Medical Science Journal for Advance Research

Vol. 6, No. 2, June 2025
e-ISSN: 2774-4892

Carbapenemase Genes *bla*$_{OXA23}$ and *bla*$_{OXA58}$ Distribution among *Acinetobacter baumanii* Isolated from Clinical Isolates

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ABSTRACT

Background: *Acinetobacter baumannii is one of the most prevalent Gram-negative microbes associated with serious and fatal nosocomial infections. It is routine to use carbapenems as a treatment for A. baumannii infections. The dissemination of carbapenem resistance offers a major challenge to the treatment of life-threatening infections caused by these bacteria.* ***Aim of Study:*** *Determine which isolate of carbapenem-resistance A. baumannii have higher prevalence of the bla*$_{OXA23}$ *and bla*$_{OXA58}$ *gene at Al-Najaf city.* ***Settings and Design:*** *Cross-Sectional study.* ***Methods and Materials:*** *From December 2023 to July 2024, a total of 220 samples were collected in this cross-sectional study including urine (n=85,39%), wounds (n=55,25%) burns (n=45,20 %), sputum (n=20, 9%), blood (n=15, 7%) from patients at Al-Sadder Medical City, burn center unit, and private laboratories in Al-Najaf city. The identification and antibiotic susceptibility profile of A. baumannii were done by vitek2 compact system. The isolates were treated for PCR assays with specific primers for (bla*$_{OXA-23}$ *gene, bla*$_{OXA-58}$ *gene).* ***Results****: The recovery rate of A. baumannii isolates was (n= 16, 7.27%) from different clinical samples. Antibiotic-susceptibility patterns demonstrated that isolate of A. baumannii these isolates were multi-drug resistant (MDR). The MDR isolates were resistant to all 11 antibiotics classes tested in this study. The prevalence of bla*$_{OXA-23}$ *gene (75%) was higher than blaOXA-*$_{58}$ *gene (31.25%) in A.baumannii isolates****. Conclusion:*** *Increased resistance to carbapenem antibiotic in Al-Najaf Hospitals serves to highlight how critical this issue is when treating multidrug-resistant A. baumannii infections that are life-threatening.*

Keywords: Carbapenems Resistance, MDR A. baumannii, bla$_{OXA23}$ gene, bla$_{OXA58}$ gene.

Article Information

Received: April 30, 2025; Revised: May 30, 2025; Online: June, 2025

INTRODUCTION

The genus *Acinetobacter* is a major cause of nosocomial infections; it is currently contributing to numerous outbreaks and has been a cause of concern in an extensive spectrum of hospitals worldwide. Multi-antibiotic resistant *Acinetobacter baumannii* is now recognized as having important therapeutic significance (1, 2). *Acinetobacter baumannii* can cause a variety of illnesses, including bacteremia, meningitis, urinary tract, bloodstream or surgical wound infections, and ventilator-associated pneumonia (3). However, the development of antibiotics-resistant *A. baumannii*, especially multi-resistant forms, severely challenges the management of these infections (4,5). Resistance to antibiotic is an important problem for global public health (6). Due to strong resistance of *Acinetobacter baumannii* to numerous antibiotics,

https://doi.org/10.46966/msjar.v6i2.273

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notably last-option treatments like carbapenems. Mobile genetic elements (MGEs) play a significant role in the spread and expression of antibiotic resistance genes (ARGs), including the mobilization of ARGs within and between species (7).

Acinetobacter baumannii develops carbapenem resistance by multiple mechanisms, including oxacillinases (OXAs) and occasionally metallo-β-lactamases (MBLs). (8). However, it is typically discovered that *Acinetobacter baumannii* exhibit multidrug resistance to variety of drugs, including aminoglycosides, fluoroquinolones and third generation of cephalosporins (9). *Acinetobacter baumannii* is one of the most common sources of infections acquired in hospitals and community. Multidrug resistance *A. baumannii* has been increasingly reported worldwide. Carbapenem WHO, 2017, identified carbapenem-resistance *A. baumannii* as top pathogen on the critical priority of the "Global Priority Pathogen List". The Polymerase Chain Reaction (PCR) technique of *bla*$_{OXA}$-like-carbapenemase gene identification is practical molecular tool for identifying isolates of *A. baumannii* (10). *Acinetobacter baumannii* that have evolved resistance to recently developed antimicrobial drugs over the previous 30 years are reffered to as MDR *A. baumannii* strains. It spread to many medical centers worldwide and is now known to be major nosocomial pathogen (11, 12).

Multi-drug resistant *Acinetobacter spp*. are defined as having resistance to at least three categories of antibiotics, such as aminoglycosides, fluoroquinolones, and all forms of pencillin and cephalosporins. A contributing factor to that problem is increasing prevalence of resistance to carbapenems and other broad-spectrum antibiotics. Traditionally, carbapenems have been the drug of choice for treating *Acinetobacter spp.* however, an increasing number of isolates are now resistant to these drugs (13,14). One significance mechanism of carbapenem resistance in *A. baumannii* is the production of carbapenemase. Carbapenemase production is an important mechanism of carbapenem resistance in *Acinetobacter* species. This study investigated the existence of the genes *bla*$_{OXA-23}$ and *bla*$_{OXA-58}$, which encode class D β-lactamases that hydrolyze carbapenem, and their correlation with the spread of multi-drug resistance *A. baumannii* in Al-Najaf hospitals.

This research aimed to determine which isolate of carbapenem-resistance *A. baumannii* have higher prevalence of the *bla*$_{OXA23}$ and *bla*$_{OXA58}$ gene at Al-Najaf city

1. Isolation and identification of *A. baumannii* from different clinical samples.
2. Study the antibiotic resistance patterns of *Acinetobacter baumannii* isolates by Vitek-2 system.
3. Genomic DNA extraction.
4. Molecular detection of *bla*$_{OXA23}$ and *bla*$_{OXA58}$ genes by using PCR technique

SUBJECTS AND METHODS

In total, 220 clinical samples were taken from patients distributed as urine 85 (39%), wound 55 (25%), burns 45 (20%), sputum 20 (9%), and blood 15 (7%). The patients were attended at Al-Sadder Medical City, burn center unit, and private laboratories during the period from December 2023 to April 2024. *A. baumannii* was isolated from urine two (0.9%), wound seven (3.18%), burns 5 (2.27%), sputum two (0.9%), and 0 (0%) blood clinical samples. Under safety handling conditions, the clinical samples were obtained from patients with sterile swabs of transport media and instructed with patients' information, then transported to the laboratory. All samples were streaked based on standard procedures by using differential and selective media (MacConkey, blood, chromogenic agar) for detection of *A. baumannii* and incubated at 37^0 C for 24 hours aerobically (15). Confirmative diagnosis of isolates was achieved using conventional biochemical tests and confirmed by the Viteck2 compact system (Biomerieux, France).

Acinetobacter baumannii isolates were subjected to antibiotics susceptibility test by Vitek2 compact system (Biomerieux, France). All isolates were examined against ten antibiotic agents related to six antibiotic classes. According to Clinical and Laboratory Standards Institute 2021 (16) recommendations, all results were interpreted and all *A. baumannii* isolates were classed as sensitive, intermediate, or resistance to each tested antibiotics agent.

Genomic DNA was extracted from *A. baumannii* isolates according to instructions of the manufacturer Genomic DNA Extraction Kit (FavorPrep, Austria). The purity and concentration of DNA for each isolate were measured by the Nanodrop instrument (THERMO, USA). Polymerase Chain Reaction (PCR) was employed for screening the carbapenem resistance genes: bla_{OXA23} genes, bla_{OXA58} genes. In this study, all primers were provided by the Promega company, USA. Primers details are tabulated in Table (1).

Table 1: Carbapenem resistance genes of *A. baumannii.*

N.	Gene	Primer Sequence		Amplicon size (bp)	Annealing Temp./time	Reference
1	bla_{OXA-23}	F	GATCGGATTGGAGAACAGA	501 bp	55 ºC/45 sec	17
		R	ATTTCTGACCGCATTTCCAT			
2	bla_{OXA-58}	F	AAGTATTGGGGCTTGTGCTG	599bp	55 ºC /45sec	
		R	CCCCTCTGCGCTCTACATAC			

RESULTS

Out of 220 collected samples only 144 (65 %) samples gave positive results for culturing, and 76 (35%) negative culturing samples, only 16 isolates (7.27%) were identified to be *A. baumannii* depending on culture characteristics and biochemical tests as shown in Table 2.

Table 2: Distribution of isolates of *A. baumannii* depending on sample source.

Sample source	Total number	Bacterial culture results No. (%)			
		No growth	Bacterial growth		
			A. baumannii isolate	Other Gram-negative bacteria	
Urine	85 (39%)	44(51.7%)	2 (2.3%)	39 (45.8%)	< 0.0001*
Wound	55(25%)	4 (7.3%)	7 (12.7%)	44 (80%)	< 0.0001*
Burn	45(20%)	6 (13.3%)	5 (11.1%)	34 (75.5%)	< 0.0001*
Sputum	20(9%)	8 (40%)	2 (10%)	10 (50%)	< 0.0001*
Blood	15(7%)	14 (93.3%)	0 (0%)	1 (6.7%)	< 0.0001*
Total	220(100%)	76 (34.5%)	16 (7.3%)	128 (58.1%)	< 0.0001*
P-value (P ≤0.05)		< 0.0001*	0.0014*	< 0.0001*	
*Significant differences at (P ≤0.05) by chi Squair test					

All strains were Extensive Drug Resistant (XDR) except four (25%) isolates were multidrug resistance (MDR).

Table 3: Antibiotics susceptibility patterns of *A. baumannii* isolates

Antibiotics classes	Antibiotics	Resistant (R)		Sensitive (S)		P-value (P ≤0.05)
		Isolates No.	%	Isolates No.	%	
Beta-lactam combination agents	Piperacillin/ Tazobactam	16	100%	0	0%	-------
Cephalosporins	Cefazolin (3rd G)	12	75%	4	25%	< 0.0001*

	Ceftriaxone (3rd G)	16	100 %	0	0%	-------
	Ceftazidime (3rd G)	16	100 %	0	0%	-------
	Cefepime (4th G)	16	100%	0	0%	-------
Carbapenem	Meropenem	16	100%	0	0%	-------
Aminoglycoside	Amikacin	16	100%	0	0%	-------
	Gentamicin	16	100%	0	0%	-------
Folate pathway antagonsits	Trimethoprim/ Sulfamethoxazole	13	81.25%	3	18.75 %	< 0.0001*
Quinolone	Ciprofloxacin	16	100 %	0	0%	--------
P-value (P ≤0.05)		0.5053*		< 0.0001*		
*Significant differences at (P ≤0.05) by chi Squair test						

Table 4 Showed that 12 from 16 (75%) CRAB isolates had *bla*$_{OXA23}$ carbapenemase gene while four isolates (25%) had *bla*$_{OXA58}$ carbapenemase genes and three of 16 (18.75%) PCR confirmed the presence of both *bla*$_{OXA23}$ and *bla*$_{OXA58}$.

Table (4): Antibiotic resistance of *Acinetobacter baumannii* isolates.

Gene	**Result No. (%) n=16**		**P-value (P ≤0.05)**
	Positive	Negative	
***bla*$_{OXA23}$**	12 (75%)	4 (25%)	< 0.0001*
***bla*$_{OXA58}$**	5 (31.3%)	11 (68.7%)	0.0002*
Both genes	3 (18.7%)	13 (81.3%)	< 0.0001*
P-value (P ≤0.05)	< 0.0001*	< 0.0001*	
*Significant differences at (P ≤0.05) by chi Squair test			

Subsequently, all of the 16 *A. baumannii* isolates were evaluated for the presence of selected carbapenem resistance genes including bla_{OXA23} and bla_{OXA58}. The distribution rate of carbapenem resistance genes in 16 *A. baumannii* isolates is shown in (Figure 1, 2), were the rate of bla_{OXA23} was to be found 12 (75%) and bla_{OXA58} was to be 5 (31.25%).

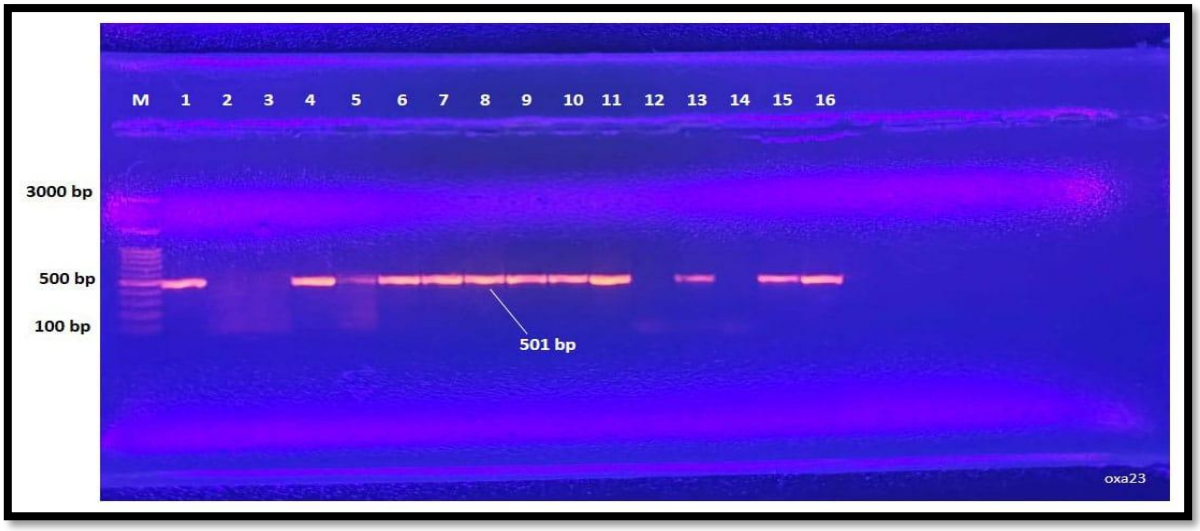

Fig.1: Electrophoresis of PCR products for the resistance genes bla_{OXA23} on agarose gel (1.5%) stained with ethidium bromide (5volt/cm for 2hr). M: 100bp DNA ladder; number (1, 4, 5, 6, 7, 8, 9, 10, 11, 13, 15, 16) positive *Acinetobacter baumannii* isolates while number (2, 3, 12, 14) were negative.

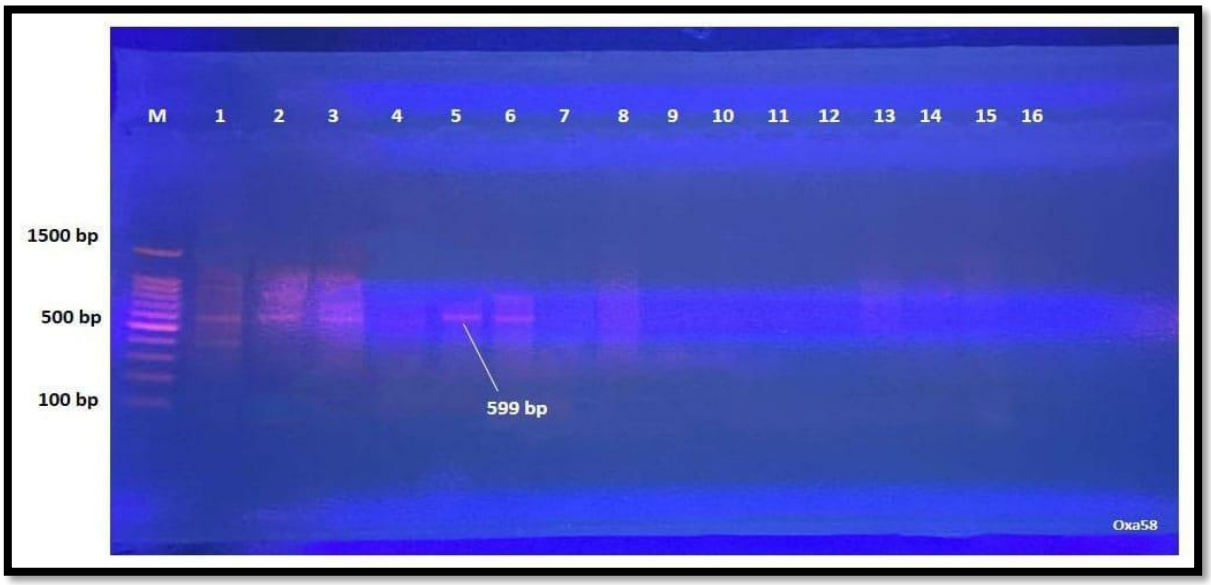

Fig. 2: Electrophoresis of PCR products for the resistance genes $bla_{OXA\text{-}58}$ on agarose gel (1.5%) stained with ethidium bromide (5volt/cm for 2hr). M: 100bp DNA ladder; number (1, 2, 3, 5, 6) positive *Acinetobacter baumannii* isolates while number (4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16) were negative.

DISCUSSION

The worldwide public health is at hazard due to the prevalence of carbapenem-resistant *A. baumannii*. The current study showed that 16 (7.27%) of isolates were *A. baumannii*, from 220 different clinical samples. Twenty isolates (3.8%) out of 530 clinical samples were identified as *A. baumannii* in a previous study (18).

According to this study, the majority of *A. baumannii* isolates were from wound samples (3.18%), which were followed by burn (2.27%), and finally urine and sputum samples (0.9%) for each. Similar finding was made by Tawfeeq *et al*., (19), who discovered that 42.59% of *A. baumannii* isolates were form wound then urine samples 31.48%, and 5.55% from sputum samples. All isolates of *A. baumannii* were carbapenem resistant (100%) and these results agree with finding of study in Egypt (10). Some among these isolates harboring several genes together and these consistent with the findings of Al-Wahid and AL-Azawi in Iraq (20) and study in Iran (21).

Many mechanisms can confer *Acinetobacter spp.* resistance to carbapenem, but the production of carbapenemase- particularly those from Ambler class D, also known as oxacillinases (*OXA*) is thought to be the most important one (22, 23). This is may be generated by the accumulation of mutations chosen by different antibiotics prior to introduction of carbapenems, as well as the numerous mechanisms of carbapenem resistance in *A. baumannii* giving simultaneous resistance to antibiotics of different classes (24, 25).

In the current study, demonstrated that the isolates that carry bla_{OXA-58} reveled lower rate of carbapenem resistance compared with bla_{OXA23}-carrying isolates and these finding similar to previous study in China (26) and study in Egypt (10). Present study, it was found that *A. baumannii* carrying bla_{OXA23} gene 75%. Research in Iraq discovered that two (40%) *A. baumannii* isolates had bla_{OXA-23} gene positive in Al-Najaf hospitals (27). Based to the current results, the bla_{OXA-23} gene is the major carbapenem resistance gene in *A. baumannii* isolates. This might be explained by the fact that bla_{OXA23} is found on both chromosome and plasmid in a variety of bacteria other than *Acinetobacter*. As a result, bacteria can acquire this gene more easily than other genes (28, 29,30).

CONCLUSION

Increased resistance to carbapenem antibiotic in Al-Najaf Hospitals serves to highlight how critical this issue is when treating multidrug-resistant *A. baumannii* infections that are life-threatening.

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