The microorganism isolated were mostly obtained from patients over 50, with a larger percentage of male cases, especially among those with respiratory illnesses, according to a study conducted by Liu *et al*. (2022) [11]. Additionally, since it could direct infection control initiatives and risk assessment, this finding has clinical significance. It highlights the significance of focused monitoring and preventative measures for elderly male patients who are at a higher risk of contracting severe or multidrug-resistant bacterial infections. Since antibiotic resistance is becoming a bigger concern worldwide, knowing demographic trends might help develop more effective empirical therapy and antimicrobial stewardship strategies.

Table.4.1: Mean age of patient group

	Males		Females	
Mean age	Mean	±SD	Mean	±SD
	45.92	14.58	37.38	13.22

The data in Table (4.2) illustrated how the distribution of efflux pump activity in isolated of *A. baumanni*i was ascertained by phenotypic expression and the adeA gene, which was an

essential component of the AdeABC efflux pump system. Of the isolated, 78.13% (25/32) exhibited phenotypic indications of active efflux pump production, while 84.38% (27/32) proved positive for the adeA gene by genotypic analysis. This high prevalence indicates the role of efflux pump systems, particularly the AdeABC family, in mediating multidrug resistance (MDR) in the microbe. The resistance-nodulation-cell division (RND) family includes the AdeABC efflux pump, which was controlled by the two-component system adeRS and encoded by the adeA, adeB, and adeC genes. Resistance to a number of antibiotics, including as aminoglycosides, fluoroquinolones, β-lactams, and chloramphenicol, is closely linked to overexpression of this mechanism [3]. Even while the majority of isolates have the adeA gene, not all of them may be actively expressing the efflux system, as evidenced by the fact that a larger number of isolates are genotypically positive (84.38%) than those displaying the phenotypic (78.13%). Environmental factors that impact gene expression levels or regulatory alterations in the adeRS system may be the cause of this disparity [4]. These findings align with earlier studies. According to Roca *et al*. (2012) [12], for example, more than 80% of clinical isolates of *A. baumanni*i included at least one AdeABC efflux pump gene, and the presence of these genes was highly associated with patterns of multidrug resistance [12]. The adeA gene was also found to be present in 87% of MDR *A. baumanni*i isolates investigation by Abbasi *et al*. (2024) [13], confirming its significance as a primary contributor to the resistance phenotype in clinical settings [13]. The significant prevalence of efflux pump-related resistance emphasizes the necessity of ongoing monitoring and the creation of efflux pump inhibitors (EPIs) as possible supplements to current antibiotic treatments. It also highlights how crucial it is to integrate genotypic and phenotypic investigations in order to completely comprehend the mechanisms of resistance and inform the choice of the best course of treatment as depicted in Fig 1,1.

Table 4.2: Comparison of phenotypic and genotypic expression of Efflux pump

Efflux pump production	Phenotype expression		Genotype expression (adeA)	
	No.	%	No.	%
Positive	25	78.13	27	84.38
Negative	7	21.88	5	15.63
Total	32	100.00	32	100.00

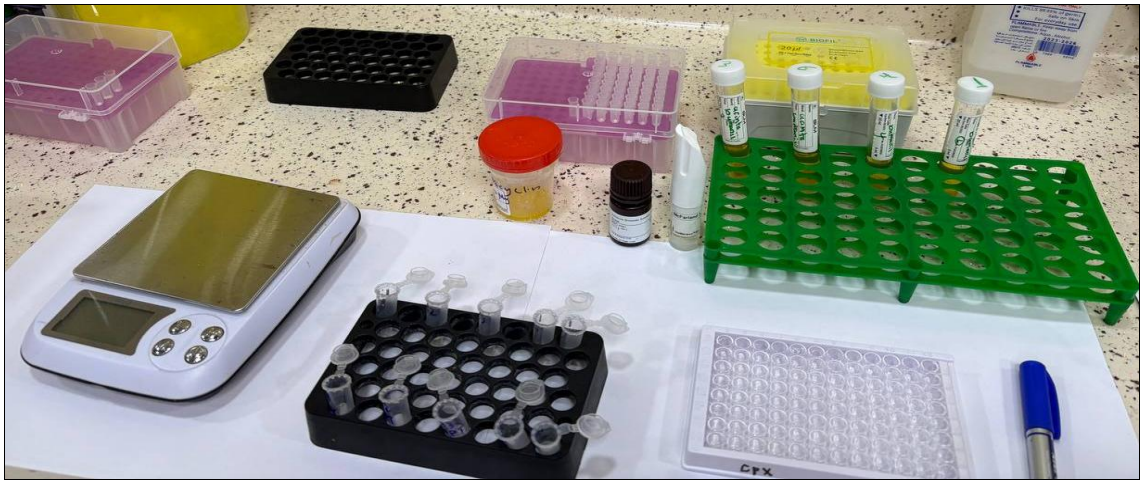

Fig 1.1: Ethidium bromide Efflux pump inhibition protocol on *A. baumanni*i isolates.

Regarding to the data in Table (4.4) illustrated that 84.38% (27/32) of *A. baumanni*i isolated had the adeA gene (Fig 1.1), a crucial part of the AdeABC efflux pump system. This suggests that the virulence-associated gene is highly prevalent among clinical strains. Of the isolated, only 15.63% (5/32) tested negative for the gene. The membrane fusion protein encoded by the adeA gene is essential for the formation of the resistance-nodulation-division (RND) family's functioning AdeABC efflux pump. It was increasingly recognized that this system was essential for antibiotic resistance, particularly to aminoglycosides, fluoroquinolones, and β-lactams, and also plays a part in virulence, biofilm formation, and environmental persistence [3]. The high percentage of adeA gene positive in this sample in line with the results of other recent investigations. According to Haeili *et al*. (2019), for example, adeA was detected in 87% of the bacteria isolated [19], whereas Ali *et al*. (2019) [20] discovered the gene in about 80% of isolates, especially those with multidrug resistance (MDR) profiles [20]. Because mutations in the adeRS two-component system frequently regulate the overexpression of the AdeABC efflux system, there is a strong link between the existence of adeA and resistance phenotypes. Overexpression of this pump is a dual role in resistance and pathogenicity, since it not only lowers intracellular concentrations of drugs but also increases bacterial survival under stress [4]. Furthermore, adeA expression has been linked to a greater capacity for biofilm formation, which is a key virulence mechanism in *A. baumanni*i. According to Eze *et al*. (2018) [15], biofilms allow bacteria to survive on surfaces like wounds, endotracheal tubes, and catheters, which leads to persistent infections and resistance to both immunological responses and medications [15]. The significance of incorporating efflux pump genes in molecular surveillance programs for virulence and antibiotic resistance is highlighted by the prevalence of the adeA gene among clinical isolates. It also implies that in order to increase efficacy and prevent the emergence of resistance, future therapeutic strategies may benefit from the combination of efflux pump inhibitors (EPIs) and conventional antibiotics.

Table.4.4. Virulence genes of *A. baumanni*i

Virulence Genes	Positive		Negative		Total	
	No.	%	No.	%	No.	%
adeA gene	27	84.38	5	15.63	32	100.00

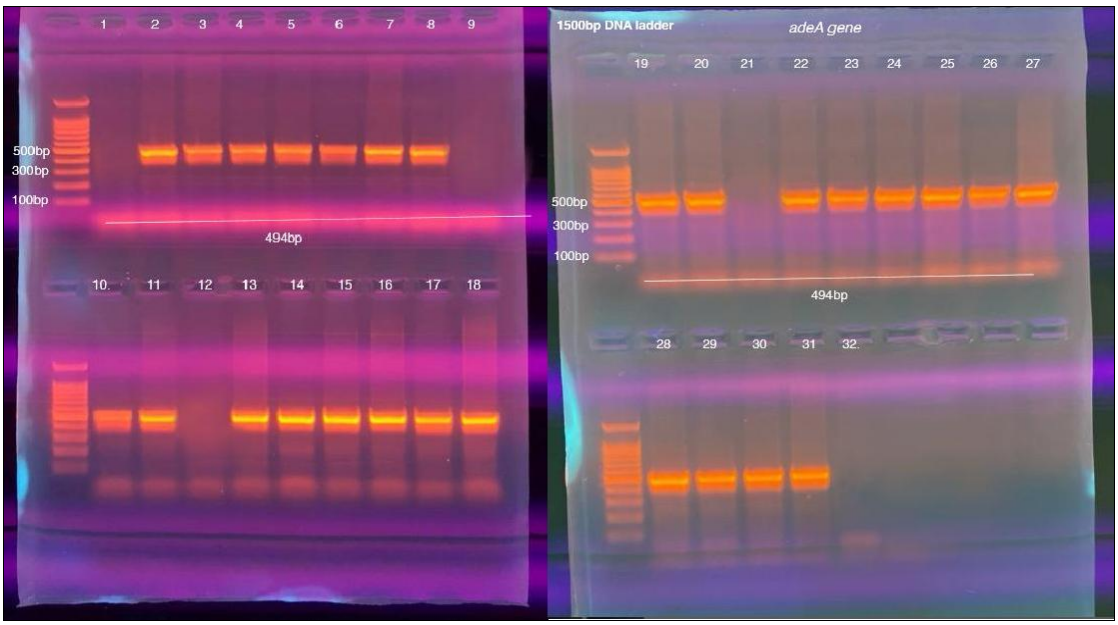

Fig 1.2: PCR amplification of Efflux adeA gene, PCR products run on 1.5% agarose gel at 80v for 1 hour. 100pb control DNA ladder was used as control for band size.

References

1. Harding CM, Hennon SW, Feldman MF. Uncovering the mechanisms of *Acinetobacter baumannii* virulence. Nature Reviews Microbiology,2018:16(2):91–102.
2. Ahuatzin-Flores OE, Torres E, Chavez-Bravo E. *Acinetobacter baumannii*, a multidrug-resistant opportunistic pathogen in new habitats: a systematic review. Microorganisms, 2024, 12(4).
3. Coyne S, Courvalin P, Périchon B. Efflux-mediated antibiotic resistance in Acinetobacter spp. Antimicrobial Agents and Chemotherapy,2011:55(3):947–953.
4. Yoon EJ, Jeong SH. Mobile carbapenemase genes in Pseudomonas aeruginosa. Frontiers in Microbiology,2021:12:614058.
5. Hood MI, Skaar EP. Nutritional immunity transition metals at the pathogen–host interface. Nature Reviews Microbiology,2012:10(8):525–537.
6. Kubin CJ, Garzia C, Uhlemann A-C. *Acinetobacter baumannii* treatment strategies a review of therapeutic challenges and considerations. Antimicrobial Agents and Chemotherapy,2025:0(0):01063-24.
7. Lee C-R, Lee JH, Park M, Park KS, Bae IK, Kim YB, *et al*. Biology of *Acinetobacter baumannii*, pathogenesis, antibiotic resistance mechanisms, and prospective treatment options. Frontiers in Cellular and Infection Microbiology, 2017, 7.
8. Tasdogan AM. Risk factors of mortality from *Acinetobacter baumannii* infection in intensive care units. Eurasian Journal of Medical Investigation, 2019.
9. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii* emergence of a successful pathogen. Clinical Microbiology Reviews,2008:21(3):538–582.
10. Weiner LM, Webb AK, Limbago B, Dudeck MA, Patel J, Kallen AJ, *et al*. Antimicrobial-resistant pathogens associated with healthcare-associated infections summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. Infection Control and Hospital Epidemiology,2016:37(11):1288–1301.
11. Liu C, Qin S, Xu H, Xu L, Zhao D, Liu X, *et al*. Epidemiological and genetic characteristics of clinical carbapenem-resistant *Acinetobacter baumannii* strains collected countrywide from hospital intensive care units' ICUs in China. Emerging Microbes and Infections,2022:11(1):1730–1741.
12. Roca I, Espinal P, Vila-Farrés X, Vila J. The *Acinetobacter baumannii* oxymoron. commensal hospital dweller turned pan-drug-resistant menace. Frontiers in Microbiology,2012:3:148.
13. Abbasi A, Hajihashemi B, Shokri D. Molecular mechanisms of colistin resistance mediated by pmrCAB genes in *Acinetobacter baumannii* isolated from patients hospitalized in Isfahan medical centers. Postępy Higieny i Medycyny Doświadczalnej,2024:78(1):52–57.
14. Gedefie A, Demsis W, Ashagire M, Shiferaw A, Belachew F, Tarko S, *et al*. *Acinetobacter baumannii* biofilm formation and its role in disease pathogenesis a review. Infection and Drug Resistance,2021:14:3711–3719.
15. Eze EC, Chenia HY, El Zowalaty ME. *Acinetobacter baumannii* biofilms. effects of physicochemical factors, virulence, antibiotic resistance determinants, gene regulation, and future antimicrobial treatments. Infection and Drug Resistance,2018:11:2277–2295.
16. Javadi K, Soleimani M, Farahani A, Shojaei H, Douraghi M, Karami P. Investigating the link between biofilm formation and antibiotic resistance in clinical isolates of *Acinetobacter baumannii*. International Journal of Microbiology,2025:2025(1):1009049.
17. Pormohammad A, Mehdinejadiani K, Ghotaslou R, Nasiri MJ, Dabiri H, Azimi T, *et al*. Global prevalence of colistin resistance in clinical isolates of *Acinetobacter baumannii* a systematic review and meta-analysis. Microbial Pathogenesis,2020:139:103887.
18. Gaddy JA, Actis LA. Regulation of *Acinetobacter baumannii* biofilm formation. Future Microbiology,2009:4(3):273–278.
19. Haeili M, Kafshdouz M, Feizabadi MM. Molecular mechanisms of colistin resistance among pandrug-resistant isolates of *Acinetobacter baumannii* with high case-fatality rate in intensive care unit patients. Microbial Drug Resistance,2018:24(9):1271–1276.
20. Ali MH, Abdelrahim SS, Alnour TM, Ali AM, Adam IA, Koko WS, *et al*. Predictors of multidrug-resistant tuberculosis MDR-TB in Sudan. Antibiotics, 2019, 8(3).