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Unmasking the Resistance: Detecting Carbapenem Genes in *Acinetobacter baumannii* Isolated from some Hospitals in Najaf and Baghdad

Muhammad Kareem Jasim[1], Zainab Jaber Hadi[2], *Hashim Ali Abdualmeer Al-sherees[3], Aaedah Annooz[4]

[1-4]*Department of Microbiology , Faculty of Medicine , University of Kufa, Najaf, Iraq.*

***Email.** hashimaa49@yahoo.com

ABSTRACT

Background: *Acinetobacter baumannii is a multidrug-resistant bacterium associated with nosocomial infections and known for its ability to develop resistance rapidly. Carbapenem-resistant A. baumannii (CRAB) is a top priority pathogen according to the World Health Organization (WHO). We focused on evaluating the susceptibility of A. baumannii to antibiotics, detecting carbapenemase enzymes using the modified Hodge test, and characterizing the presence of specific carbapenem resistance genes using PCR analysis.This cross-sectional study took place at Al-Sader Medical City and Baghdad Teaching Hospitals from October 2022 to February 2023. It involved 59 A. baumannii isolates collected from patients. The isolates were obtained and processed for accurate diagnosis using morphological techniques, biochemical tests, and Vitek2 systems. The Kirby-Bauer method was employed to assess the susceptibility of the isolates to 24 antibiotics. DNA extraction and PCR analysis were conducted to detect carbapenem resistance genes, 59 specimens from patients, including sputum, wound swabs, blood, and inguinal swabs were analyzed. The majority of isolates were from in-patients, showing a significant difference compared to outpatients. Our finding revealed that among the studied isolates, blaSPM was the most prevalent gene, detected in 50% of the isolates. This indicates a significant presence of blaSPM-mediated carbapenem resistance among A. baumannii strains in our study population.Furthermore, our findings demonstrated alarmingly high resistance rates against the majority of antibiotics commonly prescribed to treat A. baumannii infections. A striking 95% of the isolates were classified as extensively drug-resistant, indicating resistance to multiple classes of antibiotics. This poses significant challenges for effective treatment options and underscores the urgent need for alternative strategies in managing A. baumannii infections.*

Keywords: Acinetobacter baumannii, Carbapenem-resistant A. baumannii (CRAB), Carbapenem Genes, Extensive Drug-Resistant (XDR), Modified Hodge test(MHT).

Article Information

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INTRODUCTION

The global rise in antibiotic resistance has reached alarming levels, posing a serious threat to the effective treatment of common infectious diseases[1]. Among the bacterial pathogens contributing to this problem, *A. baumannii* is a significant member of the ESKAPE group, which includes six major pathogens known for their antibiotic resistance, such as *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiellapneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter* species [2]. *A. baumannii*, a Gram-negative bacterium, is an opportunistic nosocomial pathogen frequently

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associated with pneumonia, as well as infections in burn patients and other types of wounds [3], [4]. Its ability to endure harsh environmental conditions, including desiccation and extreme pH levels, presents a substantial challenge in managing infections, particularly in intensive care units and burn wards within hospitals[5].

Since the 1970s, *A. baumannii* has been recognized as a significant threat due to its rapid development of resistance against a broad range of antibiotics, including last-resort options like carbapenems[6], [7]. Treatment options for *A. baumannii* infections are often extremely limited or nonexistent[3], [8]. In response to this growing concern, the In this study, we aimed to evaluate the susceptibility of *Acinetobacter baumannii* to various antibiotics, detect the presence of carbapenemase enzymes using the modified Hodge test, and characterize the presence of specific carbapenem resistance genes using PCR analysis.

First, we performed susceptibility testing of *A. baumannii* isolates against a panel of antibiotics to determine their sensitivity or resistance. The antibiotics tested included commonly used drugs as well as carbapenems, which are considered last-resort treatments. The results of this testing provided valuable information about the effectiveness of different antibiotics against *A. baumannii.*

To identify the presence of carbapenemase enzymes, we employed the modified Hodge test, a phenotypic screening method. This test helps detect the production of carbapenemase enzymes by *A. baumannii* strains, which contribute to carbapenem resistance. The modified Hodge test used in this study as a tool for initial screening to indicate the presence of carbapenemase-producing strains.

Furthermore, we employed PCR analysis to characterize the presence of specific carbapenem resistance genes in the *A. baumannii* isolates. PCR allows for the amplification and detection of targeted gene sequences associated with carbapenem resistance. By using specific primers designed for known carbapenemase-encoding genes, we were able to identify the presence of these genes in the isolates

World Health Organization (WHO) designated carbapenem-resistant *A. baumannii* (CRAB) as the highest priority among a list of 12 antibiotic-resistant bacteria, emphasizing its critical clinical significance and the global burden it imposes on healthcare systems[9].

MATERIALS AND METHODS

Specimen collection and bacterial isolates

This cross-sectional study was conducted at Al-Sader Medical City and Baghdad Teaching Hospitals, located in Najaf and Baghdad, respectively, from October 2022 to February 2023. The study population comprised 59 *A. baumannii* isolates from patients who attended or were admitted to these hospitals. The collected isolates included blood, sputum, wound exudates, and inguinal swab. All specimens utilized in this investigation underwent accurate diagnosis by specialized microbiologists and well-trained medical laboratory personnel using morphological techniques, biochemical tests, and Vitek2 systems.

Antibiotic Susceptibility

The Disc Diffusion method, commonly known as the Kirby-Bauer method, was employed in this study to assess the susceptibility of bacteria to various antibiotics, following the protocol described by[10]. A total of 24 different antibiotics, as

per CLSI 2022 guidelines, were tested using this method.

The antibiotics and their respective concentrations used in the study were as follows: Ampicillin(10µg), Pipracillin(100 µg), Amoxicillin-clavulanic acid(30µg), Ampcillin-sulbactam(20 µg), Pipracillin-tazobactam(10 µg), Ticarcillin-clavulanic acid(75 µg), Ceftazidime(30 µg), Cefotaxime(30 µg), Ceftriaxone (30µg), Cefepime (30 µg), Cefepime- clavulanic acid(40µg), Imipenem(10µg), Meropenem(10µg),Meropenem-EDTA(10+730µg), Amikacin(30µg), Gentamicin(10µg), Tobramycin(10µg), Netilmicin(30µg),Doxycycline(30µg), Minocycline(30µg), Tetracycline(30µg), Ciprofloxacin(5µg),Levofloxacin(5µg),Gatifloxacin(5µg),Trimethoprim-Sulphamethazol(1.25-23.75µg), Polymyxin(10µg), Colistin(10 µg).

Modified Hodge Test

The modified Hodge test (MHT) was employed to assess the presence of carbapenemase in imipenem and/or meropenem-resistant*A. baumannii* isolates, following the protocol developed by[11]. A bacterial suspension of the *E. coli* ATCC 25922 was prepared and diluted, and the diluted suspension was spread on a Mueller-Hinton agar plate. After placing an imipenem disk at the center of the plate, the test isolate was streaked outward from the disk's edge. Incubation of the plates at 37°C for 18-24 hours allowed for the observation of positive results, indicated by a clover-leaf indentation within the inhibitory zone surrounding the imipenem susceptibility disk, exhibited by both the test isolate and the *E. coli* 25922 strain.

Molecular Characterization of Carbapenem Resistance Genes

The DNA extraction step was conducted following the manufacturer's guidelines (Favorgen,Taiwan), which involved isolating DNA from bacterial cells for subsequent analysis or experiments. To assess the concentration and purity of DNA, a Biophotometer Plus (Eppendorf, Germany) was employed by measuring the optical density at 260nm and 280nm. The primer sequence used was based on the published work of [12]. Table 1

For the PCR reaction, a mixture was prepared in a total volume of 25 µl, consisting of 10µl of PCR master mix (Solg™mix), 2µl of each primer, and 5µl of the extracted DNA. The volume was adjusted to 25µl with sterile deionized distilled water, the Biometra PCR system (T3000 Thermocycler) was utilized to detect the presence of carbapenem resistance genes.

Table 1: The Primers used in the study. Macrogen/ Korea

Primer Name		Primer sequence	Product(bp)	References
bla_{IMP}	$_{IMP}$-F	TAATGCTTTGATCGGCCTTG	139	[12]
	$_{IMP}$-R	GATYGAGAATTAAGCCACYCT		
bla_{VIM}	$_{VIM}$ (F)	GATGGTGTTTGGTCGCATA	390	
	$_{VIM}$ (R)	CGAATGCGCAGCACCAG		
bla_{NDM}	bla_{NDM}(F)	CCCGGCCACACCAGTGACA	129	
	bla_{NDM}(R)	GTAGTGCTCAGTGTCGGCAT		
bla_{SPM}	$_{SPM}$(F)	GGGTGGCTAAGACTATGAAGCC	447	
	$_{SPM}$ (R)	GCCGCCGAGCTGAATCGG		

Data analysis

Statistical analysis was performed using Graph Pad Prism version 6 software and Microsoft Office Excel 2013 for certain calculations. A significance threshold of 0.05 was used, and p-values below this threshold were deemed statistically significant.

RESULTS

Bacterial Isolates

A total of 59 specimens were included in this study, comprising 28(47%) sputum samples, 28(47%) wound swabs, 2(3%) blood samples, and 1(1%) inguinal swab. The highest percentage of specimens was observed in the 60-69 age group (n=22; 37%), while the lowest percentage was observed in the 20-29 age group (n=1; 1.6%). The male-to-female ratio was higher among males, with a ratio of 2:1 with a p-value of ≤0.0004. The majority of isolates (n=51; 86%) were obtained from in-patients, while a smaller proportion was obtained from outpatients (n=8; 13%) with a *p-value* of ≤0.00001, indicating a notable disparity in the distribution of *A. baumannii* isolates between in-patients and outpatients.

Assessing the Antibiotic Susceptibility Patterns of *A.baumannii*

The susceptibility results of *A. baumannii* to 24 antibiotics from 9 antimicrobial categories was Listed in Table 2. The results demonstrated complete resistance of all isolates to ampicillin and 96.6% resistance to pipracillin. The resistance rates for beta-lactam combinations were 57.6% for ampicillin-sulbactam, 93.2% for pipracillin-tazobactam, and 100% for ticarcillin-clavulanate. Furthermore, high resistance rates were observed for third-generation cephalosporins, with rates of 88.1% for ceftazidime, 100% for cefepime, 98.3% for cefotaxime, and 94.9% for ceftriaxone. However, carbapenem antibiotics exhibited significantly higher resistance rates, with 88.1% susceptibility. Regarding

aminoglycosides, the resistance rates were 77.9% for gentamicin, 61% for tobramycin, 86.4% for amikacin, and 64.4% for netilmicin. Tetracycline resistance rates were 61% for doxycycline, 96.6% for tetracycline, and only 5% resistance was observed for minocycline. The resistance rates for fluoroquinolones were 94.9% for ciprofloxacin and 76.2% for levofloxacin. Trimethoprim/sulfamethoxazole displayed a resistance rate of 84.7%, while polymyxin and colistin demonstrated resistance rates of 13.5% and 11.8% respectively. Among the 59 isolates only 5% were categorized as MDR, while the remaining 95% were classified as XDR. No isolates exhibited pan-drug resistance (PDR).

Table 2: Antibiotic susceptibility expressed by *A.baumannii* isolates (n= 59)

Antibiotic name	Sensitivity Test Results		
	R(No./%)	I(No./%)	S(No./%)
Ampicillin	59/100%	0	0
Pipracillin	57/96.6%	1 /1.6%	1 /1.6%
Ampcillin-sulbactam	34/57.6%	9/15.2%	16/27.1%
Pipracillin-tazobactam	55/93.2%	0	4/6.7%
Ticarcillin-clavulanic acid	59/100%	0	0
Ceftazidime	52/88.1%	2/3.39%	5/8.4%
Cefotaxime	58/98.3%	0	1/1.6%
Ceftriaxone	56/94.9%	0	3/5%
Cefepime	59/100%	0	0
Cefepime- clavulanic acid	59/100%	0	0
Imipenem	52/88.1%	0	7/11.8%
Meropenem	52/88.1%	0	7/11.8%
Meropenem-EDTA	52/88.1%	0	7/11.8%
Amikacin	51/86.4%	1/2.17%	7/11.8%
Gentamicin	46/77.9%	6/10.1%	7/11.8%
Tobramycin	36/61%	0	23/38.9%
Netilmicin	38/64.4%	8/13.5%	13/22%
Doxycycline	36/61%	1/2.17%	22/37.2%
Minocycline	3/5%	3/5%	53/89.8%
Tetracycline	57/96.6%	2/4.35%	0
Ciprofloxacin	56/94.9%	0	3/5%
Levofloxacin	45/76.2%	7/11.8%	7/11.8%

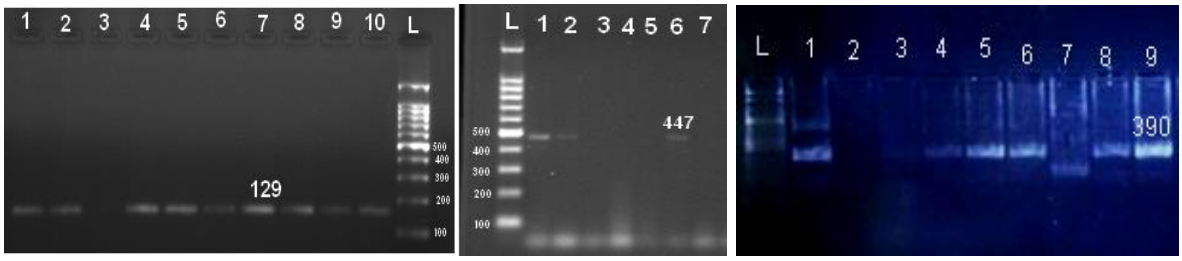
Trimethoprim-Sulphamethazol	**50/84.7%**	**0**	**9/15.2%**
Polymyxin	**8/13.5%**	**1/2.17%**	**50/84.7%**
Colistin	**7/11.8%**	**2/4.35%**	**51/**

MHT Results

A significant proportion of isolates that demonstrated resistance to imipenem and meropenem in the disk diffusion method exhibited negative results when subjected to the modified Hodge test. Specifically, only 13.4% of the isolates exhibited positive results, while the majority, comprising 76.2% of the isolates, yielded negative results.

Detection of Carbapenem Resistence Genes in *A.baumannii*

PCR Analysis revealed the following gene distributions: bla_{SPM} was detected in 50% of the isolates figure 1, bla_{NDM} in 23% of the isolates, bla_{VIM} in 18% of the isolates and notably, none of the isolates were found to carry the bla_{IMP} gene. These findings highlight the presence and distribution of carbapenem resistance genes among the *A. baumannii* isolates studied.



(A) (B) (C)

Figure 1. Agarose gel electrophoresis of PCR ampilified products from extracted DNA of *A. baumannii* **(A)** amplified with *bla $_{NDM}$ genes primers.* L: size marker (100-1500bp), Lanes (1,2,4,5,6,7,8,9,10) illustration positive results with bla $_{NDM}$ (129 bp). **(B)**: *bla_{SPM} genes primers.* L: size marker (100-1500bp), Lanes (1,2,6) illustration positive results with *bla_{SPM}* (447 bp). *). **(C)**: bla_{VIM} genes primers. L: size marker (100-1500bp), Lanes (1,3,4,5,6,8,9) illustration positive results with bla_{VIM} (390 bp).*

DISCUSSION

The distribution of *A.baumannii* across different age ranges was examined in this study. The highest percentage of specimens was observed in the 60-69 age group, comprising 37% of the total. This finding suggests a potential age-related susceptibility to *A. baumannii* infections or colonization in the studied population. On the other hand, the 20-29 age groups had the lowest representation, accounting for only 1.6% of the specimens. Furthermore, the male-to-female ratio was higher among males, which is consistent with findings from previous studies [13]–[17].

Moreover, a notable predominance of isolates was obtained from in-patients (86%) compared to outpatients (13%), indicating a higher likelihood of *A. baumannii* acquisition in a hospital or healthcare facility setting. This finding aligns with previous studies [18]–[20]emphasizing the nosocomial nature of *A.baumannii* infections and highlighting the importance of implementing effective infection control measures within healthcare institutions to prevent its spread.

The susceptibility of *A. baumannii* to a wide range of antibiotics revealed high levels of resistance to several antibiotics, which limited the available treatment options. Notably, all isolates were completely resistant to

ampicillin, while a significant proportion (96.6%) exhibited resistance to pipracillin. These resistance patterns are consistent with previous studies[21-23]reporting similar findings for ampicillin. However, there were discrepancies in the resistance rates for pipracillin compared to studies conducted by [21, 24-26]. Resistance rates for beta-lactam combinations varied as well. Ampicillin-sulbactam exhibited a resistance rate of 57.6%, contradicting the findings of[23] but closely resembled the result of [24]. For pipracillin-tazobactam, the resistance rate was 93.2%, which was consistent with the findings of [23]. Ticarcillin-clavulanate showed a resistance rate of 100%, in line with [23], but differing from [26]. Regarding third-generation cephalosporins, high resistance rates were observed. Ceftazidime displayed a resistance rate of 88.1%, which was consistent with the findings of [13, 23, 27]. Cefepime exhibited a resistance rate of 100%, similar to [27], but differing from [24, 28]. Cefotaxime demonstrated a resistance rate of 98.3%, consistent with [13, 25, 27], but differing from [24]. Similarly, ceftriaxone showed a resistance rate of 94.9%, with slight variations compared to other studies such as [25, 29]. Carbapenem antibiotics, including imipenem and meropenem, displayed significantly high resistance rates. Our study found an 88.1% susceptibility rate for carbapenems, resembling[29], but differing from [23, 27], which reported higher resistance rates. Among the aminoglycosides, varying resistance rates were observed. Gentamicin exhibited a resistance rate of 77.9%, which aligned with [27, 28]. Tobramycin showed a resistance rate of 61%, differing from [23, 24, 28]. Amikacin displayed a resistance rate of 86.4%, with variations compared to [25-27]. Netilmicin showed a resistance rate of 64.4%. The resistance rates for tetracyclines varied as well. Doxycycline exhibited a resistance rate of 61%, while tetracycline showed a resistance rate of 96.6%. These findings differed from [26, 27], highlighting the importance of considering specific tetracycline agents and their local resistance profiles. Notably, minocycline displayed a remarkably low resistance rate of 5%, contrasting with [24, 26]. The resistance rates for fluoroquinolones were relatively high. Ciprofloxacin showed a resistance rate of 94.9%, resembling [23] and differing from [13, 26, 28]. Levofloxacin exhibited a resistance rate of 76.2%, with variations compared to [13,26]. Trimethoprim/sulfamethoxazole displayed a resistance rate of 84.7%, closely resembling [26] but differing from [24]. Polymyxin and colistin demonstrated relatively lower resistance rates of 13.5% and 11.8%, respectively.

Furthermore, the prevalence of multi-drug resistance (MDR), extensive drug resistance (XDR), and pan-drug resistance (PDR) among the 59 isolates was investigated. The results indicated that 5% of isolates were categorized as MDR, while the remaining 95% were classified as XDR. These findings contradict the reports of [13], [22], [23], [30] which reported higher MDR rates and relatively lower XDR rate.

The low sensitivity observed in our study for the modified Hodge test, with only 13.4% of isolates showing positive results, contradicts previous studies. Our findings are in contrast to the results reported by[22], who reported a sensitivity of 60% for the MHT. Similarly, [31, 32]reported sensitivities of 33% and 20%, respectively, which are more consistent with our study's findings.

In terms of carbapenemase-encoding genes, bla_{VIM} was detected in 18% of the isolates, resembling the findings of [32] but differing significantly from the studies conducted

by[33–35]. Interestingly, *bla*$_{SPM}$ was found in 50% of the isolates, contradicting the findings of [34], where none of the tested isolates carried this gene. This discrepancy suggests regional variations in the prevalence of *bla*$_{SPM}$ and highlights the need for further investigation into the factors contributing to its distribution. Furthermore, *bla*$_{NDM}$ was identified in 23% of the isolates, contradicting the findings of [36]. The presence of *bla*$_{NDM}$ emphasizes the importance of this gene in conferring carbapenem resistance and underscores the need for effective infection control measures to prevent its spread. Notably, *bla*$_{IMP}$ was not detected in any of the isolates, resembling the studies conducted by [33, 35, 37, 38]. However, our findings differ from[35], which reported the presence of *bla*$_{IMP}$. This discrepancy might be due to regional variations or the circulation of different carbapenemase-encoding genes in different settings.

CONCLUSION

In conclusion, our study revealed that *bla*$_{SPM}$ was the most prevalent gene among the isolates of *Acinetobacter baumannii*. Additionally, our findings indicate alarmingly high resistance rates against a wide range of commonly prescribed antibiotics for *A. baumannii* infections. The classification of 95% of the isolates as extensively drug-resistant further emphasizes the challenges in effectively treating these infections due to resistance to multiple classes of antibiotics.

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