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Carbapenem Resistance Mechanisms In *Acinetobacter Baumannii*: Spotlight On OXA Genes

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ABSTRACT

Background: *Acinetobacter baumannii is a highly problematic bacterium due to its multidrug-resistant nature and ability to rapidly develop resistance. Carbapenem-resistant A. baumannii (CRAB) is particularly concerning, recognized as a top priority pathogen by the World Health Organization (WHO). In this study, we aimed to assess the susceptibility of A. baumannii to antibiotics and characterize the presence of specific carbapenem resistance genes using PCR analysis. The study was conducted as a cross-sectional investigation at Al-Sader Medical City and Baghdad Teaching Hospitals between October 2022 and February 2023. A total of 59 A.baumannii isolates were collected from patients. To ensure accuracy in diagnosis, the isolates underwent thorough processing using morphological techniques, biochemical tests, and Vitek2 systems. The susceptibility of the isolates to 24 antibiotics was evaluated using the Kirby-Bauer method. Additionally, DNA extraction and PCR analysis were performed to detect the presence of carbapenem resistance genes. The study included analysis of 59 specimens collected from patients, including sputum, wound swabs, blood, and inguinal swabs. The majority of the isolates were obtained from in-patients, demonstrating a significant difference compared to outpatients. Among the studied isolates, the most prevalent carbapenem resistance gene detected was blaOXA51, present in 97% of the isolates. This highlights the substantial presence of blaOXA-mediated carbapenem resistance among A. baumannii strains in our study population. Furthermore, our findings revealed alarmingly high resistance rates against the majority of antibiotics commonly used to treat A. baumannii infections. Approximately 95% of the isolates were classified as extensively drug-resistant, indicating resistance to multiple classes of antibiotics. This poses significant challenges in terms of effective treatment options and emphasizes the urgent need for alternative strategies to manage A. baumannii infections.*

Keywords: A. baumannii, Carbapenem-Resistant A. baumannii (CRAB), Beta lactamase Genes, Oxacillinase Genes, Extensive Drug-Resistant (XDR).

Article Information

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INTRODUCTION

Acinetobacter baumannii is an opportunistic pathogen that can survive for long periods on both dry and moist surfaces. *A. baumannii* is prevalent in healthcare facilities, colonizes different surfaces and survives on the hair or skin of patients and hospital staff as a commensal bacterium. The ability of *A. baumannii* to survive in hospital environments for a long time and its ability to gain many virulence factors have led it to emerge as an important nosocomial pathogen[1,2]. *Acinetobacter baumannii*is classified as an ESKAPE organism, alongside *Enterococcus faecium*,

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Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterobacter spp. These bacteria possess a remarkable ability to rapidly acquire antimicrobial resistance, reshaping our understanding of pathogenesis, transmission, and resistance in infectious diseases[3]Among the ESKAPE organisms, *A. baumannii* holds a critical position, posing a significant threat in hospital settings, especially in critically ill individuals[4].

Intrinsic resistance to various antibiotics is a prominent characteristic of *A. baumannii*, and its propensity to acquire antibiotic resistance genes further contributes to the escalating patterns of antimicrobial resistance[5]. This evolutionary process gives rise to novel and more complex pathogens, presenting formidable challenges for healthcare providers[6]. The World Health Organization (WHO) recognizes antibiotic resistance as a major global health issue[7], Projections suggest that by 2050, infections caused by resistant strains could lead to 300 million premature deaths[8]. In 2018, carbapenem-resistant *A. baumannii* (CRAB) was identified by the WHO as the top priority for antibiotic research and development. The selection of carbapenem resistance as a marker stems from its association with broad-spectrum co-resistance to other classes of antibiotics[9]. In this study, we aimed to evaluate the susceptibility of *A. baumannii* to various antibiotics and characterize the presence of specific carbapenem resistance genes using PCR analysis. Initially, susceptibility testing of *A. baumannii* isolates was conducted to assess their susceptibility or resistance to a range of antibiotics, including both commonly used drugs and carbapenems, which are recognized as essential treatment options. These testing results yielded crucial insights into the efficacy of various antibiotics against *A. baumannii*. Furthermore, we employed PCR analysis to investigate the presence of specific carbapenem resistance genes, namely bla_{OXA}, bla_{OXA23}, and bla_{OXA51}, bla_{OXA48} within the *A. baumannii* isolates. This molecular approach allowed for the identification and characterization of these genes, which are known to be associated with carbapenem resistance in *A. baumannii*.

MATERIALS AND METHODS

Study Design and Specimen Collection

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 testing

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).

Detection of Oxacillinase Genes in *A. baumannii*

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 [11, 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*$_{OXA}$**	$_{OXA}$-F	GGCACCAGATTCAACTTTCAAG	564	[12]
	$_{OXA}$-R	GACCCCAAGTTTCCTGTAAGTG		
***bla*$_{OXA-48}$**	$_{OXA}$-48-F	TTG GTG GCA TCG ATT ATC GG	744	[11]
	$_{OXA}$-48-R	GAG CAC TTC TTT TGT GAT GGC		
***bla*OXA-23-like**	$_{OXA}$-23-F	GATCGGATTGGAGAACCAGA	501	[11]
	$_{OXA}$-23-R	ATTTCTGACCGCATTTCCAT		
***bla*$_{OXA-51-like}$**	$_{OXA}$-51-F	TAATGCTTTGATCGGCCTTG	353	
	$_{OXA}$-51-R	TGGATTGCACTTCATCTTGG		

Statistical 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 mean age of the study population was 62 years (± standard deviation). Age was categorized into five groups: 1-20, 21-40, 41-60, 61-80, over 80 years. The analysis revealed that the highest percentage of specimens was observed in the 61-80 age group (n=27; 45.7%), while the lowest percentage was observed in the over 80 age group (n=2; 3.3%) The result showed there was highly significant different among these age groups and the bacterial isolated with *p-value* 0.00001.. 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.

Antibiotic susceptibility pattern

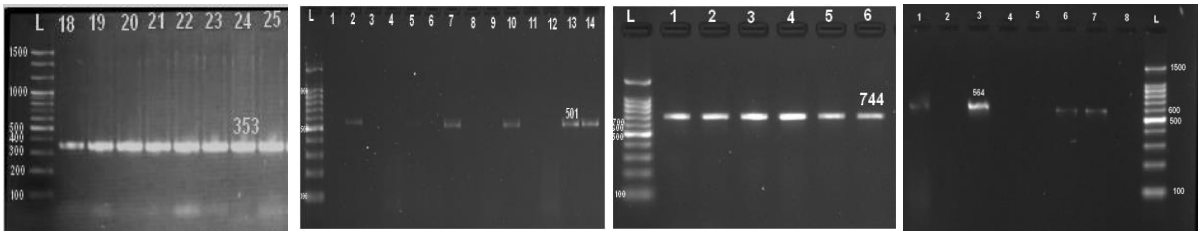
The susceptibility results of *A. baumannii* to 24 antibiotics from 9 antimicrobial categories was assessed. The results demonstrated complete resistance of all isolates to ampicillin and 96.6% resistance to pipracillin. The resistance rates for beta-lactam combinations were 57.6% for ampicillin-sulbactam, 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.

Table 2: Prevalence Rates of Multi-Drug Resistant (MDR), Extensively Drug Resistant (XDR), and Pan Drug Resistant (PDR) Strains.

Multiple resistance type	Antibiotic agents No.	Isolates	No. / %	No./ % Inpatient	Outpatient
MDR (n=3/ 5%)	4	Ab58 wound swab	1 /1.6%	0	1 /1.6%
	5	Ab57 wound swab	1 /1.6%	0	1 /1.6%
	8	Ab56 wound swab	1 /1.6%	0	1 /1.6%
XDR (n=56/ 95%)	10	Ab55 wound swab	1 /1.6%	0	1 /1.6%
	11	Ab54 wound swab	1 /1.6%	0	1 /1.6%
	12	Ab53 wound swab	1 /1.6%	0	1 /1.6%
	15	Ab47, Ab48, Ab49, Ab50, Ab51, Ab52 wound swab and Ab16	7/11.8%	5/8.4%	2/3.3%
	16	Ab1, Ab2, Ab7, Ab12 are sputum and Ab3 is wound swab	5/8.4%	5/8.4%	0
	17	Ab11 wound	1 /1.6%	1 /1.6%	0
	18	Ab10, Ab18, Ab35, Ab38, Ab43 are wound swabs and Ab33 is sputum	6/10.1%	6/10.1%	0
	19	Ab6, Ab15, Ab19 are wound swabs, Ab22, Ab30,Ab32, Ab34, Ab36, Ab37, Ab41, Ab44, Ab45, Ab46 are sputum and Ab24 is blood	14/23.7%	14/23.7%	0
	20	Ab4, Ab8, Ab9, Ab20, Ab21 are wound swabs and Ab17, Ab23, Ab25, Ab26, Ab27, Ab29, Ab39, Ab40 are sputum	13/22%	13/22%	0
	21	Ab13 sputum	1 /1.6%	1 /1.6%	
PDR	0				

Molecular Characterization of Oxacillinase Resistance Genes

The analysis of the isolates revealed the presence of several genes associated with carbapenem resistance. The most prevalent gene was *bla*$_{OXA51}$, which was detected in 97% of the isolates. This was followed by *bla*$_{OXA23}$, which was identified in 74% of the isolates. Furthermore, *bla*$_{OXA48}$ was present in 39% of the isolates, while *bla*$_{OXA}$ was detected in 10% of the isolates. These findings suggest a diverse distribution of carbapenemase-encoding genes among the studied isolates, highlighting the importance of these genes in conferring resistance to carbapenem antibiotics.



[a] [b] [c] [d]

Figure 1. Agarose gel electrophoresis of PCR ampilified products from extracted DNA of *A. baumannii* **(a)** amplified with *bla*$_{OXA51}$ *genes primers.* L: size marker (100-1500bp), Lanes (18,19,20, 21, 22,23,24,25) illustration positive results with *bla*$_{OXA-51}$ (353 bp). **(b)**: *bla*$_{OXA-23}$ *genes primers.* L: size marker (100-1500bp), Lanes (1,7,10,13,14) illustration positive results with *bla*$_{OXA-23}$ (501 bp). **(c)***: bla*$_{OXA-48}$ genes primers. L: size marker (100-1500bp), Lanes (1,2,3,4,5,6) illustration positive results with *bla*$_{OXA-48}$ (744 bp). **(d)**: *bla*$_{OXA}$genes primers. L: size marker (100-1500bp), Lanes (1, 3, 6 and 7) illustration positive results with *bla*$_{OXA}$ (564 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 61-80 age group, comprising 45.7 % 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 over 80 year's age groups had the lowest representation, accounting for only 3.3% 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.

In terms of Oxacillinase-encoding genes, the most prevalent gene identified in our study was *bla*$_{OXA51}$, which was detected in 97% of the isolates. This finding is consistent with previous studies conducted by [31-37]. The high prevalence of *bla*$_{OXA51}$ suggests its significant role in carbapenem resistance and highlights the need for targeted interventions against this gene. The second most prevalent gene identified in our study was *bla*$_{OXA23}$, detected in 74% of the isolates. This finding aligns with the studies conducted by [32, 36-38], but slightly differs from [37]. The presence of *bla*$_{OXA23}$ in a substantial proportion of isolates indicates its importance as a carbapenemase-encoding gene in the studied population. We identified *bla*$_{OXA48}$ in 39% of the isolates, which shows variation compared to [36] and contradicts the findings of[38, 39], where none of the isolates carried *bla*$_{OXA48}$. This finding suggests the emergence of *bla*$_{OXA48}$ in the studied population, emphasizing the need for ongoing surveillance and monitoring of resistance mechanisms.

CONCLUSION

In conclusion, our study determined that *bla*$_{OXA51}$ and *bla*$_{OXA23}$ were the most prevalent genes detected among the isolates of *A. baumannii*. Furthermore, our findings reveal

concerning levels of resistance against a wide range of commonly used antibiotics for the treatment of *A. baumannii* infections. Particularly noteworthy is the classification of 95% of the isolates as extensively drug-resistant, indicating their resistance to multiple classes of antibiotics.

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