







10.30699/ijmm.18.3.200

Iranian Journal of Medical Microbiology | ISSN:2345-4342



Isolation and Molecular Identification of *Acinetobacter baumannii* From Urinary Tract Infection in Diyala Province, Iraq

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ABSTRACT

Background and Aim: *Acinetobacter baumannii* is one of the most prominent opportunistic bacterial pathogens associated with hospital-acquired infections and has been associated with antibiotic resistance. The high rates of resistance have made it difficult to choose the appropriate treatment and put the lives of infected patients at risk of death. This study aimed to isolate *A. baumannii* from urinary tract infections (UTI) and detect the bacterial ability to form biofilms from clinical samples.

Materials and Methods: In this study, *A. baumannii* bacteria were isolated from several sources (UTI). The microtiter plate method revealed biofilm formation. Clinical specimens were grown on selective media. The *A. baumannii* was identified by classical techniques; the VITECK combined 2 system and 16S rRNA gene amplification.

Results: From the 130 suspected isolates, 20 isolates were obtained from *A. baumannii* multidrug-resistant (MDR) and extensively drug-resistant (XDR) types. Among them, 14 (70%) were MDR and 6 (30%) were XDR types.

Conclusion: The results showed that *A. baumannii* bacteria were more resistant to antibiotics and had strong biofilm formation.

Keywords: *Acinetobacter baumannii*, Antibiotic Resistant, Biofilm, Extensively drug-resistant, Multidrug-resistant

Received: 2024/04/24;

Accepted: 2024/08/08;

Published Online: 2024/08/18;

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Raheem Hassooni H, Ibrahim Ahmed R, Alzubaidy Z M, Hassan Alhusseiny A. Isolation and Molecular Identification of *Acinetobacter baumannii* From Urinary Tract Infection in Diyala Province, Iraq. Iran J Med Microbiol. 2024;18(3):200-8.

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1. Introduction

The most important causes of asthma, blood poisoning, meningitis, and wound and urinary tract infections (UTIs) are *A. baumannii* bacteria (1). These opportunistic bacteria evolved to multidrug-resistant (MDR) and extensively drug-resistant (XDR) (2). The emergence of resistance to different types of antibiotics has become a common cause of problems in choosing the appropriate treatment (3). These bacteria are resistant to numerous antibiotics such as amoxicillin, cephalosporin, cefotaxime, chloramphenicol, gentamicin, tobramycin,

quinolones, and macrolides (4, 5). Biofilm formation is the most important virulence factor that contributes to the adaptation of bacteria to survive and spread infections (6). Microorganisms that form biofilm can cause persistent diseases and are antibiotic-resistant (7). The poly-(1,6)-N-acytelglucose amine is an additional polysaccharide hypothesized to function as an intracellular adhesion factor for the biofilm formation of bacteria (8, 9). Resistance to antibiotics, especially aminoglycosides, has increased due to the increased use of antibiotics (10).

There are two main causes of aminoglycoside resistance: the entry of aminoglycoside antibiotics and methylation enzymes (11). They are controlled by 12 different genes (12). The *A. baumannii* may move by excreting exopolysaccharide, a film consisting of sugar chains with high molecular weight (13). The oxidase test was used by clinical microbiologists to differentiate *Acinetobacter spp.* from other *Moraxellaceae*. The *Acinetobacter spp.* are the only *Moraxellaceae* members with lack of cytochrome c oxidases (14). The ACB complex includes (*A. baumannii*, *A. calcoaceticus*, and *Acinetobacter* genomic species 13TU). Members of the ACB complex are therapeutically relevant but difficult to identify (15). Most nosocomial infections are caused by *A. baumannii*, an ESKAPE pathogen (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*) (16). In European intensive care centers, infections caused by *Acinetobacter spp.* accounted for 21.8% of pneumonia cases, 17.1% of bloodstream infections, and 11.9% of UTIs (17). The pathogenic characteristics of *A. baumannii* include pathogenicity islands that are groups of two or more genes that enhance the virulence of a disease (18, 19). They can express toxins, coagulate blood, or cause resistance to antibiotics. AbaR-type resistance islands, which lead to drug resistance, are common in *A. baumannii*. Each one is 16.3 Kb and promotes horizontal gene transfer. Transposons allow the genetic material to be moved across the genomic locations (20).

Dual efflux pumps in *A. baumannii* reduce its antibiotic susceptibility. The first pump, AdeB, is aminoglycoside resistant (21), and the second one, known as AdeDE, is responsible for the efflux of a wide variety of substrates. These substrates include tetracycline, chloramphenicol, and other carbapenems (22). This study aimed to extract *A. baumannii* from UTIs and detect biofilm-forming bacteria from the clinical specimens.

2. Materials and Methods

The bacteria were isolated from collected samples during the period of August 2022 to February 2023. The samples (130 urine samples) were obtained from Al Batoool Hospital and Al-Jumhuri Teaching Hospital. These samples were incubated directly on blood agar and MacConkey agar at 37°C for 24 hr in order to initial diagnosis and knowledge of bacterial growth conditions. Then, suspected bacteria were cultured on selective *Acinetobacter* medium (LAM) (7-8) to confirm the diagnosis. Subsequently, all samples were confirmed by VITEK2 and molecular examination.

Identification of the Isolated Bacteria

Cultural Characteristics and Microscopic Evaluation

The staining and cell arrangement were observed under the light microscope. Morphological examination was combined with biochemical testing to specifically identify the isolates. The oxidase, urease, and motility tests were conducted, as well as oxidation–fermentation (O-F) tests, catalase tests, and IMViC tests (Indole test, Methyl Red test, Voges-Proskauer test, and Citrate test), triple sugar iron Agar, and carbohydrate fermentation tests according to the instructions (9, 10).

Identification of *A. baumannii* by PCR Amplification

According to the previous studies (11, 12) the salting out approach was used to extract DNA from the isolates. DNA was taken from a patient's blood and used as a template for polymerase chain reaction (PCR) amplification. The mixtures (25 µl) consisted of GoTaq® Green master mix 2X (Promega, USA) and 10 µM of each forward (AGAGTTTGATCCTGGCTCAG) and reverse (TACCAGGGTA TCTAATCCTGTT) primers (Kapa, USA) (13), DNA template (100 µg) and PCR free ion water (Promega). The 16S ribosomal RNA (rRNA) was amplified using a thermal cycler (Gene Amp 97000, Applied Biosystem-Singapore).

To replicate 16S rRNA the cycling program below was followed: initial 95°C denaturation for 3 min, 30 cycles of 95°C for 1 min, 55°C annealing for 1 min, 72°C extension for 1 min, and a final 72°C extension of 5 min. To identify the PCR amplicons, the PCR products were exposed to agarose gel electrophoresis, and visualization was performed on the gels containing ethidium bromide.

Biofilm Formation

Tryptic soy broth (TSB) was inoculated into the sterile 96-well flat-bottomed plates loaded with bacterial culture (Hi Media India). Negative controls utilized only medium for 24 hr at 37°C. Each well was then drained, rinsed with sterile saline, and dried. The plates were then incubated for 5 min with 2% crystal violet (Hi Media India). Any residual stain on the plate was washed away with dH₂O. After that, the plates were air-dried, and the dyes conjugated to the adhering cells were solubilized with 33% glacial acetic acid (v/v). An ELISA reader was used to measure the optical density (OD) at 650 nm (10, 11).

Detection of Extended-Spectrum β-lactamases Production

The double disk synergy test (DDST), was performed in accordance with the procedures previously

described (23) to establish the level of extended-spectrum β -lactamases (ESBL) production.

Detection of Metallo β -lactamases Production

After placing two disks of 10 μ g imipenem (IMP) on the surface culture already contained the isolates, ethylenediaminetetraacetic acid (EDTA) (0.5 M) and dehydrated disodium salt (750 μ g) were added to one IMP disk. Following 16-18 hr incubation, a comparison was made between the inhibitory zones surrounding IMP and EDTA disks. A favorable result would be the rise of at least 7 mm in the zone size surrounding the IMP-EDTA disk (14).

Antimicrobial Susceptibility Tests and Examination of MDR and XDR Isolates

Disk diffusion method was used to determine the MDR and XDR levels of the local isolates of *A. baumannii* using Mueller Hinton agar and antibiotic disks ampicillin-sulbactam (20 μ g), meropenem (10 μ g), clarithromycin (15 μ g), ceftriaxone (30 μ g), azithromycin (15 μ g), erythromycin (15 μ g), nalidixic acid (30 μ g), Trimethoprim (5 μ g), levofloxacin (5

μ g), ciprofloxacin (5 μ g), amikacin (30 μ g), gentamicin (10 μ g), Piperacillin (30 μ g), imipenem (30 μ g), tetracycline (30 μ g), chloramphenicol (10 μ g), ceftiofur (30 μ g) and ceftazidime (30 μ g) as mentioned in CLSI 2019 guidelines (18, 19).

Statistical Analysis

The data from the shaking and static microtiter plates and tube techniques were compared using SPSS 20 and one-way ANOVA statistical technique. A P-value less than 0.05 in the repeated tests was considered significant.

3. Results

Only 20 isolates of *A. baumannii* from 130 patients with clinical UTI symptoms were identified and diagnosed by morphological, biochemical, and VITEK2 system criteria. All isolates were catalase-positive and oxidase-, urease-, and sugar fermentation-negative, as shown in Table 1. In addition, PCR with *A. baumannii* 16S rRNA gene primers confirmed all the isolates, as shown in Figure 1.

Table 1. The biochemical tests for *A. baumannii* identification

Biochemical test	Result
Oxidase test	-
Galactose	+
Catalase test	+
Triple sugar iron agar test (TSI)	K/K, H ₂ S -
Oxidation Fermentation Test	+
Motility test	-
Glucose	+
Urease test	-
Fructose	-
IMVIC	+ ---
Sugar fermentation test	-
Sorbitol	-
Sucrose	-
Maltose	-
Lactose	-

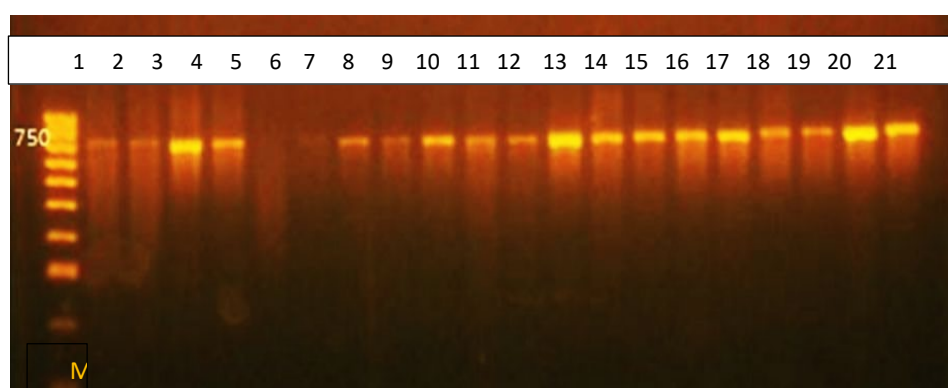


Figure 1. 16S rRNA gene PCR amplification results (product size 750 bp). Lane 1: DNA marker (1500 bp); Lanes 2-21: bacterial isolates with positive results except for Lane 6.

Antibiotic Susceptibility

[Table 2](#) shows antibiotic susceptibility of *A. baumannii* isolates. Only one (5%) isolate was responsive to ampicillin and trimethoprim-sulfamethoxazole versus 19 (95%). Just two (10%) isolates were sensitive to cefepime from cephalosporines and nitrofurantoin, while 18 (90%) were resistant to the same antibiotic. Three isolates (15%) were responsive to nalidixic acid versus 17 (85%) were resistant. Only four (20%) isolates were sensitive

to ceflazidime, while 16 (80%) were resistant to the same antibiotic. In addition, there were 6 isolates (30%) sensitive to cefotaxime, ceftaxime, gentamicin, azithromycin, and chloramphenicol, while 14 isolates (70%) were resistant to the same antibiotics. Piperacillin and tetracycline resistance were 13 (65%). In addition, the current study recorded resistance rates of 60%, 50%, and 30% to amoxicillin-clavulanate, imipenem, and meropenem, respectively. In this study, 4 isolates (20%) were resistant to ciprofloxacin and levofloxacin.

Table 2. Antibiotic susceptibility of *A. baumannii* isolates.

Antibiotics	Sensitive		Resistant	
	No	%	No	%
Ampicillin	1	5	19	95
Trimethoprim-sulfamethoxazole				
Cefepime	2	10	18	90
Nitrofurantoin				
Nalidixic acid	3	15	17	85
Ceflazidime	4	20	16	80
Cephotoxime				
Ceftaxime				
Gentamicin	6	30	14	70
Azithromycin				
Chloramphenicol				
Pipracillin	7	35	13	65
Tetracycline				
Amoxicillin – Clavulanate	8	40	12	60
Imipenem	10	50	10	50
Meropenem	14	70	6	30
Ciprofloxacin	16	80	4	20
Levofloxacin				

Multidrug-Resistance of *A. baumannii*

The current study found that 14 out of 20 (70.0%) and 6 out of 20 (30.0%) *A. baumannii* isolates were MDR XDR, respectively.

Table 3. MDR and XDR isolates.

Categories	%
Multidrug-resistant (MDR)	70.0
Extensively drug-resistant (XDR)	30.0

Association of Biofilm Formation With Variables

The results demonstrated that 11 (55%) of the 20 isolates were strong and moderate biofilm formers, while 3 (15%) were non-producers ([Table 4](#)).

Table 4. The highest rates of biofilm formation in general

Biofilm formation of total No. isolates (20)	Biofilm formation	
	No.	%
Strong and Moderate	11	55
Non-biofilm	3	15

Association of Biofilm Formation With Antibiotic Susceptibility

[Table 5](#) reveals that all strong biofilm former isolates resisted all drugs except meropenem, ciprofloxacin, and levofloxacin. No statistically significant differences were observed ($P>0.05$).

Table 5. Association of biofilm formation with antibiotic susceptibility.

Antibiotics		Biofilm formation					
		Strong		Moderate		Non-biofilm	
		No.	%	No.	%	No.	%
Ampicillin	Sensitive	1	5	-	-	-	-
	Resistant	6	30	11	55	2	10
Pipracillin	Sensitive	1	5	4	20	2	10
	Resistant	8	40	5	25	-	-
Amoxicillin-Clavunate	Sensitive	3	15	3	15	2	10
	Resistant	7	35	5	25	-	-
Cefepime	Sensitive	1	5	1	5	-	-
	Resistant	10	50	6	30	2	10
Cephotoxime	Sensitive	3	15	3	15	-	-
	Resistant	6	30	6	30	2	10
Cefoxitin	Sensitive	3	15	2	10	1	5
	Resistant	6	30	7	35	1	5
Ceflazidime	Sensitive	-	-	2	10	2	10
	Resistant	11	55	5	25	-	-
Imipenem	Sensitive	4	20	4	20	2	10
	Resistant	7	35	3	15	-	-

Antibiotics		Biofilm formation					
		Strong		Moderate		Non-biofilm	
		No.	%	No	%	No	%
Meropenem	Sensitive	5	25	7	35	2	10
	Resistant	3	15	2	10	1	5
Gentamicin	Sensitive	2	10	4	20	-	-
	Resistant	8	40	5	25	1	5
Azithromycin	Sensitive	2	10	3	15	1	5
	Resistant	9	45	5	25	-	-
Tetracycline	Sensitive	3	15	3	15	1	5
	Resistant	7	35	6	30	-	-
Ciprofloxacin	Sensitive	6	30	9	45	1	5
	Resistant	3	15	1	5	-	-
Levofloxacin	Sensitive	7	35	7	35	2	10
	Resistant	2	10	2	10	-	-
Nalidixic acid	Sensitive	1	5	2	10	-	-
	Resistant	9	45	7	35	1	5
Trimethoprim-sulfamethoxazole	Sensitive	-	-	1	5	-	-
	Resistant	7	35	9	45	3	15
Chloramphenicol	Sensitive	4	20	2	10	-	-
	Resistant	5	25	8	40	1	5
Nitrofurantoin	Sensitive	-	-	2	10	-	-
	Resistant	10	50	7	35	1	5

4. Discussion

The origin of bacterial resistance is due to the ability of the bacteria to produce beta-lactamase enzymes or due to a change in the target site and a decrease in permeability of the outer wall (24). The efflux systems may also expel anticellular agents to the outside (25). Microorganisms resistant to at least one antibacterial treatment of three or more are also referred to as multidrug-resistant (MDR). However, when a bacterial species is resistant to all but fewer than two antimicrobial antibiotics, it is called extensively drug-resistant (XDR) (26).

The current study results indicate that the majority of *A. baumannii* isolates were MDR. These strains are rapidly extended between hospitalized patients. These findings are matters of concern for the healthcare staff and society, as they make treating these bacteria complicated. It is consistent with a 2019 CDC report on antibiotic resistance in which the study classified carbapenem-resistant *Enterobacteriaceae* and the resulting risk as an urgent issue and that addressing these threats requires a collaborative global public health approach (27).

Resistance genes are passed by mobile genetic elements such as insertion sequences (IS), plasmids, transposons, and gene cassettes in integrons (28). As well as, which conjugative transposons can transport wide range of resistance genes which allowing spreading multidrug resistance between different types of bacteria (22). In *Acinetobacter*, resistance to cephalosporins and carbapenems is strongly correlated with IS, many of ISAb elements have ability to encode potent externally facing promoters which are required for expression of β -lactamases to confer clinical resistance (29).

The *A. baumannii* antibiotic resistance can be caused by inappropriate exposure to antibiotics, chronic widespread use of antibiotics, exposure to resistance genes, or lack of clinical hygiene. Also, due to the extreme consumption of quinolones and aminopyrimidine, the emergence of resistant strains has caused a lot of concern in treating transferable resistance.

The receptor of *A. baumannii* isolates may be both XDR and pan drug-resistant (PDR). The *A. baumannii* is resistant to carbapenems and other antibiotics (30).

Biofilms that are common in nature for bacterial survival are responsible for their antibiotic resistance. Biofilms contribute to the pathogenesis of bacterial diseases, especially in chronic infections (31, 32). Bacteria can cause tissue damage and severe infections by hiding from the immune system. In biofilms, bacteria adapt to hypoxia and nutritional deficiencies by altering their metabolism, protein production, and gene expression, which may reduce their metabolic rate and cell division rate (33).

Modifications such as inactivating the antimicrobial targets or reducing the requirements for cellular function can render the bacteria resistant to antimicrobial treatment (34). These are plausible explanations for the increased antibiotic resistance of most *A. baumannii* isolates in the current study, even if the increase is not statistically significant. Biofilms of microorganisms are embedded in a self-produced extracellular matrix that clings to a biotic or abiotic surface. The pathogenic biofilms can exist in high concentrations of antibiotics (35). Biofilms acquire antibiotic resistance through various processes, some of which can increase the number of MDR bacteria (36, 37). Biofilms are a reservoir of antibiotic resistance genes.

5. Conclusion

This study found that *A. baumannii* isolates more resistant to antibiotics were more strong biofilm formers, and the origin of resistance is due to the capacity of bacteria to produce broad-spectrum beta-lactamase enzymes EsβLs. We also noticed that the best antibiotics with the ability to eliminate clinical strains of *A. baumannii* are ciprofloxacin and levofloxacin. It is

recommended that physicians and health workers in Diyala use them for the treatment of the patients infected with MDR *A. baumannii*.

Acknowledgment

We appreciate our colleagues in the College of Medicine, University of Diyala for their lab facilities and the staff at Al Batool Hospital and Al-JUmhuri Teaching Hospital for their hard work, support, and help in collecting information.

Ethical approval

The Health Department in Diyala Governorate approved the research protocol, which is affiliated with the Ministry of Health in Iraq. Special consent forms were designed to obtain the patient's consent before taking the sample. The signed informed consent assured confidentiality.

Authors' Contributions

Conceptualization: Hanan Raheem Hassooni; Methodology: Raghad Ibrahim Ahmed; Statistical analysis: Hanan Raheem Hassooni; Preparation: Zainab M. Alzubaidy; Editing: Raghad Ibrahim Ahmed and Adil Hassan Alhusseiny

Funding

The author(s) received no financial support for the research or publication of this article.

Conflict of Interest

No conflicts of interest were declared by the authors.

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