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Molecular Detection of *adeL, adeR and adeS* Genes of *Acintobacter baumannii* Isolated from Different Clinical Samples

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Abstract: The emergence of multidrug-resistant *Acinetobacter baumannii* has become a global concern, particularly for patients susceptible to burn, blood bacteremia, and wound infections. In this research endeavor, was collected and analyzed 150 clinical samples from various sites within Baghdad/Iraq hospitals, specifically the Laboratory of Medical City, spanning from October 2022 to March 2023. Through meticulous culturing procedures, *A. baumannii* isolates were identified via microscopic examination and biochemical assays and Vitek-2 tests. To further validate the identification, a molecular approach was employed, confirming the presence of the *16S-rRNA* gene in (n=32) of the *A. baumannii* isolates using PCR analysis. This molecular verification reinforced outcomes obtained through conventional diagnostic techniques including culture, biochemical tests, chromagar selective media and Vitek-2 tests. An exhaustive antibiotic susceptibility evaluation was performed, revealing multi-drug resistance in 32 isolates. Significantly, all isolates exhibited the presence of the *adeL* gene, while 31 and 22 isolates carried the *adeR* and *adeS* genes respectively. This study was concluded comprehensive insights into the prevalence of multidrug-resistant *A. baumannii* in Baghdad hospitals. It was concluded highlighting the significance of molecular techniques in accurate identification and understanding antibiotic resistance patterns.

Keywords: Multi-Drug Resistance, *adeR, adeS*, *adeL* , *Acinetobacter baumannii*.

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Introduction

Acinetobacter baumannii, a nonfermenting coccobacillus, holds a significant position in the realm of human health as an oxidase-negative, aerobic bacillus and an opportunistic extracellular pathogen. Often traced back to hospital-acquired infections, it is synonymous with nosocomial infections, adding complexity to healthcare challenges. Remarkably, the Gramnegative bacterium, *Acinetobacter* baumannii, has garnered attention with the moniker "Iraqibacter," reflecting its emergence in US military treatment facilities stationed in Iraq (17).

In the global healthcare landscape, *A.baumannii* has rapidly assumed a daunting role, emerging as a pivotal concern for medical institutions. Its persistent antibiotic resistance has propelled it to the forefront of challenges requiring immediate intervention. The urgency of this issue is underscored by its current status atop the priority pathogens list for the development of novel antibiotics. (1, 2, 13). The outstanding survival properties and antibiotic resistance of *A. baumannii* are strongly associated with its efflux pumps systems. The pathogen was shown to have various efflux

pumps systems, including (MATA, RND, SMR, ABC, MF) (3). As a class of important virulence factors in bacteria These pumps have been shown to be involved in resistance to a broad range of antibiotics, as well as other toxic compounds such as detergents, disinfectants, and heavy metals (4). proving the importance of *adeL,adeR, adeS* regulatory genes of efflux pumps systems in resistance development Thus, targeting *adeL,adeR, adeS genes* would be a novel therapeutic strategy for combating the threat of drug resistant *A. baumannii* strains. (5).

Materials and methods

Bacterial isolation and identification

A total of one hundred and fifty samples were gathered within the vicinity of Baghdad, Iraq, specifically from Medical City's Center Health Laboratory. These samples, spanning the period from October 2022 to March 2023, underwent a meticulous cultivation process on appropriate culture mediums. Subsequent microscopic scrutiny and biochemical testing led to the identification of 32 isolates belonging to *A. baumannii*. To further validate the identification, both the VITEK-2 system and a molecular technique centered around the *16s-rRNA* gene were employed. Through this combined approach, all the 32 isolates confirmed as *A. baumannii* isolates, ensuring precision and consistency in the identification process.

Antibiotic susceptibility test

The assessment of antibiotic sensitivity involved the utilization of AST-N222 cards in conjunction with the VITEK 2 Compact Instrument. This comprehensive analysis encompassed a range of antimicrobial agents, including: Amikacin (AK), Aztreonam (AZT), Cefepime(CPM),Ceftazidime(CAZ),Trimethoprim/Sulfamethoxazole(TMP/SMX), Gentamicin (GM), Imipenem (IMI), Ticarcillin/clavulanate (TIM), Tobramycin (TM), Rifampicin (RA), Colistin (CS), Pefloxacin (PEF), Ciprofloxacin (CIP), Meropenem (MEM), Piperacillin (PRL), Minocycline (MNO), and Piperacillin/tazobactam (PTZ).(6).

Molecular method DNA extraction

The extraction of genomic DNA from bacterial growth was achieved through the application of the Transgene Genomic DNA Purification Kit method. This kit's versatility extends to facilitating DNA isolation from diverse sample types. Specifically, the DNA extraction process adhered to the bacterial protocol designed for gram-negative bacteria, as stipulated by the kit's instructions. This ensured the successful isolation of genomic DNA from the bacterial specimens under investigation.

Estimation of extracted DNA and concentration

The estimation of the concentration of the extracted DNA was done by using the Quantus™ Fluorometer Single-Tube Format Protocol (7).

Conventional PCR for *16S-rRNA* and *adeL, adeR, adeS* genes

The identification of each target gene was achieved through the utilization of the standard polymerase chain reaction (PCR) procedure, facilitated by specific primers. Notably, the specific primers employed were designed for the 16S-rRNA gene, as well as the adeL and *adeR, adeS* genes as outlined in (Table 1). Each PCR reaction was composed of a 50µl volume, incorporating nuclease-free water to reach the final volume. The reaction mixture comprised 1µl of each primer, with a concentration of 10 picomoles/µL, along with 25µl of Go Taq® green master mix 2X sourced from Transgene (China). Furthermore, 5 µl of DNA template at a concentration of 100 ng/µl was included. Following

vortexing and centrifugation of the extracted DNA, the samples were maintained at a temperature of 4°C to ensure stability. Negative control experiments, devoid of DNA, consisted of the same components as the standard PCR reaction. The PCR protocols were executed on the Thermal cycler gradient PCR system from Thermo Fisher (USA). The amplification conditions were as follows: an initial denaturation step at 94°C for five minutes, succeeded by 35 cycles of denaturation at 94°C for 30 seconds, followed by a series of annealing steps temperature of (adeR, adeL) at 56°C and annealing steps temperature of (16s-rRNA, and *adeR, adeS*) is 51°C for 30 seconds each. A subsequent extension steps was conducted at 72°C for one minute (repeated for 30 cycles), followed by a final extension at 72°C for 10 minutes as shown in (Table 2).

The resultant PCR products were then visualized through agarose gel electrophoresis utilizing a 2% agarose gel.

Table (2): Conventional PCR thermal program.

Thermal cycler PCR Program			
Steps	**Temp. °C**	**M : s**	**Cycle**
Initial Denaturation (hold)	94	05:00	1
Denaturation	94	00:30	35
Annealing	51for (adeR, adeL) and 56 for (adeS,16s-rRNA)	00:30	
Extension	72	01:00	
Final extension	72	10:00	1

Results and discussion

Acinetobacter baumannii distribution

Based on type of sample **(**Table 5) shows that out of a total of 150 clinical samples, (32) *A. baumannii*. Wounds, burns, sputum, and peritoneal fluid from hospitalized patients were among the sources for the collected samples (Table 3) This result was close to previous study in 2022 by Sara Y. AL-falahat (16).

Identification of *A. baumannii*

A total of 32 clinical isolates were validated as *A. baumannii* through a dual verification process involving the Gram-negative strain identification card integrated within the VITEK 2 system, chromagar selective media(12), as well as the analysis of the 16s-rRAN gene (10) as shown in (figure 1).

Susceptibility to antibiotic test

Utilizing the automated VITEK 2 Compact system, were conducted a comprehensive assessment of antibiotic resistance in the context of all 32 *A. baumannii* isolates. Each individual isolate was cultivated within a McFarland 0.5 standard (11) suspension comprising 0.45% sodium chloride on MacConkey agar plates. The VITEK apparatus was subsequently charged with a liquid solution containing the entirety of the isolated substances. Leveraging the Gram-negative susceptibility card inherent to the VITEK 2 Compact instrument, were scrutinized the efficacy of 14 antibiotics in targeting distinct bacterial strains, evaluating their potential to counteract bacterial growth (11) refer to (Table 4).

Table (3): Prevalence of *A. baumannii* isolates among clinical samples. No (150).

Source	No. of sample	No. and % of *A. baumannii isolates*
Blood	80 (53.3%)	21(14%)
Wound	40 (26.4%)	7(4.6%)
Burns	30 (20%)	4(2.6%)
total	150 (100%)	32 (21.3 %)

Table (4): Antimicrobial Susceptibility of 41 *A. baumannii* Isolates to different Antimicrobial Agents.

Antibiotic	Resistant	Intermediate	Sensitive
Ticarcillin	32(100%)	0 (0.0%)	0 (0.0%)
Ticarcillin /clavulanic acid	32(100%)	0 (0.0%)	0 (0.0%)
Piperacillin (PRL)	32(100%)	0 (0.0%)	0 (0.0%)
Piperacillin/tazobactam(PTZ)	32(100%)	0 (0.0%)	0 (0.0%)
Ceftazidime(CAZ)	32(100%)	0 (0.0%)	0 (0.0%)
Cefepime(CPM)	32(100%)	0 (0.0%)	0 (0.0%)
Imipenem	28(87. 5%)	0 (0.0%)	4(12.5%)
Meropenem	26(81.25%)	0 (0.0%)	6(18.75%)
Amikacin	32(100%)	0 (0.0%)	0 (0.0%)
Gentamicin41	29 (90.3%)	3(9.7%)	0 (0.0%)
Tobramycin	29(90%)	3(9.7%)	0 (0.0%)
Ciprofloxacin	32(100%)	0 (0.0%)	0 (0.0%)
Pefloxacin	32(100%)	0 (0.0%)	0 (0.0%)
Minocycline	0 (0.0%)	19(58.5%)	13(41.5%)
Colistin	0 (0.0%)	0 (0.0%)	32(100%)
Trimethoprim Sulfamethoxazole	32(100%)	0 (0.0%)	0 (0.0%)

Presence of *adeL* and *adeR,adeS* .

Culture, biochemical, and Vitek-2 assays all agreed that all 32 (100%) are *A. baumannii* isolates, but PCR analysis proved it by *16s-rRAN* gene. Amplicons of this gene, which were generated during the electrophoresis method (8), ran clearly at 137 bp on agarose (Figure 1). All 32 multidrug-resistant *A. baumannii* isolates were tested with a PCR technique to identify their efflux pump regulatory gene. A PCR experiment using specific primers was performed to identify a single gene. The findings showed that 22, 31isolates possessed the (***adeS, adeR***) respectively as shown in (Figure 2), Results were reported in a study by Hassan *et al.* (6) the presence of adeR,adeS gene in their isolates were 96.8% ,63.4% respectively (6). Results were in this study The presence of genes in their isolates were adeR 31(96.8%) and adeS 22(68.7%) respectively. adeL was confermed in all 32 isolates (Figure 3).

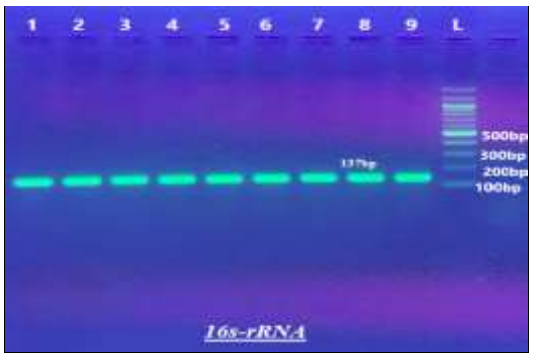

Figure (1): The results of the amplification of 16s-rRNA gene of *Acinetobacter baumannii* samples were fractionated on 2% agarose gel electrophoresis stained with red-save stain. L: 100bp ladder marker. Lanes 1-9 resemble 137 bp PCR product.

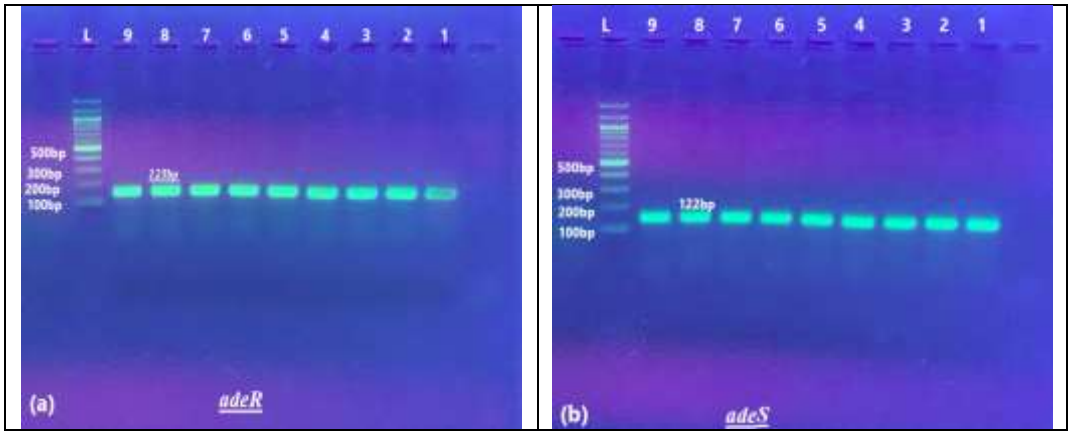

Figure (2): The results of the amplification of (a)*adeR*, (b) *adeS* gene of *Acinetobacter baumannii* samples were fractionated on 2% agarose gel electrophoresis stained with red-save stain. L: 100bp ladde marker. Lanes 1-9 resemble bp PCR product.

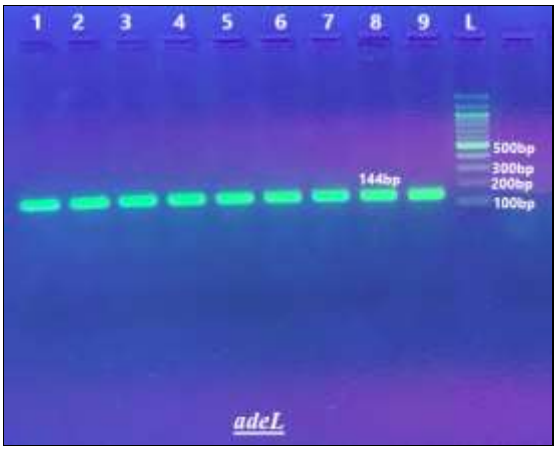

Figure (3): The results of the amplification of *adeL* gene of *Acinetobacter baumannii* samples were fractionated on 2% agarose gel electrophoresis stained with red-save stain. L: 100bp ladder marker.
Lanes 1-9 resemble 144 bp PCR product

Conclusion

This study demonstrated the role of 16srRNA genes for molecular detection *A.baumannii* at the level of genus and species respectively. This study revel the existence and distribution of *adeL, adeR, adeS* genes in *A.bumannii* isolates in Iraqi hospitals.

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