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EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF THE AQUEOUS EXTRACT OF POMEGRANATE PEEL AGAINST THE BACTERIA ACINETOBACTER BAUMANNII ISOLATED FROM IMAM AL-SADIQ HOSPITAL IN BABYLON GOVERNORATE

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Abstract. Background: Acinetobacter baumannii is one of the most prominent causes of opportunistic infections associated with healthcare, and has gained wide resistance to antimicrobials, making it a global threat. This study aimed to assess the efficacy of Punica Granatum as an alternative natural antibiotic against the Acinetobacter baumannii, highly antibiotic-resistant and isolated patients at Imam Al-Sadiq Hospital.
Methods of action: 40 clinical samples (blood, urine, sputum, and swabs of intensive care) were collected from the hospital. The blaoxa-51 gene use as a species-specific marker. Antibiotic resistance testing was performed with 5 antibiotics (Imipenem, Merobenim, Ceftazidem, Ciprofloxacin, and Gentamicin) according to the CLSI 2026 Standards. Pomegranate and Evaluation Efficacy of Minimum Inhibiting (MIC) against super-resistance isolates.
Results: Of the 40 samples, 33 samples (82.5%) showed positive growth for A. baumannii. 9 samples (27.3% of the positive samples) showed a pattern of multiple resistance (MDR), which included all five tested antibiotics, especially carbapenems (Imipenem and Meropenem). The aqueous extract of the pomegranate peels showed clear effectiveness, with MIC ranged between 2.5-10 mg/ml, confirming its ability to inhibit these resistant strains.
Conclusion: The aqueous extract of pomegranate peels has showed effective as a natural antibiotic against A. baumannii, which is resistant to multiple drugs, which opens the way for it to be used as an adjuvant therapy or as a preservative in hospitals after further studies on its mechanisms of action and toxic effects.

Keywords: Acinetobacter baumannii, antibiotic resistance, pomegranate peels, blaOXA-51

1. Introduction

Over the past three decades, Acinetobacter baumannii has emerged as a serious global challenge for healthcare facilities due to its exceptional ability to acquire and regulate resistance determinants, making it one of the most dangerous threats in the current antibiotic era. A. baumannii is recognized as a leading drug-resistant pathogen worldwide, primarily affecting the most vulnerable hospital patients (WHO, 2017). This bacterium possesses multiple drug-resistance mechanisms such as drug-inactivating enzymes, efflux pumps, and target-site mutations and can survive for up to a month on dry surfaces by forming biofilms, particularly in settings like hospitals, restaurants, and water treatment plants; infections caused by the organism can lead to severe conditions such as sepsis, pneumonia, and urinary tract infections (McConnell et al., 2013).

In Iraq, local studies have demonstrated a widespread prevalence of this resistant bacterium, especially in ICUs and burn and haematology patients; high rates of resistance have been recorded against imipenem and meropenem (carbapenems)-drugs that often serve as the last line of treatment (Hussein et al., 2013). This resistance is mainly due to the ability of bacteria to produce beta-lactamase, the most common is the blaOXA-51 gene, which is an intrinsic gene that is characteristic of the species, as well as Genes such as blaOXA-23 responsible for the acquired resistance (Lellouche et al., 2024).

This resistance leads to higher healthcare costs to cover the costs of phenotyping tests, medicines and long treatment periods the situation is further aggravated by the reduced efficacy and toxicity of manufactured drugs (Ferri et al., 2017). Scientific be more attention towards plant-based treatments as potential alternatives to antimicrobials as natural products are rich in bioactive secondary metabolites that show promising antimicrobial activity (Gorlenko et al., 2020).

Pomegranate peels are rich in phenolic compounds like tannins, flavonoids and gallic acid, that have anti-bacterial and antioxidant properties. Studies indicate that these compounds inhibit bacteria through multiple mechanisms, including disruption of the cell wall, inhibiting bio-enzymes, and interfering with the formation of the biofilm (Cruz-Valenzuela et al., 2022; Makharita et al., 2020). The novel studies show that pomegranate peel extract show stronger antimicrobial activity compared to other parts of the plant. The antimicrobial effect has been linked to the content of tannins and flavonoids, knowing that the extraction method may greatly affect its effectiveness (Yassin et al., 2021). A recent Iraqi study conducted in Basra Governorate showed a clear effectiveness of pomegranate extract 100% against the bacteria A. baumannii resistance (Mohammed and Al-Hamdani, 2024). Therefore, this study aimed to isolate the bacteria A. baumannii from the patients of Al-Imam Al-Sadiq Hospital, determine its resistance patterns to the common antibiotics, and then evaluate the potential therapeutic efficacy of the aqueous pomegranate extract against these resistant strains.

2. Materials and Methods

2.1. Sample Collection

Forty clinical samples were collected from Imam Al-Sadiq Hospital patients, between April and August 2026. The samples included blood (10 samples), urine (10 samples), sputum (15 samples), and swabs from mechanical ventilators in the intensive care unit (5 sample).

2.2. Bacterial Isolation and Identification

Samples were cultured on MacConkey agar and blood agar and incubated at 37°C for 24 hours. Isolates were identified based on phenotypic and microscopic characteristics (Gram staining) and biochemical tests (oxidase and catalase tests). Out of 40 samples, 33 tested positive for A. baumannii. The isolates were maintained on nutrient agar at 4°C for subsequent use.

2.3. Antibiotic Susceptibility Testing

The disk diffusion method (Kirby-Bauer) was employed in accordance with CLSI 2025 guidelines. Five antibiotics representing first-line treatments were selected: imipenem (IPM-10 µg), meropenem (MEM-10 µg), ceftazidime (CAZ-30 µg), ciprofloxacin (CIP-5 µg), and gentamicin (CN-10 µg). The plates were incubated at 37°C for 18 hours, then the inhibition zones were measured.

2.4. Genetic detection (PCR) using the blaOXA-51 gene

DNA was extracted from all 33 isolates using a commercial kit (Geneaid, Taiwan). PCR used to confirm the isolates by amplifying the intrinsic blaOXA-51gene.

Table 1. PCR primers and their conditions used in this study

Primer	Sequence (5----------›3)	Product (bp)	Condition		Cycle No.	references
			Temp °C	Time sec		
blaOXA-51	F: 5'-TAA TGC TTT GAT CGG CCT TG-3'	353	94	30	35	(10)
			57	40		
	R: 5'-TGG ATT GCA CTT CAT CTT GG-3'		72	40		

Table 2. Preparation of the Polymerase Chain Reaction (PCR)

material	quantity
Master mix	12.5 µg/ml
Primer Forward	1 µg/ml
Primer Reverse	1 µg/ml
Sample DNA	3 µg/ml
d. H2O	7.5 µg/ml
Final volume	25 µg/ml

2.5. Preparation of the Aqueous Extract of Pomegranate Peels

Fresh pomegranate peels were collected, at room temperature do shade-dried, then make a fine powder by ground it. 100 g of the powder was soaked in 500 mL of sterile distilled water for 24 hours with continuous shaking. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was subsequently freeze-dried (lyophilized) to obtain the crude extract. The extract was stored at -20°C until use.

2.6. Determination of Minimum Inhibitory Concentration (MIC)

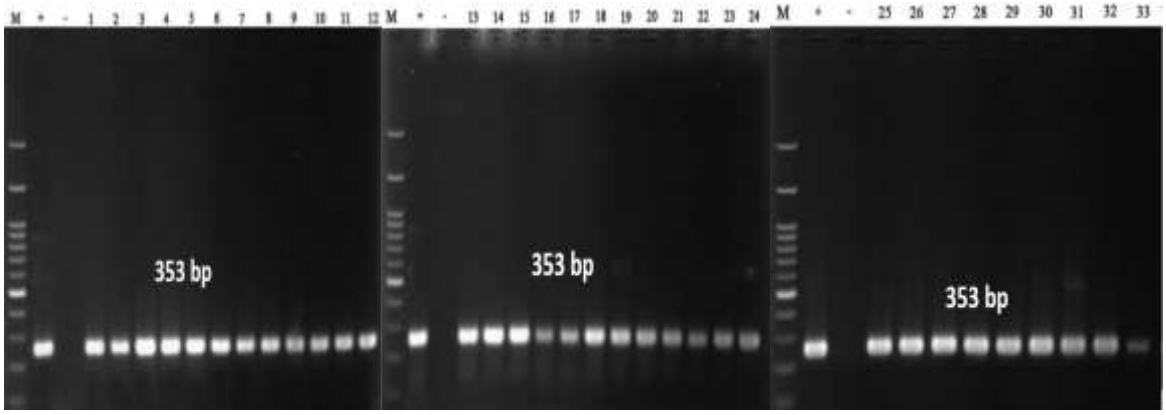
The broth microdilution method was employed in accordance with CLSI guidelines. A series of twofold dilutions of the aqueous extract was prepared (with concentrations

ranging from 0.625 to 20 mg/mL). Then, 100 µL of a bacterial suspension (adjusted to a 0.5 McFarland standard) was added to the wells containing the extract. The plates were incubated at 37°C for 24 hours. MIC was defined as the lowest concentration of the extract that prevents the visible growth of bacteria.

3. Results

The percentage of positive isolates was 82.5% (33/40), as the 33-isolate showed pale pink growth on Macconkey Agar and gray growth with no haemolysis (γ-hemolysis) on Blood Agar. All were Gram-negative short bacilli, catalase-positive, and oxidase-negative characteristics typical of Acinetobacter baumannii. The molecular confirmed via the presence of the blaOXA-51 gene. The highest isolation rate was from sputum samples (45%), followed by samples from the intensive care unit (27%).

Figure 1. PCR product for blaOXA-51 (353 bp) in 33 A. baumannii isolates.



Antibiotic susceptibility testing results for the five antibiotics (imipenem, meropenem, ceftazidime, ciprofloxacin, and gentamicin) revealed that the highest resistance rate was observed for imipenem at 93.9% (31/33 samples), followed by meropenem at 90.9% (30/33 samples), ceftazidime at 87.9% (29/33 samples), and ciprofloxacin at 84.8% (28/33 samples). Conversely, the lowest resistance rate was observed for gentamicin at 72.7% (24/33 samples), as shown in Table (2). This distribution confirms the widespread prevalence of carbapenemase-producing bacteria (such as OXA-23 and OXA-51) at Imam Al-Sadiq Hospital and suggests that gentamicin may be the "least bad" option compared to carbapenems, although its resistance rates remain concerning.

Table 3. Percentages and numbers of resistance to five antibiotics among 33 A. baumannii isolates.

Antibiotic	**class**	**Number of resistan samples(out of 33)**	**(%)**
(IPM)	Carbapenem	31	93.9%
(MEM)	Carbapenem	30	90.9%
(CAZ)	Cephalosporin	29	87.9%
(CIP)	Fluoroquinolone	28	84.8%

(GN)	Aminoglycoside	24	72.7%

Nine bacterial isolates, all resistant to the antibiotics under study, were selected. Minimum Inhibitory Concentration (MIC) testing was performed on the aqueous extract of pomegranate peels against these nine highly resistant isolates; the extract demonstrated high inhibitory efficacy (Table 3).

Table 4. MIC values of the aqueous extract of pomegranate peels against resistant isolates.

Isolate number	MIC value (mg/mL)
Ab-3	5
Ab-7	5
Ab-11	2.5
Ab-15	10
Ab-19	5
Ab-22	10
Ab-25	2.5
Ab-28	5
Ab-31	10

The minimum inhibitory concentration (MIC) ranged (2.5 - 10 mg/mL). Isolates Ab 11 through Ab 25 (obtained from the intensive care unit) were the most sensitive to the extract. These results confirm that the aqueous extract of pomegranate peel has the ability to destroy bacteria resistant to carbapenems and other antibiotics.

4. Discussion

Acinetobacter baumannii represents a major global healthcare threat due to the limited availability of effective antibiotics for treating infections caused by these strains, driven by their high rates of antibiotic resistance. This bacterium is responsible for numerous hospital-acquired infections, including bloodstream infections, urinary tract infections, wound infections, ventilator-associated pneumonia, other respiratory tract infections, meningitis, and bacteraemia (Jawad, 2026).

In present study, the prevalence of Acinetobacter baumannii isolates were 82.5% (33/40). These isolates exhibited high levels of antibiotic resistance, showing resistance to imipenem and meropenem (93.9% and 90.9%, respectively), as well as high resistance to the other antibiotics studied. These results reflect the concerning healthcare situation in Iraq, where multidrug-resistant A. baumannii strains are widespread. The high percentage of imipenem and meropenem resistance recorded isolates are consistent with what local studies have indicated in Najaf and Karbala, which found that the resistance to imipenem exceeded 90% in some clinical samples, may be attributed to the appearance of antibiotic resistance

due to multiple to overuse, biofilm formation, decomposed enzyme production, metabolic state change, decreased bacterial permeability, antibiotic targets, increased efflux-pumps, and gene transfer mechanisms. (Alwohaili and Awade, 2025). This confirms that carbapenems are no longer effective as a first treatment option in these hospitals, which is a health crisis.

When comparing the effect of synthetic antibiotics with natural extracts, the essential difference lies in the mechanism of action. While antibiotics target the bacterial wall or folic acid pathway (that makes it easier for bacteria to develop resistance via point mutations or enzymes), pomegranate peels contain a "constellation" of phenolic compounds (such as tannins and flavonoids) Synergism to destroy the cell in several ways (Debib et al., 2022). The punica granatum fruit has many medicinal benefits and healing properties, whether the fruit is complete, its seeds and even its peel. Fruit bodies and seeds are tonic for the heart and stomach. As for the juice of this plant, it is very rich in vitamins and minerals that are necessary for the body. As for the pomegranate peels, it turns out to be one of the most powerful medicines that kill worms and treat amoebic dysentery. It is noted that the outer shell of the pomegranate contains tannic acid, which is astringent, and its powder is an excellent antidote to the treatment of diarrhea. A decoction of the peels acts as an anthelmintic (worm-expelling agent), particularly against tapeworms, due to the presence of various alkaloids and flavonoids, including pelletierine. The material contained various alkaloids, including N-Ethyl, Methylisopellelierine, Ethylpelleticrin, and Isopellefierine pseudopelleticrin, along with other compounds (Mohammed and Al-Hamdani, 2024).

Our MIC values (2.5-10 mg/mL) were within the effective range indicated by previous studies, where Debib et al. (2022) found that the extract of pomegranate peels was effective against resistance bacteria at 512 µg/mL in other species (Cruz-Valenzuela et al., 2022). The superiority of the aqueous extract in this study, specifically, may refer that water was more efficient in extracting polar compounds such as water-soluble tannins, compared to organic solvents that extract fat more. This hypothesis is supported by a previous study that showed that the aqueous extract contains a higher percentage of total phenolic compounds compared to ethanol (Akhtar et al., 2015).

5. Conclusion

This study confirms the clinical risk of extensive antibiotic-resistant Acinetobacter baumannii

(XDR) strains at Imam Al-Sadiq Hospital, particularly in intensive care units and respiratory specimens. The results showed a remarkable treatment failure of carbabinem (Imipenem and meropenem), indicating a decline in the efficiency of the last lines of defence. In contrast, the water extract of pomegranate peels (Punica granatum) proved a promising efficiency in inhibiting the growth of these resistant strains in Vitro. This effect is due to its richness in phenolic and flavonoid compounds with multiple inhibitory mechanisms. These results support the potential development use of the extract as an adjuvant or as a natural antiseptic for medical surfaces and devices, However, further studies assessing chemical stability, toxicity, and in vivo efficacy are required before clinical implementation can be recommended.

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