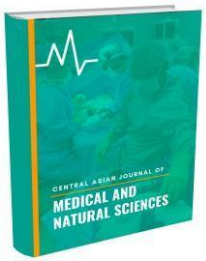

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Phenotypic and Molecular Characterization of Acinetobacter Baumannii and its Ability to Produce Beta-Lactamase Isolated from Different Clinical Samples in Diwaniyah Governorate

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Abstract: Acinetobacter baumannii is an opportunistic bacterial pathogen responsible for a significant proportion of nosocomial infections, particularly in intensive care units. The bacterium's multidrug resistance and ability to survive in hospital environments contribute to its persistence and pathogenicity. In Diwaniyah Governorate, limited studies have explored the phenotypic and molecular characteristics of A. baumannii, particularly its beta-lactamase production and antibiotic resistance. Identifying genetic markers associated with drug resistance is crucial for developing targeted antimicrobial strategies. Although previous research has investigated A. baumannii in different hospital settings, data on its molecular characterization and resistance profile in this region remain scarce. Understanding the prevalence of resistance genes, such as TEM-2 and blaOXA-51, can provide valuable insights into infection control measures. This study aimed to isolate and identify A. baumannii from clinical samples, determine its antibiotic resistance profile, and analyze the presence of beta-lactamase-producing genes using molecular techniques. Results: Of the 60 clinical samples, 20 (33%) were confirmed as A. baumannii. The highest isolation rate was from sputum (55%), followed by burns (45%) and wounds (15%). The rapid iodine test and Vitek 2 system confirmed that 85% of isolates produced beta-lactamase, exhibiting resistance to most antibiotics except Tigecycline. PCR analysis revealed that all isolates carried the 16S rRNA gene, 85% harbored the TEM-2 gene, and 50% contained the blaOXA-51 gene. This study is among the first to document the molecular profile of A. baumannii in Diwaniyah Governorate, highlighting its high resistance levels and genetic determinants of beta-lactamase production. Implications: The findings underscore the urgent need for continuous surveillance, stringent infection control protocols, and antimicrobial stewardship programs to combat the spread of drug-resistant A. baumannii in healthcare settings.

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1. Introduction

Acinetobacter baumannii is a bacterial species characterized by its pathogenicity, as more than 80% of human disease cases are associated with infection with this bacteria during the patient's admission and hospitalization. The aforementioned bacteria are considered an opportunistic pathogen due to their presence in hospitals [1]. The danger of this bacteria increases with its rapid spread in the environment and its tolerance to dry conditions for a period of up to more than a month and a half. This bacteria causes many infections acquired in hospitals, including pneumonia, respiratory tract infections, soft

tissue infections, blood poisoning, and skin inflammation. There are several factors that contribute to the occurrence of infection with this bacteria, including the patient's age, the length of the patient's hospitalization, and people with immunodeficiency [2]. The reason for the pathogenicity of A. baumannii bacteria is due to its possession of many virulence factors, including the endotoxin that includes lipopolysaccharides (LPS), and its production of iron siderophores and necrosis factor. Cytotoxic factor necrotizing, capsule production, biofilm formation, thin membrane formation, and protease production [4]. Many Gram-negative bacterial species such as E. coli, Klebsiella, and Pseudomonas are resistant to antibiotics because they have (LPS) in cell wall [4]. These bacteria are characterized by their high resistance to antibiotics, including the group of beta-lactamases such as penicillins, cephalosporins, aminoglycoside antibiotics, fluoroquinolones, and Sulphamethoxazole-Trimethprim antibiotics, due to their production of broad-spectrum beta-lactamases [5].

2. Materials and Methods

2.1.Sample collection

60samples were collected from hospitals and health centers in Diwaniyah Governorate, namely (Women's and Children's Hospital, Diwaniyah General Teaching Hospital, and Public Health Laboratory) for the period from August 2023 to November 2023, as the study included different clinical samples, collected from different areas of the body for hospitalized patients of all ages and both sexes, as samples were taken from (wounds, burns, and sputum).

2.2 Isolation and identification

Swabs and samples were transferred directly to the laboratory, and the cultures were incubated under aerobic conditions at a temperature of 37°C for 24 hours to diagnose some cultural, microscopic and biochemical characteristics. Bacterial species were isolated using culture media and diagnosed based on the morphological and microscopic characteristics of the isolates growing on the plate and through a series of biochemical tests (catalase, oxidase, IMVC and TSI tests). The diagnosis of A. baumannii isolates was confirmed as reported [6] and also by the Vitek2 Compact.

2.3. Rapid standard iodine method for detecting the ability of bacteria to produce beta-lactamase enzymes

a. A number of newly grown colonies of (18-24) hours old, grown on the heart-brain infusion medium, were transferred using sterile wooden sticks to a microdilution plate containing 100 microliters of penicillin G solution in each hole of the plate, mixed well and incubated at 37 °C for 30 minutes.
b. Add 50 microliters of starch solution, mixed using wooden sticks and add 20 microliters of iodine solution, mixed well with sticks, to ensure homogeneity of the contents. The formation of the blue color occurs as a result of the reaction of iodine with starch, and the rapid color change from blue to white within 5 minutes is a positive result [7]. (table 1)

Table 1. Primers used in this study with their nucleotide sequence and amplification size.

Gene name		Oligo sequence (5'-3') (primer)	Product Size(bp)
16srRNA	F	TAAGCCGTGGAGGGTCATTG	**560 bp**
	R	GTATGTCACCGGCAGTCAAC	
TEM-2	F	ACAGTTCACATGCCAAAGAGT	**450bp**
	R	TACCGAAAGCAGCAGGTGTT	
bla *OXA-51*	F	TAATGCTTTGATCGGCCTTG	**343bp**
	R	**TGGATTGCACTTCATCTTGG**	

2.4.Genomic DNA extraction

NA was extracted from Acinetobacter baumannii using the ready-made Genomic DNA Mini Kit provided by Geneaid USA and according to the instructions of the company.

2.5 Preparation of Agarose Gel

It was prepared according to the method of Sambrook and his group (15) as follows:

a. Dissolve 1.5 g of Agarose gel in 100 ml of TBE buffer solution at a concentration of (1X) and use a magnetic hot plate stirrer for 15 minutes.
b. The gel was left to cool at 50°C, then (3) microliters of the radioactive DNA dye Ethidium bromide were added and mixed well with the gel.
c. The acro gel was poured into the migration mold ((Tray containing the comb, then the gel was left to solidify at room temperature for 15 minutes, the comb was carefully removed from the gel for the purpose of making and defining the holes (wells) in the gel necessary for injecting the amplified samples.

2.6 PCR Master Mix Preparation

1. Prepare this mixture in PCR tubes according to the instructions of the company that supplied Wizpure PCR FDmix and as shown in the following table 2:

Table 2. Components of the PCR master mix.

Mixture Ingredients	Size
PCR FD mix	1Tube
10mM Forward Primer	1.5 µL
10mM Reverse Primer	1.5 µL
Template DNA	5 µL
PCR water	12 µL
Total	20 µL

2. After completing the process of preparing the mixture tubes, they were transferred to the Vortex device for 5 seconds, after which the tubes were transferred to the PCR

thermocycler device to perform the DNA amplification process according to the ideal conditions for thermal cycles.

3. The thermal cycle program required for DNA amplification
   The Thermocycler PCR device was used and programmed for the genes under study according to the reaction.

3. Results and Discussion

3.1 Numbers and percentages of Acinetobacter baumannii isolation

20 isolates were diagnosed (33% isolation rate). The highest isolation rate of Acinetobacter baumannii was from sputum (55%), followed by burn infections (45%), while the lowest isolation rate was in wounds (15%). The reason may be attributed to the ability of this bacterium to adhere to the mucosal cells of the respiratory system and produce inhibitors such as bacteriocins. This study was consistent with what was mentioned by [8], who explained that the highest infection rate was with the respiratory system, which amounted to 26 isolates out of a total of 37 samples, at a rate of 72%, while it differed with what was explained by [9], as the lowest isolation rate was from sputum samples at a rate of 10%. This may be due to the difference in the time of sample collection, environmental conditions, the number of samples taken, and the health conditions in which the patients live. (Figure 1)

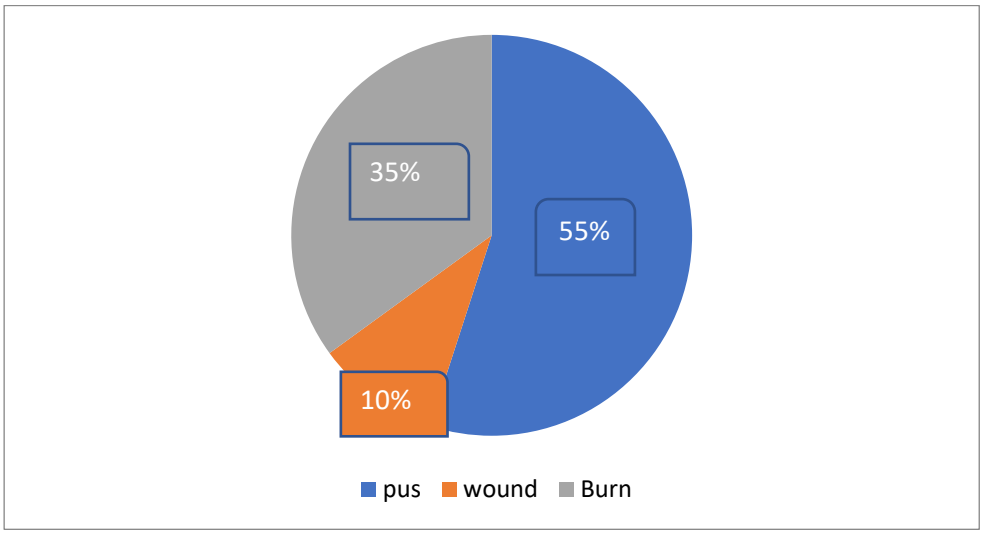


Figure 1. Distribution of Acinetobacter baumannii isolates according to sample source.

The results of the current study showed that the percentage of isolation of Acinetobacter baumannii bacteria from people aged 21-30 is 80% as shown in Table 2. This is due to the activity of this age group in social and professional life, which increases the risk of exposure to bacterial infection in different environments, while the lowest percentage of isolation of Acinetobacter baumannii bacteria from people aged (51-60) and (61-70) was 10%. Table No. (3) Distribution of infection with Acinetobacter baumannii bacteria according to the age groups of the infected and their percentage.

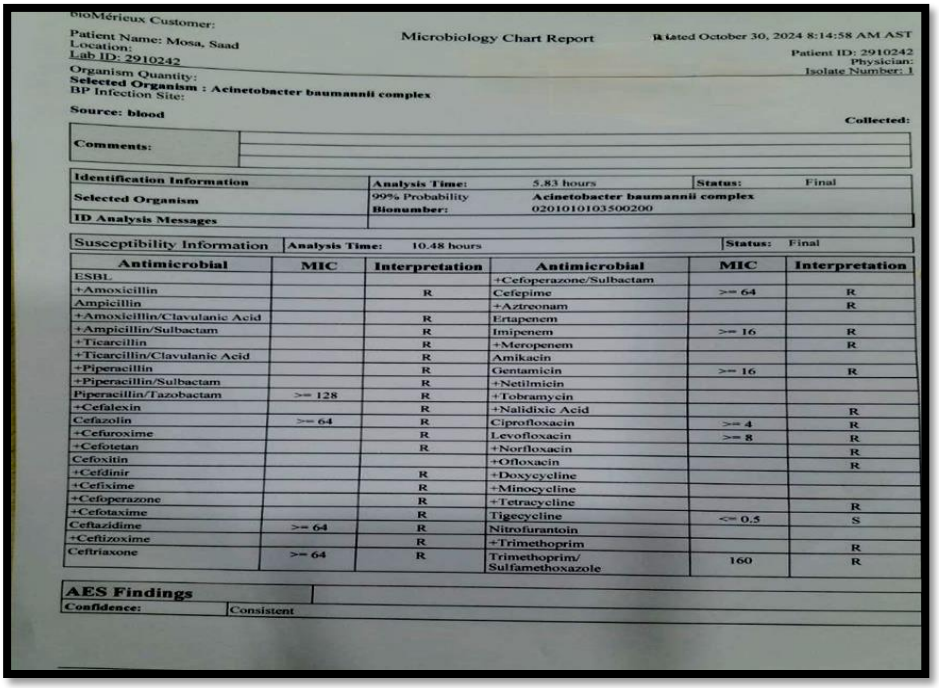
Table 3. Distribution of infection with Acinetobacter baumannii bacteria according to age groups of infected persons and their percentage.

Age groups	Number of samples	Number of isolates	Percentage (%)
11-20	10	3	**0%3**
21-30	10	8	**%80**
31-40	10	5	**%50**

	41-50	10	2	**%20**
	51-60	10	1	**%10**
70	- 61	10	1	**%10**
	Total	60	20	**% 33.3**

Ability A.bummanii isolates to antibiotic resistant and produce beta-lactamase enzymes.

The results showed that 17 isolates out of 20, at a rate of 85%, had the ability to produce beta-lactamase enzymes and the resistance shown by these bacteria to some antibiotics such as penicillins and cephalosporins that contain a beta-lactam ring in their structure. This is consistent with what was mentioned [10]. Based on the results of the Vitek, the majority of the isolates showed resistance to the antibiotics under study with a resistance rate of 100%, except for the Tigecycline antibiotic, which showed its effectiveness and inhibition of bacteria, as the sensitivity rate of bacteria to this antibiotic reached between (0.5- 1), as shown in the attached image of the Vitek device down. (Figure 2)



bioMérieux Customer:
Patient Name: Mosa, Saad
Location:
Lab ID: 2910242
Organism Quantity:
Selected Organism : Acinetobacter baumannii complex
BP Infection Site:
Source: blood

Microbiology Chart Report

Patient ID: 2910242
Physician:
Isolate Number: 1
Collected:

Comments:

Identification Information	Analysis Time:	5.83 hours	Status:	Final
Selected Organism	99% Probability	Acinetobacter baumannii complex		
ID Analysis Messages	Bionumber:	0201010103500200		

Susceptibility Information — Analysis Time: 10.48 hours — Status: Final

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL			+Cefoperazone/Sulbactam		
+Amoxicillin		R	Cefepime	>= 64	R
Ampicillin			+Aztreonam		R
+Amoxicillin/Clavulanic Acid		R	Ertapenem		
+Ampicillin/Sulbactam		R	Imipenem	>= 16	R
+Ticarcillin		R	+Meropenem		R
+Ticarcillin/Clavulanic Acid		R	Amikacin		
+Piperacillin		R	Gentamicin	>= 16	R
+Piperacillin/Sulbactam		R	+Netilmicin		
Piperacillin/Tazobactam	>= 128	R	+Tobramycin		
+Cefalexin		R	+Nalidixic Acid		R
Cefazolin	>= 64	R	Ciprofloxacin	>= 4	R
+Cefuroxime		R	Levofloxacin	>= 8	R
+Cefotetan		R	+Norfloxacin		R
Cefoxitin			+Ofloxacin		R
+Cefdinir		R	+Doxycycline		
+Cefixime		R	+Minocycline		
+Cefoperazone		R	+Tetracycline		R
+Cefotaxime		R	Tigecycline	<= 0.5	S
Ceftazidime	>= 64	R	Nitrofurantoin		
+Ceftizoxime		R	+Trimethoprim		R
Ceftriaxone	>= 64	R	Trimethoprim/ Sulfamethoxazole	160	R

AES Findings
Confidence: Consistent

Figure 2. the Vitec device proving the accurate diagnosis of Acinetobacter baumannii and its resistance to antibiotics.

The table 4 lists the incidence of ability to produce beta-lactamase enzymes in isolates of Acinetobacter baumannii of various source isolations. The isolates were beta-lactamase producer in 85% and non producer in 15% and they were 10, 5, and 2 isolates from pus while that from burn and wound sources respectively.

Table 4. Ability A.bummanii isolates to produce beta-lactamase enzymes according source isolated.

Ability to produce beta-lactamase enzymes	**Source Isolated**			**Percentage %**
	Pus	**Burn**	**Wound**	
Producing isolate	10	5	2	**85**
Non-producing isolate	1	2	0	**15**

As shown in the figure 3, white represents positive in the rapid standard iodine method and dark represents negative. Distinct color variations observed in the microplate assay reflect specific enzymatic activity of the tested samples for bacterial starch hydrolysis or other biochemical properties assessment.

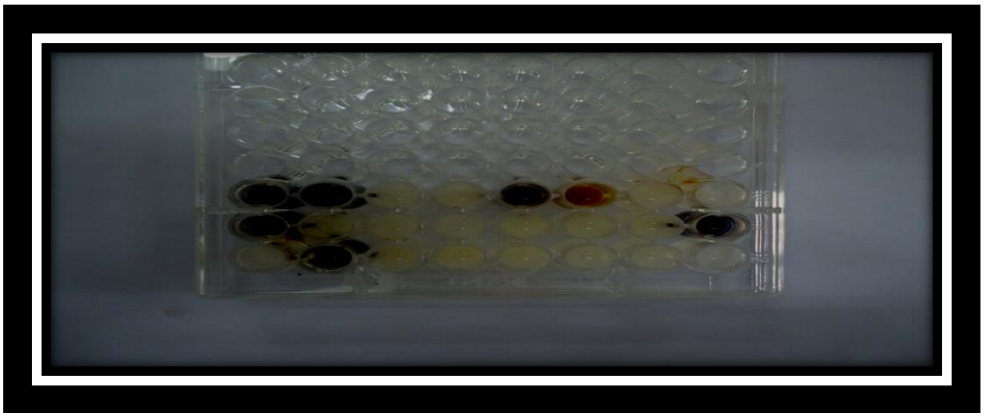


Figure 3. Shows the rapid standard iodine method (White is positive, Dark is negative).

3.2.Molecular diagnosis of A. bumannii using PCR

All 20 bacterial isolates under study were tested for the purpose of identifying and diagnosing the 16srRNA gene, and all isolates, at a rate of 100%, contained the 16srRNA gene. The virulence gene (bla oxa-51 gene) was also investigated at a rate of 85%. This may be due to the production of beta-lactamase enzymes by these bacteria and their resistance to antibiotics of this group by changing the outer membrane proteins and efflux pumps [11].

Acinetobacter baumannii is considered one of the bacteria that are multi-resistant to antibiotics and has several mechanisms that make it resistant to these drugs, such as its production of beta-lactamase enzymes that nullify the effectiveness of antibiotics and the expulsion pumps that expel antibiotics out of the cell, as well as its possession of some genes carried on the chromosome or plasmid of this bacteria that enable it to resist antibiotics, as shown in Figure (4). These are among the main reasons for the resistance of the bacteria under study to antibiotics [12], [13], [14].

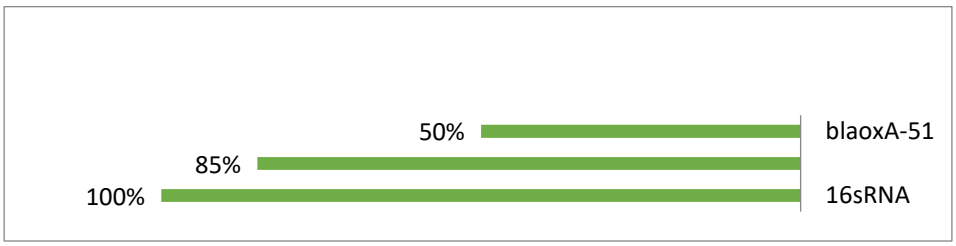


Figure 4. The percentage of gene prevalence in Acinetobacter baumannii bacteria under study.

4. Conclusion

It reveals noteworthy prevalence of Acinetobacter baumannii in clinical samples from Diwaniyah governorate, with the notable resistance against most of the antibiotics but sensitive to Tigecycline. All the isolates were found to carry 16S rRNA gene with 85% carrying the blaTEM-2 gene and 50% such isolates containing blaOXA-51 gene, indicating high potential to produce beta lactamase. These findings highlight the urgency for strengthened infection control measures, antibiotic stewardship programs and continuous molecular surveillance of the spread of multidrug resistant A. baumannii in health care settings. Future research should therefore identify alternative therapeutic

strategies targeting, for example, novel antimicrobial agents or targeted modes of gene inhibition for treatment against this persistent pathogen.

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