

**MOLECULAR DETECTION OF BLAOXA-23 GENES IN ACINETOBACTER BAUMANNII ISOLATED FROM URINARY TRACT INFECTIONS IN IRAQI PATIENTS****Ahmed Khalid Ahmed Alhamrah<sup>1\*</sup>, Güner Ekiz Dinçman<sup>2</sup>, Hassan Ali Hussein Al-Saadi<sup>3</sup>**<sup>1</sup>Department of Medical Laboratory Technique, Dejlah University, Iraq.<sup>2</sup>Department of Pharmaceutical Microbiology, Near East University Faculty of Pharmacy, Nicosia, North Cyprus.<sup>3</sup>College of Applied Medical Sciences/University of Kerbala, Iraq.

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**ABSTRACT**

This study aimed to identify risk factors for urinary tract infections (UTIs) among hospitalized patients in Iraq, with a particular focus on antibiotic-resistant *Acinetobacter baumannii* isolates, and to determine the presence of virulence factors and resistance genes, especially *blaOXA-23*. A total of 250 clinical urine samples from UTI patients were collected, and bacterial identification was performed using conventional microbiological techniques. Antibiotic susceptibility testing and PCR analysis for virulence genes were conducted, along with biofilm formation assays and sequencing. *A. baumannii* was isolated from 20% (50/250) of the samples, with all isolates exhibiting Gram-negative coccobacilli morphology and typical biochemical characteristics. PCR confirmed the presence of the *blaOXA-23* gene in all isolates. Most beta-lactam antibiotics had resistance rates above 95%, and imipenem had resistance rates as high as 60%. However, 95% of the isolates were still sensitive to colistin. Biofilm formation was detected in 72% of isolates, with 22% demonstrating strong biofilm-forming capacity. These findings reveal a high prevalence of multidrug resistance and virulence traits in *A. baumannii* isolates from UTI patients in Iraq, emphasizing the need for urgent infection control interventions and the development of targeted therapeutic strategies.

**KEYWORDS:** *Acinetobacter baumannii*, *blaOXA-23*, urinary tract infections, multidrug resistance, biofilm, Iraq.**1. INTRODUCTION**

UTIs are the most common bacterial infections that befall people all over the world especially hospitalized and immunocompromised populations. These infections are a major problem of public health because they are associated with a high incidence, recurrence and the increasing cases of multidrug-resistant (MDR) pathogens. Clinical management of UTIs has been made more complex by the fact that resistance against antibiotics that are usually used in the treatment of this condition has been on the increase particularly in Gram-negative bacteria.<sup>[1]</sup> *Acinetobacter baumannii*, a non-fermentative, opportunistic, Gram-negative coccobacillus is one of the most frequent pathogens related to nosocomial UTIs that has attracted the attention of the

world population as a leading cause of healthcare-associated infections.<sup>[2]</sup>

The key feature of *Acinetobacter baumannii* is its outstanding capacity to survive in the hospital setting, withstand desiccation and disinfectants, and develop resistance determinants by horizontal gene transfer. These characteristics have led to its continued presence in the clinical environment and its roles in numerous infections, such as pneumonia, bacteremia, wound infections, and especially in the urinary tract infections. Increasing cases of *A. baumannii* infection in Iraq and other developing nations have been of concern because of a limited treatment regimen and an emerging trend of carbapenem-resistant strains.<sup>[3]</sup>

The resistance of *A. baumannii* to carbapenem is primarily due to the synthesis of OXA-type carbapenemases, which are a category of 21 6-lactamases that are able to hydrolyze carbapenem antibiotics. One of the most common and clinically meaningful carbapenem resistance determinants in the world, and one of these, is the blaOXA-23 gene.<sup>[1]</sup> It has been reported that the blaOXA-23 has spread to numerous areas such as Middle East and this is a fundamental challenge to infection control interventions. The gene is frequently linked with portable genetic components including plasmids and transposons that make the transfer of the gene across the bacterial strains easier, promoting the transmission of the resistance.<sup>[4]</sup>

Besides antibiotic resistance *A. baumannii* is able to possess various virulence factors that contribute to its pathogenicity. These are biofilm formation, surface motility and generation of outer membrane proteins which enhance adherence and a persistence within host tissues. Particularly, biofilm formation is a major cause of chronic infections and antimicrobial drug resistance because bacteria cells in biofilms are much more resistant to antibiotics and immune-based responses.<sup>[3]</sup>

As the clinical importance of the *A. baumannii* is on the rise in the context of hospital-acquired infections, there is an immediate need to comprehend its resistance patterns and virulence patterns, in particular in the regions where surveillance data are scarce. In Iraq, there are limited studies on the molecular detection of resistance genes, including blaOXA-23, in the *A. baumannii* isolates in urinary tract infections. Thus, the current research was implemented to identify the *A. baumannii* prevalence in UTIs among the Iraqi patients, to examine their pattern of antibiotic resistance, biofilm formation, as well as to identify the presence of blaOXA-23 gene by the use of molecular tests. The results should add some valuable information regarding the epidemiology of multidrug-resistant *A. baumannii* and contribute to the effective work on the prevention of infection and antibiotic stewardship.<sup>[5]</sup>

## 2. MATERIALS AND METHODS

### 2.1. Sample Collection

A total of 250 clinical urine samples were collected from hospitalized patients diagnosed with a urinary tract infection (UTI) from January 2024 to May 2024. The study was conducted at Al-Yarmouk Teaching Hospital and Baghdad Medical City Hospital in Iraq. Midstream urine was selected for sample collection as it is the most reliable representation of bacterial presence in UTIs. All samples were collected in sterile containers and transported immediately to the laboratory, where they were processed within two hours to preserve sample integrity. The samples included individuals from various demographic groups and age ranges, with a focus on UTIs caused by multidrug-resistant *Enterobacteriaceae*.<sup>[6]</sup>

### 2.2. Isolation and Identification of *A. baumannii*

Urine samples were inoculated onto MacConkey agar and blood agar, followed by incubation at 37 °C for 24 hours. Suspected colonies were subjected to Gram staining and standard biochemical tests including oxidase, catalase, urease and motility assays, as well as IMViC tests (Indole test, Methyl red test, Voges-Proskauer test, and Citrate test) and lactose fermentation test. Isolates that were non-motile, oxidase-negative, and Gram-negative coccobacilli were preliminarily identified as *A. baumannii*. Identification was confirmed through additional biochemical profiling and molecular methods. Subsequently, all the samples were confirmed by VITEK2.<sup>[7]</sup>

### 2.3. Antimicrobial Susceptibility Testing

The VITEK® 2 Compact system was used to evaluate the antimicrobial susceptibility of 100 *A. baumannii* isolates against 14 different antibiotics.<sup>[8]</sup>

### 2.4. Biofilm Formation Assay

Biofilm formation was assessed using the microtiter plate method. Overnight cultures of isolates were diluted and inoculated into sterile 96-well polystyrene microtiter plates containing tryptic soy broth supplemented with 1% glucose. After incubation at 37 °C for 24 hours, wells were washed, fixed, stained with crystal violet, and the absorbance was measured at 570 nm to quantify biofilm biomass. Isolates were classified as strong, moderate, or weak biofilm producers based on optical density values.<sup>[7]</sup>

### 2.5. DNA Extraction and PCR Amplification

Genomic DNA was extracted from confirmed isolates using a commercial bacterial DNA extraction kit (e.g., Qiagen, Germany), following the manufacturer's instructions. PCR amplification was performed to detect the blaOXA-23 gene using specific primers (forward: 5'-GATCGGATTGGAGAACAGA - 3', reverse: 5'-ATTTCTGACCGCATTCCAT -3') targeting a 501-bp region. The PCR conditions consisted of initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at appropriate

temperature (e.g., 55 °C) for 30 s, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min. PCR products were analyzed using agarose gel electrophoresis.<sup>[9]</sup>

## 2.6. Sequencing Analysis

Selected PCR-positive samples were sent for DNA sequencing to confirm the identity of the amplified blaOXA-23 gene. The resulting sequences were compared with known sequences in the NCBI GenBank database using BLAST analysis for confirmation.<sup>[10]</sup>

## 2.7. Statistical Analysis

The study results were statistically analyzed using IBM SPSS Statistics version 27.0 (Armonk, NY, USA). Graphs were generated with GraphPad Prism version 9.4.1 (San Diego, CA, USA). Categorical variables were expressed as numbers and percentages, while continuous variables were presented as mean  $\pm$  standard deviation (SD). An independent *t*-test was used to compare two groups. One-way analysis of variance (ANOVA) was performed to compare multiple groups. A *p*-value of less than 0.05 was considered statistically significant, and a *p*-value of less than 0.01 was considered highly significant.<sup>[11]</sup>

## 3. RESULTS

### 3.1. Sample Collection and Initial Culturing

A total of 250 urine samples were collected from patients clinically diagnosed with urinary tract infections (UTIs).

**Table 1: Identification of *A. baumannii* by Biochemical Tests.**

No.	Results	Biochemical Test
1	Negative	Gram Stain
2	Coccobacilli	Microscopic Shape
3	Positive	Growth at 44°C
4	Negative	Lactose Fermentation
6	Negative	Oxidase Test
7	Positive	Catalase Production Test
8	Positive	Methyl Red
9	Positive	Citrate Utilization
10	Negative	Voges-Proskauer
11	Negative	Indole Production
12	Variable	Urease Production

+ = Positive result, - = Negative result

**Table 2: Results of the biochemical test to *Acinetobacter baumannii* by using the VITEK-2 system.**

Test Type	Result										
APPA	-	ADO	-	PyrA	-	IARL	-	Dcel	+	BGAL	-
H2S	-	BNAG	-	AGLTp	-	dGLU	+	GGT	-	OFF	-
BGLU	-	dMAL	-	dMAN	-	dMNE	+	BXYL	-	BAlap	-
ProA	-	LIP	-	PLE	-	TyrA	+	URE	-	dSOR	-
SAC	-	dTAG	-	dTRE	-	CIT	+	MNT	-	5KG	-
ILATk	+	AGLU	-	SUCT	+	NAGA	-	AGAL	-	PHOS	-
GlyA	-	ODC	-	LDC	-	IHISe	+	CMT	+	BGUR	-
O129R	+	GGAA	-	IMLTA	-	ELLM	-	ILATa	-		

\*+ = Positive result, \*- = Negative result

Among these, 150 samples (60%) yielded positive bacterial growth, while 100 (40%) showed no growth. Of the positive cultures, 50 isolates (20% of total samples) were identified as *Acinetobacter baumannii*, and the remaining 100 samples revealed growth of other bacterial species.

## 3.2. Identification of *A. baumannii*

Initial identification of the 50 *A. baumannii* isolates was carried out using standard cultural, morphological, and microscopic techniques. On MacConkey agar, colonies appeared pale pink and non-lactose fermenting, while blood agar revealed smooth, round, cream-colored colonies with increased mucoid appearance after prolonged incubation. Microscopic examination using Gram staining confirmed Gram-negative coccobacilli arranged singly, in pairs, or short chains.

Biochemical characterization (Table 1) demonstrated that all isolates were catalase-positive, oxidase-negative, and capable of growing at 44°C. The isolates tested negative for indole, Voges-Proskauer, and urease production (variable results), while positive for citrate utilization and methyl red. These conventional tests were corroborated by confirmatory identification using the VITEK® 2 Compact GN ID card, which classified all isolates as *A. baumannii* with over 99% confidence (Table 2).

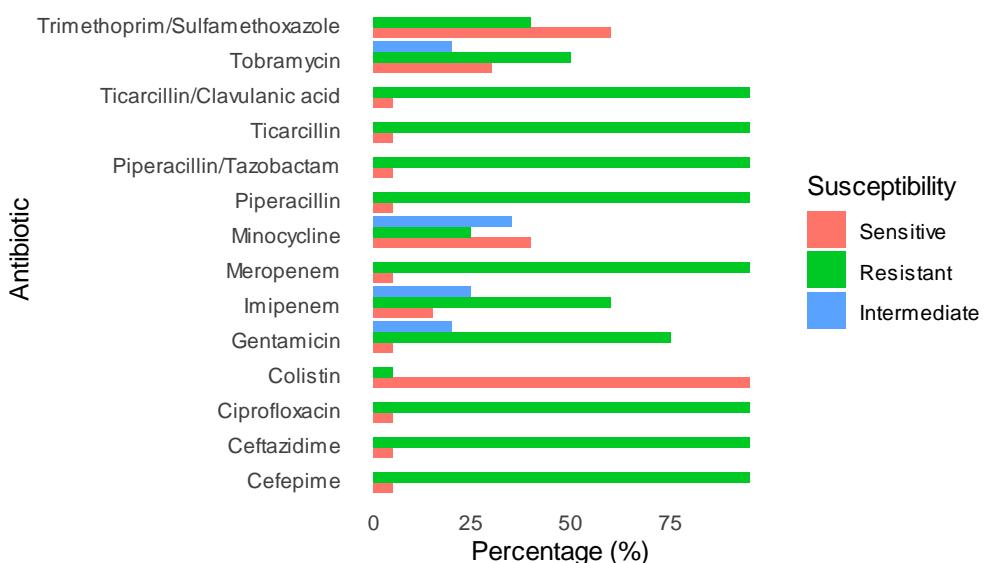
### 3.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing using the VITEK® 2 Compact system was performed against 14 antibiotics (Table 3, Fig. 1). High resistance rates (95%) were observed for beta-lactams including Ticarcillin, Piperacillin, and Meropenem, as well as for fluoroquinolones (Ciprofloxacin). Imipenem showed 60% resistance, 25% intermediate resistance, and 15%

susceptibility. Among aminoglycosides, resistance to Gentamicin was 75%, while Tobramycin had lower resistance (50%) and 30% susceptibility. Colistin exhibited the highest efficacy, with 95% of isolates being sensitive. Trimethoprim/Sulfamethoxazole showed a moderate profile, with 60% sensitivity and 40% resistance.

**Table 3: Percentage distribution of antimicrobial susceptibility test results for *A. baumannii* isolates against 14 antibiotics.**

Antibiotic	Sensitive (%)	Resistant (%)	Intermediate (%)
Ticarcillin	5%	95%	0%
Ticarcillin/Clavulanic acid	5%	95%	0%
Piperacillin	5%	95%	0%
Piperacillin/Tazobactam	5%	95%	0%
Ceftazidime	5%	95%	0%
Cefepime	5%	95%	0%
Imipenem	15%	60%	25%
Meropenem	5%	95%	0%
Gentamicin	5%	75%	20%
Tobramycin	30%	50%	20%
Ciprofloxacin	5%	95%	0%
Minocycline	40%	25%	35%
Colistin	95%	5%	0%
Trimethoprim/ sulfamethoxazole	60%	40%	0%



**Fig. 1: Antimicrobial susceptibility tests of *A. baumannii* isolates against 14 antibiotics.**

### 3.4. Multidrug Resistance Profiling

The isolates were classified into multidrug-resistant (MDR) and extensively drug-resistant (XDR) categories based on CDC guidelines (Table 4). Nine isolates (45%) were identified as MDR, exhibiting resistance to at least

three classes of antibiotics. Eleven isolates (55%) were classified as XDR, showing resistance to all tested antibiotics except one or two classes, highlighting a significant clinical challenge.

**Table 4: Distribution of isolates according to multidrug-resistant categories.**

Categories	No. of isolates	No. and percentage %
<b>MDR</b>	AK17, 6, 8, 15, 9, 11, 2, 13, 1	n=9 (45%)
<b>XDR</b>	AK 5,3,4,18,16,14,19,10,20,7,12	n=11(55%)

### 3.5. Biofilm Formation Capacity

Biofilm production was observed in all 50 isolates using OD measurements at 630 nm (Table 5). Based on absorbance values, 22% of isolates were classified as

strong biofilm producers, 72% as moderate, and 6% as weak. The robust biofilm-forming ability among most isolates may contribute to their persistence in clinical environments and resistance to antimicrobial therapy.

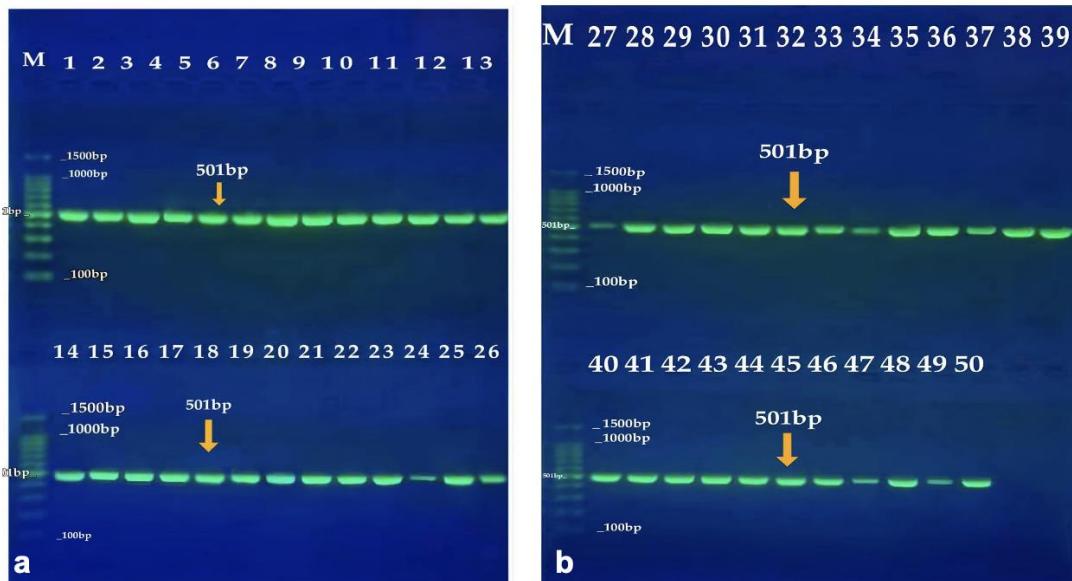
**Table 5: The results of biofilm production for isolates.**

Biofilm intensity	Frequency (%) No.=100
Strong	22%
Moderate	72%
Weak	6%

### 3.6. Detection of *blaOXA-23* Gene by PCR

Conventional PCR amplification targeting the *blaOXA-23* gene was performed using specific primers. All 50 isolates (100%) produced a 501 bp PCR product, confirming the presence of the gene in every tested strain. Electrophoresis was conducted using 1.5% agarose in 1× TBE buffer at 5 volts/cm<sup>2</sup> for 90 minutes. The PCR products were compared against a 100 bp DNA

ladder, and all positive isolates exhibited a single band at the expected size of 501 bp (Fig. 2). These findings demonstrate universal carriage of the *blaOXA-23* gene among the clinical *A. baumannii* isolates included in this study, corroborating earlier regional and global reports of its widespread dissemination (Al-Agamy et al., 2014; Ghaima, 2016).



**Fig. 2: a.** PCR product with a band size of 501 bp. The product was electrophoresed on 1.5% agarose at 5 volts/cm<sup>2</sup> for 1:30 hours in 1x TBE buffer. N: DNA ladder (100 bp). **b.** PCR result with a 501 bp band size. The product was electrophoresed in 1x TBE buffer on 1.5% agarose for 1:30 hours at 5 volts/cm<sup>2</sup>. N: 100 bp DNA ladder.

### 3.7. Sequencing and Phylogenetic Analysis

Selected *blaOXA-23* PCR products were purified and subjected to direct sequencing. Sequence alignment using BLAST analysis showed 100% identity with reference *blaOXA-23* sequences deposited in GenBank, thereby confirming their specificity and genetic conservation.

Subsequently, phylogenetic analysis was conducted using the MEGA software platform. A neighbor-joining phylogenetic tree was constructed to determine the genetic relationships between the clinical isolates and other known sequences from various geographic regions. The resulting tree revealed that the study isolates

clustered tightly with previously reported *blaOXA-23* sequences from clinical *A. baumannii* strains, indicating close genetic relatedness and supporting the hypothesis of global dissemination (Fig. ???).

These results underscore the evolutionary conservation of the *blaOXA-23* gene and highlight its diagnostic reliability for molecular identification of *A. baumannii*. Moreover, they emphasize the gene's critical role in carbapenem resistance and the necessity for continuous genetic surveillance in hospital settings.<sup>[14]</sup>

#### 4. DISCUSSION

*Acinetobacter baumannii* is increasingly recognized as a critical nosocomial pathogen, particularly in urinary tract infections (UTIs), due to its remarkable capacity for antibiotic resistance and biofilm formation. In this study, *A. baumannii* was isolated from 20% of the UTI samples collected from patients in Iraqi hospitals, confirming its substantial prevalence. The midstream urine collection method used ensured accurate representation of urinary pathogens and minimized contamination, thus increasing the reliability of findings.<sup>[15]</sup>

Conventional biochemical methodologies and VITEK® 2 Compact system analysis were employed to verify the phenotypic identification of *A. baumannii*. The isolates displayed characteristic features, such as Gram-negative coccobacilli morphology, non-lactose fermentation on MacConkey agar, and growth at elevated temperatures (44°C), in line with previously reported descriptions in literature.<sup>[16]</sup>

Antimicrobial susceptibility testing revealed alarming resistance levels among isolates. Particularly, 95% resistance was noted against  $\beta$ -lactam antibiotics such as Ticarcillin, Piperacillin, and Meropenem, and fluoroquinolones like Ciprofloxacin. Imipenem resistance was also high (60%), which is consistent with previous global reports (Peleg et al., 2008; Dijkshoorn et al., 2007). Only colistin retained high effectiveness, with 95% susceptibility, although its known nephrotoxicity limits widespread use.<sup>[17,18]</sup>

The blaOXA-23 gene, which is present in 100% of *A. baumannii* isolates, is of particular concern due to its high prevalence. This gene is recognized for its ability to confer resistance to carbapenems, and its presence in the Iraqi clinical context serves to confirm its dominant role in resistance mechanisms (Gaddy et al., 2012; Zarrilli et al., 2013). The genetic analysis demonstrated that these strains are closely related to those found worldwide, and PCR amplification and sequencing confirmed accurate detection.<sup>[19,20]</sup>

Furthermore, 72% of the isolates exhibited moderate to strong biofilm-forming capabilities, which exacerbates persistence in hospital environments and inhibits antibiotic efficacy. These findings are consistent with the literature that underscores the significance of biofilms in the development of antibiotic resistance and chronic infections (Flores-Mireles et al., 2015; Kenyon et al., 2013).<sup>[21,22]</sup>

The classification of isolates into MDR (45%) and XDR (55%) groups further underscores the public health threat posed by *A. baumannii*. These resistance patterns warrant immediate intervention via improved infection control, antibiotic stewardship, and development of targeted therapeutics. Importantly, the study highlights the role of molecular tools such as PCR in identifying

resistance determinants, supporting their integration into routine clinical diagnostics.<sup>[23,24,25]</sup>

#### 5. CONCLUSION

This study investigates the molecular characterization of virulence factors in *Acinetobacter baumannii* isolated from UTI patients in Iraqi hospitals. The results confirm the universal presence of the blaOXA-23 gene (100%), which plays a central role in carbapenem resistance and pathogenicity. High resistance levels observed underscore the urgent need for stronger infection control measures. The findings also emphasize the contribution of outer membrane proteins, biofilm-related genes, and resistance determinants to diagnostic and therapeutic strategies. These molecular insights address gaps in the regional epidemiology of *A. baumannii* and highlight the necessity for further research to support clinical management and public health policies against antibiotic-resistant infections.

**Ethical Approval:** The Near East Scientific Research Ethics Committee approval was obtained for this study (approval number: NEU/2025/131-1932, date: 27.02.2025).

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