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Original Article

Mapping Resistance and *ompA*/*epsA* Genes in Hospital *Acinetobacter baumannii*

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ABSTRACT

Acinetobacter baumannii is a dangerous hospital-acquired pathogen. It shows strong antibiotic resistance and produces biofilms that create challenging treatment conditions. The pathogen exists on hospital surfaces while it rapidly moves between patients to cause nosocomial infections. Methodology: This research was conducted between October 2024 and July 2025 in different hospitals located in the Kurdistan Region of Iraq. 126 environmental samples were taken from different high-touch surfaces located throughout various hospital wards. The VITEK 2 system was used to identify bacteria, and subsequent *16S rRNA* gene sequencing was used to confirm the results. The biofilm-related genes *ompA* and *epsA* were detected using polymerase chain reaction. Antimicrobial susceptibility was tested by the minimum inhibitory concentration test (MIC). Biofilm formation was evaluated using the microtiter plate assay. Results: Overall, 16 *Acinetobacter baumannii* isolates were identified. The *ompA* gene was found in every isolate, whereas *epsA* was present in 31.25%. All isolates were biofilm producers, though no strong biofilm formation was observed; 25% were moderate and 75% weak biofilm producers. Furthermore, 93.75% of the isolates were extensively drug-resistant. Conclusion: All isolates showed biofilm-forming ability, which enhances environmental persistence. These insights show the need for strict infection control and continuous surveillance in health facilities.

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Keywords: A. baumannii, Hospital environment, ompA, epsA gene, XDR.

1 Introduction

Acinetobacter baumannii is a gram-negative multi-drug resistant bacterium. A common cause of hospital infections, especially in ICUs, and in people with central vein catheters or weak immunity [1]. Infections caused by *A. baumannii* are blood, urinary tract, ventilator-related, and other respiratory pneumonias, surgical site, GI, meningitis, eye infection, and device-related bacteremia [2]. The capabilities of *A. baumannii* to grow under altered pH and salinity and starvation have notable advantages in therapeutics. This bacterium can live in environmental contamination on surfaces, and also can undergo genetic modification [3]. As per the WHO Report 2017 and 2024, this is a critically important pathogen that will require new antibiotics because of its extremely high level of antibiotic resistance [4]. According to the WHO, *A. baumannii* is a critical nosocomial pathogen, and it is one of the most dangerous ESKAPE pathogens [5]. Other ESKAPE pathogens group includes *Staphylococcus aureus, Enterococcus faecalis, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterobacter species* [6]. *A. baumannii* is responsible for ventilator-associated pneumonia at varying rates around the world. The percentage in the US and Europe is 8-14% while much higher 19-50%+ is seen in Asia, Latin America, and Middle Eastern countries [7]. Genomic analysis has identified several virulence factors, including outer membrane porins, phospholipases, pili, protein secretion systems, proteases, capsular polysaccharides, lipopolysaccharides, and iron-chelating systems. Some strains have genes that enable them to stick to cells, get inside, and live. They can also form biofilms on non-living surfaces [8]. Cell invasion and apoptosis are mediated by *A. baumannii*'s primary outer membrane protein *ompA* [7]. One of the roles performed by *ompA* in *A. baumannii* is resistance to complement killing and biofilm formation [9]. *A. baumannii* is capable of invading and surviving within host cells. It first attaches to host cells and then invades the cells and translocates to the nucleus. When host cells die, the bacteria enter the blood and other tissues. *OmpA* helps bacteria to stick to and invade epithelial cells [10]. The *epsA* gene encoded exopolysaccharide (EPS) the outer membrane protein required for polysaccharide export. EPS builds up on the outside of cells and protects them from the harsh outside environment [11]. *A. baumannii*'s capsular polysaccharide (CPS) shell influences its survival and spread of

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infections [12]. Extracellular polymeric substances enhance the adhesion of microorganisms on surface material. It is also involved in intercellular connections and horizontal gene transfer. It also protects strains against environmental stressors like nutrient and water availability [13]. It is necessary that we learn more about the survival and persistence of this microorganism in healthcare settings, as many infections happen there [14]. *A. baumannii* is an indicator of environmental contamination, as well as a major reservoir of HAI [15].

Based on the available studies, a few studies have examined *A. baumannii* in hospital environments, especially in the Kurdistan region of Iraq. Although it can form biofilms, little is known about the genes responsible. There is also limited information on the presence of *epsA* and *ompA* genes in hospital environments and the places where the bacterium survives and spreads. More research is needed to understand how *A. baumannii* persists and causes repeated outbreaks.

This study aims to examine *A. baumannii* collected from different areas and surfaces in hospitals in the Kurdistan Region of Iraq, with a focus on the biofilm-related genes *ompA* and *epsA*. It also looks at which hospital surfaces and devices are more likely to carry and spread this bacterium.

2 Methodology

2.1. Ethical approval

The study obtained approval from the Ethical Committee of the Faculty of Science and Health, Koya University (Approval No. DMMB-2-24).

2.2. Biosafety measurement

Biosafety level 2 precautions were strictly followed throughout all experimental procedures.

2.3. Sample collection and culture conditions

Environmental samples were collected using sterile gel-based swabs pre-moistened with their own gel medium. Sampling was conducted across different hospitals in the Kurdistan Region of Iraq from October 2024 to February 2025. Samples were taken from 8 units, including the Emergency Unit, Surgical Unit, Pediatric Unit, Burn Unit, Internal Medicine Unit, Operation Unit, Intensive Care Unit (ICU), and Respiratory Care Unit (RCU). The sampling targeted high-touch surfaces and commonly used medical devices, which were targeted due to their frequent contact with healthcare workers and patients, increasing the risk of microbial transmission and surface contamination, such as sinks, hospital beds, the surfaces of the devices, surfaces of the air conditioners, pulse oximeter monitors, infusion pumps, patient file boards, benches, IV fluid stands, floors, doors and door handles, as well as suturing instruments. In addition, environmental sources such as water were included. Sterile swabs that were not used on any surface were included as negative controls to make sure no contamination occurred during sampling or culturing. A total of 126 samples were collected based on the availability of high-touch surfaces in the hospital wards. The first 42 samples were directly cultured on MacConkey agar for primary isolation. Due to poor growth of bacteria, the other 84 samples were enriched first in BHI broth and incubated for 18–24 hours at 37°C. After incubation, they were cultured on MacConkey agar. Colonies were subcultured onto blood agar, tryptic soy agar, and HiCrome *Acinetobacte*r agar for selective isolation of *Acinetobacter* spp. The media were incubated at 37°C for 24 hours.

2.4. Identification of the bacteria

Bacterial isolates were tested biochemically and through a series of other tests. The biochemical tests to check oxidase, Simmon citrate, and motility were done for initial characterization. Then, Gram staining was performed to determine the cell morphology and Gram reaction. Further confirmation of isolates was done using the VITEK 2 Automated Identification System (bioMérieux/USA).

2.5. Molecular identification

2.5.1 Genomic DNA extraction

A Falcon tube that was free of contamination had five mL of BHI broth and a loopful of specific bacteria was added to it. The bacteria were left to grow overnight. Genomic DNA was extracted with the help of a commercial kit (Genesand Company, China). A NanoDrop spectrophotometer (Cytiva, USA) was used to estimate purity and concentration. A260/A280 ratio of 1.8 to 2.0 was within a pure range.

2.5.2. *16S rRNA* gene detection

Polymerase Chain Reaction (PCR) was used to amplify the *16S rRNA* gene performed using primer sequences (Macrogen, Korea), with details provided in (Table 1). The amplification protocol followed the thermal cycling parameters outlined in (Table 2). Following successful amplification, the resulting PCR products were submitted to (Macrogen, Korea) for Sanger sequencing. The results were analyzed using BLAST.

2.5.3. Identification of *epsA* and *ompA* genes

The *ompA* and *epsA* genes were detected using the primers (Macrogen, Korea) listed in (Table 1). PCR amplification was performed with a commercially available master mix (Genesand, China), following the conditions outlined in (Table 2), using a Bio-Rad PCR system (USA). Each reaction was carried out in a final volume of 25 µL, consisting of 12.5 µL of master mix, 0.7 µL each of forward and reverse primers (10 pmol), three µL of DNA template, and 8.1 µL of nuclease-free water. A negative control was included in all PCR reactions, containing the master mix, forward and reverse primers, and nuclease-free water,

except DNA, to check for any contamination. A 1.5% agarose gel (Genesand, China) was prepared in 1× TBE buffer at 80 V for 60 minutes. Three µL of ethidium bromide was used to stain the gel. After that, an imaging system (Syngene, UK) was utilized to visualize DNA bands in UV light. The amplification products formed via PCR were finally Sanger sequenced (Macrogen, Korea). Using BLAST, the sequences were analyzed for confirmation.

Table 1: Primers that were used in this study to detect the *16S rRNA*, *epsA*, and *ompA* genes.

Primers	Primer Sequence	Product size(bp)	References
16S rRNA	F: CACCTTCCGATACGGCTACC R: GTTGACTGCCGGTGACAAAC	372 bp	[46]
ompA	F: 5′- CAACGGTAACTTCTATGTTA -3′ R: 5′- CGTGCAGTAGCGTTAGGGTA -3′	496 bp	Designed in this study using SnapGene tool.
epsA	F: 5′- AAGCAAGTGGTTATCCAATC -3′ R: 5′- ACCAGACTCACCCATTACAT -3′	453 bp	Designed in this study using SnapGene tool.

Table 2. PCR conditions in the gene amplification of *16S rRNA*, *ompA*, and *epsA*.

PCR Phase	Condition	*16S rRNA*	*ompA*	*epsA*
Predenaturation	Temperature/Time	95 °C/5 min	95 °C/3 min	95 °C/3 min
Denaturation	Temperature/Time	95 °C/ 35 sec	94 °C/ 25 sec	94 °C/ 25 sec
Annealing	Temperature/Time	59 °C/40 sec	51 °C/25 sec	54.2 °C/ 25 sec
Extension	Temperature/Time	72 °C/45 sec	72 °C/15 sec	72 °C/15 sec
Final Extension	Temperature/Time	72 °C/7 min	72 °C/5 min	72 °C/5 min
No. of Cycles	Number	40	28	30

2.6. Antimicrobial susceptibility

The determination of the minimum inhibitory concentration (MIC) of each isolate was carried out using the VITEK 2 system (bioMérieux, USA) for assessing antibiotic resistance. Various antibiotics were tested, including Ampicillin/Sulbactam, Piperacillin/Tazobactam, Cefotaxime, Ceftazidime, Meropenem, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Colistin, and Trimethoprim/Sulfamethoxazole. The CLSI 2023 guidelines were used to interpret the result. Isolates were classified as multidrug-resistant (MDR) if they were resistant to at least one agent in three different antimicrobial classes; extensively drug-resistant (XDR) if resistant to all but two or fewer classes; and pandrug-resistant (PDR) if resistant to all agents across all classes [16].

2.7. Detection of biofilm formation

The standard 96-well plate assay was used to assess biofilm formation under static conditions. Surface adhesion of each strain was tested in microtiter plates. Fresh bacterial colonies were inoculated into test tubes containing Tryptic Soy Broth (Scharlau, Spain) and incubated at 37 °C for 24 hours. Bacterial cultures were then adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/mL). From each standardized suspension, 100 µL was transferred into individual wells of a 96-well plate. The sterile medium was included as the negative control. The plates were incubated at 37 °C for 24 hours, then inverted to remove any unattached bacteria and residual medium. Each well was washed twice with distilled water. The plates were allowed to dry at room temperature for 15 minutes before the next step. To preserve the biofilm structure, each well was treated with 125 µL of methanol and left for 15 minutes. After removing the methanol, the plates were left to air-dry at room temperature for 10–15 minutes. After that, a crystal violet solution (1% (v/v)) was added (125 µL). After staining, excess dye was discarded. Distilled water was used to wash the wells 3-4 times to remove unbound stain. The plates were thoroughly dried by placing them on paper towels to remove any residual debris and excess stain and then left to dry overnight. Once dry, each well received 125 µL of 30% (v/v) acetic acid to dissolve the bound crystal violet. This was done to quantify the biofilm mass. Following 10-15 minutes of incubation at room temperature. The optical density measurements were taken using a microplate reader (BioTek, USA). Optical density was measured at 630 nm (OD630 nm). The OD of the negative control wells was arithmetically averaged, and a standard deviation of +3 was added to get the optical density cut-off value (ODc). Samples were classified as negative if their optical density was less than the cut-off value and as positive if their OD was more than the ODc. The following criteria were used to classify strains: non-biofilm producer if $OD \leq ODc$; weak biofilm producer if $ODc < OD \leq 2 \times ODc$; moderate biofilm producer if $2 \times ODc < OD \leq 4 \times ODc$; strong biofilm producer if $OD > 4 \times ODc$ [17].

2.8. Statistical analysis

The data analysis was carried out using IBM SPSS version 27.0.1. The values are presented as mean ± standard deviation; the continuous values and percentiles are calculated using descriptive statistics. The p-value of < 0.05 was considered statistically significant.

3 Results

3.1. Detection and identification of the bacteria

A total of 126 samples were collected. The first 42 samples were cultured directly on MacConkey agar, only nine samples showed growth. The remaining 84 samples were first enriched in BHI broth before being cultured on MacConkey agar, 74 samples showed growth (Figure 1). The detection of the isolates was first based on colony morphology (Figure 2), Gram staining (Gram-negative coccobacilli), and biochemical tests (Simmons citrate positive, oxidase negative, and non-motile). After identification by the VITEK 2 system, all results showed excellent identification. In total, 16 isolates of *A. baumannii* were detected, accounting for 19.3% (16/83) of the cultured samples that showed growth. All *A. baumannii* isolates were found in the ICU and RCU. Out of the 16 isolates, seven (43.75%) were from hospital beds, which showed the highest contamination and seemed to be the most common contact points. Three isolates (18.75%) were obtained from pulse oximeter monitors, another three (18.75%) from surface devices, two (12.5%) from surface of infusion tubes, and one (6.25%) from an IV fluid stand. The location significantly affected the number of isolates (p = 0.012). Based on the results of post hoc Tukey tests conducted in this study, the isolates on hospital beds segregated significantly from those on IV fluid stands (p = 0.01) as well as surface of infusion tubes (p = 0.03).

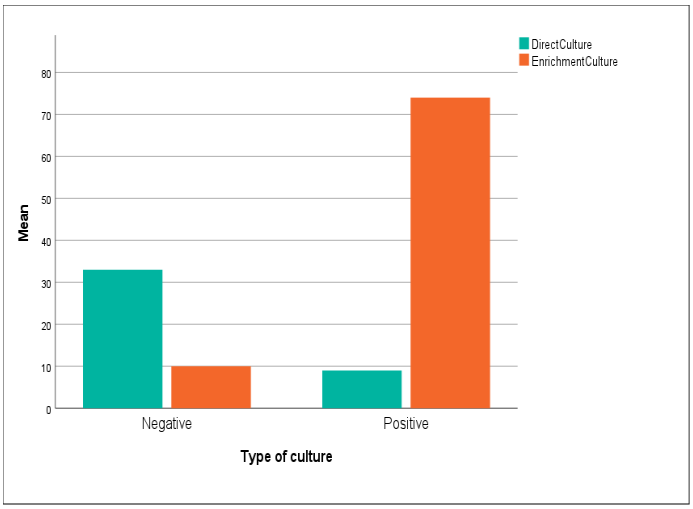

Figure 1: Comparison of the growth rate of the environmental bacterial isolates using direct culture on MacConkey agar and enrichment in BHI broth before culturing on MacConkey agar. Positive = samples that showed growth, Negative = samples that did not show growth.

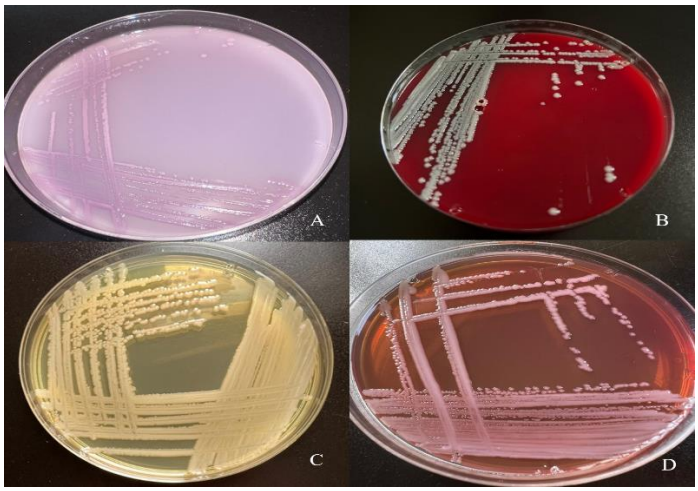

Figure 2: Colony morphology of *A. baumannii* on different culture media. (A) *A. baumannii* colonies on HiCrome™ *Acinetobacter* agar are pink to purple colonies. (B) On blood agar and (C) tryptic soy agar, colonies are smooth and greyish-white. (D) On MacConkey agar, colonies are light pink, indicating partial lactose fermentation.

3.2. Molecular identification

3.2.1. Detection of *16S rRNA* gene by PCR

The identification was confirmed by amplifying the *16S rRNA* gene from all 16 *A. baumannii* isolates, each producing a 372 bp fragment (Figure 3). The PCR products were sequenced to confirm their identity. BLAST results showed that all sequences aligned with the *A. baumannii* reference strain. The obtained accession numbers were PX461830, PX461831, PX461832, PX461833, PX461834, PX461835, PX461836, PX461837, PX461838, PX461839, PX461840, PX461841, PX461842, PX113341, PX113342, and PX113343. Finally, a phylogenetic tree was built using these sequences (Figure 4).

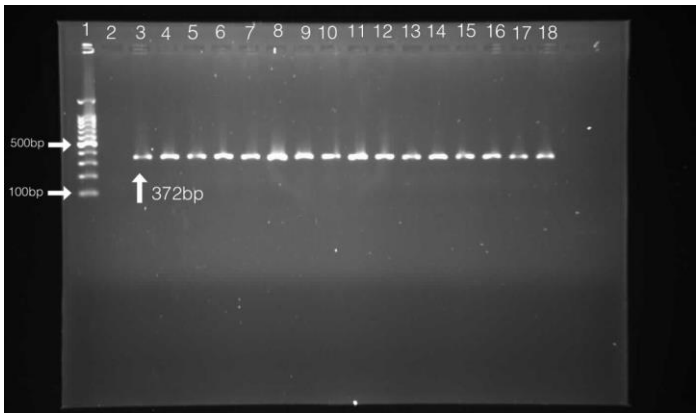

Figure 3: Gel electrophoresis of PCR products for the *16S rRNA* gene. The first lane = 100 base pair DNA ladder. Lane 2 = negative control (no template DNA added). Lanes 3 through 18 = DNA bands from successful amplification of the *16S rRNA* gene.

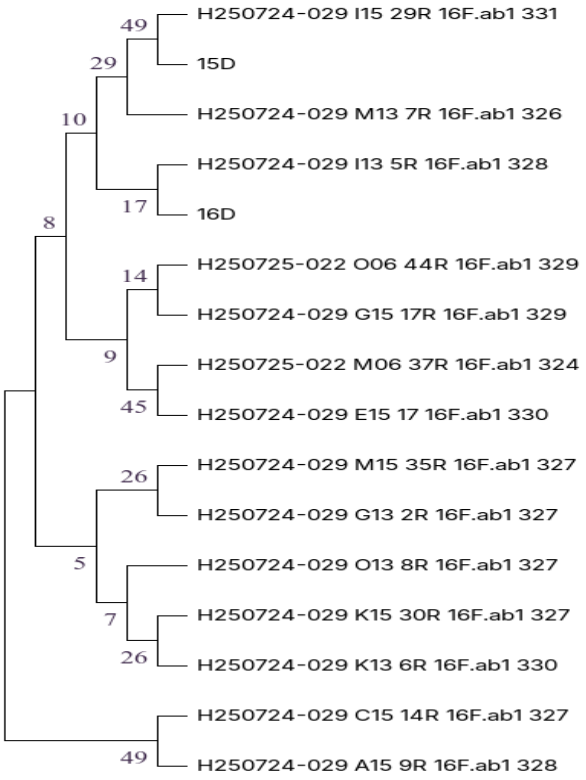

Figure 4: Unrooted phylogenetic tree showing relationships among the 16 *A. baumannii* isolates based on *16S rRNA* gene sequences.

3.2.2 Detection of biofilm-related genes

A 496 bp fragment of the *ompA* gene was amplified from all isolates, confirming that they carried the gene (Figure 5). The PCR products were sequenced, and their identity was confirmed via BLAST. For the *epsA* gene, a 453 bp fragment was amplified and detected in five isolates. The PCR products were sequenced, and their identity was confirmed via BLAST.

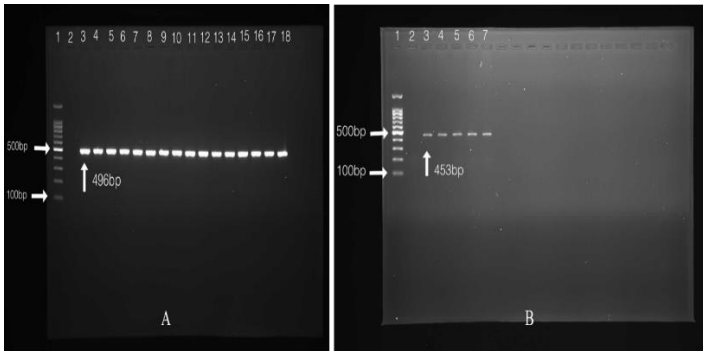

Figure 5: A. Gel electrophoresis of PCR products for the *ompA* gene. Lane 1: 100-bp DNA ladder; Lane 2: negative control; Lanes 3–18: amplicons from the 16 isolates positive for the gene. B. Gel electrophoresis of PCR products for the *epsA* gene. Lane 1: 100-bp DNA ladder; Lane 2: negative control; Lanes 3–7: isolates showing the presence of the gene.

3.3. Antimicrobial susceptibility

3.3.1. Minimum inhibitory concentration

The MIC was determined using the VITEK 2 automated system, and all isolates showed intermediate susceptibility to colistin. Among the 16 isolates, 15 (93.75%) were classified as extensively drug resistant (XDR), while only 1 (6.25%) was highly susceptible (Table 3).

Table 3: Minimum inhibitory concentration **(**MIC) results of *A. baumannii* isolates were determined using the VITEK 2 Compact system.

Antimicrobial Agent	No(%). of isolates		
	Susceptible	Intermediate	Resistant
Amikacin	1 (6.25%)	0	15 (93.75%)
Gentamicin	1 (6.25%)	0	15 (93.75%)
Piperacillin/Tazobactam	1 (6.25%)	0	15 (93.75%)
Imipenem	1 (6.25%)	0	15 (93.75%)
Meropenem	1 (6.25%)	0	15 (93.75%)
Ciprofloxacin	1 (6.25%)	0	15 (93.75%)
Ceftazidime	1 (6.25%)	0	15 (93.75%)
Ampicillin/Sulbactam	1 (6.25%)	0	15 (93.75%)
Cefepime	1 (6.25%)	0	15 (93.75%)
Cefotaxime	0	0	16 (100%)
Colistin	0	16 (100%)	0
Trimethoprim/Sulfamethoxazole	6 (37.5%)	0	10 (62.5%)

3.3.2. Assessment of correlations among antibiotic resistance profiles

The correlation analysis showed that certain antibiotics were strongly related in their resistance patterns. A strong and significant correlation ($r = 1$, $P < 0.05$) was seen between imipenem and meropenem, meaning the isolates resistant to one were also resistant to the other. The same strong correlation ($r = 1$, $P < 0.05$) was found between gentamicin and piperacillin, ciprofloxacin and cefepime, and also between ampicillin and amikacin, which suggests a possible cross-resistance among them. On the other hand, a weak and non-significant correlation ($r = 0.293$) was found between cefotaxime and trimethoprim, showing that there was little or no relation between their resistance patterns (Table 4). No clear correlation was observed between ceftazidime and colistin. These findings indicate that the *A. baumannii* isolates in this study may have related resistance mechanisms, particularly to carbapenems, aminoglycosides, and β-lactam antibiotics (Figure 6).

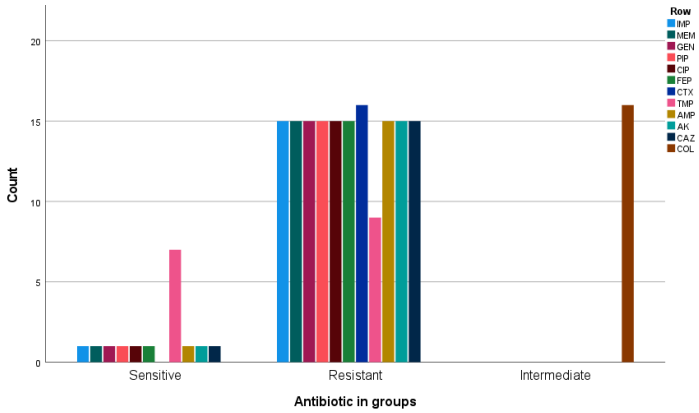

Figure 6. Correlations between different antibiotic resistances. GEN (Gentamicin), PIP/TAZ (Piperacillin/Tazobactam), IPM (Imipenem), MEM (Meropenem), CIP (Ciprofloxacin), FEP (Cefepime), CTX (Cefotaxime), COL (Colistin), TMP/SMX (Trimethoprim/Sulfamethoxazole), AMP/SUL (Ampicillin/Sulbactam), AK (Amikacin), and CAZ (Ceftazidime).

Table 4. Pearson's correlation coefficients (r) for antibiotic pairs in the 16 *A. baumannii* isolates. r values of 1* indicate a strong significant positive correlation.

Antibiotic pair	Correlation coefficient (r)	Correlation type
IMP and MEM	1*	Strong significant positive correlation
GEN and PIP	1*	Strong significant positive correlation
CIP and FEP	1*	Strong significant positive correlation
CTX and TMP	0.293	Non-significant weak positive correlation
AMP and AK	1*	Strong significant positiv

*= significant at level of 0.05.

3.4. Biofilm formation detection

Among the 16 bacterial samples tested, all produced biofilms. Twelve isolates showed weak biofilm formation, while four isolates produced moderate biofilms. None of the isolates produced strong biofilms (Table 5). The classification based on optical density (OD) values was as follows: $OD \leq 0.07725$: Non-biofilm formation, $0.07725 < OD \leq 0.1545$: Weak biofilm formation, $0.1545 < OD \leq 0.309$: Moderate biofilm formation, $OD > 0.309$: Strong biofilm formation.

Table 5. Testing of biofilm production among the 16 *A. baumannii* isolates using microtiter plate assay.

Strains	OD 630 nm (mean ± SD)	Biofilm formation ability	P - value	t-test	Standard error	Significance Level
1.	0.152±0.031	Weak	0.0071	5.069	0.018	Significant
2.	0.1±0.0143	Weak	0.0107	4.5201	0.009	Significant
3.	0.126±0.023	Weak	0.0084	4.84	0.014	Significant
4.	0.236±0.0119	Moderate	0.0001	23	0.008	Significant
5.	0.086±0.0096	Weak	0.015	4.056	0.006	Significant
6.	0.082±0.0066	Weak	0.011	4.38	0.005	Significant
7.	0.126±0.0191	Weak	0.004	5.75	0.012	Significant
8.	0.122±0.0203	Weak	0.0069	5.11	0.012	Significant

9.	0.127±0.125	Weak	0.4	0.93	0.072	Non-Significant
10.	0.141±0.0339	Weak	0.0149	4.09	0.02	Significant
11.	0.113±0.0133	Weak	0.003	6.3	0.008	Significant
12.	0.168±0.0143	Moderate	0.0003	12.1	0.009	Significant
13.	0.173±0.0426	Moderate	0.01	4.56	0.025	Significant
14.	0.147±0.00693	Weak	0.0001	16.66	0.005	Significant
15.	0.148±0.00878	Weak	0.0001	14.49	0.006	Significant
16.	0.21±0.0177	Moderate	0.0002	13.9	0.011	Significant
17. Negative control	0.05967 ±0.00586					

3.5. Relationship between biofilm strength and the presence of *ompA* with *epsA* genes

Among the 16 isolates of *A. baumannii*, the *ompA* gene was found in all the isolates, of which 12 isolates were categorized as weak biofilm producers while 4 isolates were moderate biofilm producers. Only five isolates possessed the *epsA* gene and showed weak biofilm production expression. The result of Fisher's Exact Test returned a p-value of 0.2381, which suggests no statistically significant relationship between the strength of biofilm producer and the presence of the *ompA* or *epsA* genes.

3.6. Correlation between OD values and the presence of *ompA* with *epsA* genes

No statistically significant correlation was found between OD values and the presence of the *ompA* and *epsA* genes, since *ompA* was present in all isolates p = 0.0633. However, this relationship was not statistically significant.

3.7. Association between biofilm strength with antibiotic resistance pattern and correlation with detected genes

Fifteen of the 16 isolates were found to be XDR while one was fully sensitive. Within XDR group, 11 isolates were weak biofilm formers, and 4 were moderate biofilm formers; no strong biofilm formers were detected. Biofilm formation strength did not correlate with the resistance phenotype. While there were more XDR isolates with weak biofilm formation ability, this difference was not significant ($p > 0.05$). Likewise, the analysis of Fisher's Exact Test identified no significant relationship between the presence of genes *ompA* and *epsA* with the XDR phenotype ($p > 0.05$).

4 Discussion

In our study, *A. baumannii* isolates exhibited significant antimicrobial resistance across various antibiotic classes. Significantly, 93.75% isolates showed resistance to both amikacin and gentamicin. A similarity is found in Iran, where resistance rates of *A. baumannii* isolates are 96.7% for amikacin and 95% for gentamicin, respectively [18]. It's also higher than a previous finding in Saudi Arabia, where one study reported resistance rates of 52.5% for gentamicin and 27.5% for amikacin [19] and one conducted in Egypt found 60.5% and 53% resistance to gentamicin and amikacin [20]. In this study, 93.75% of isolates show resistance to piperacillin/tazobactam and ampicillin/sulbactam. The strains had similar patterns of resistance as observed in Baghdad where 95.2% of isolates were resistant to ampicillin/sulbactam [21]. And in Erbil almost complete resistance to piperacillin/tazobactam was seen [22]. In contrast, lower resistances rates were reported in Tehran, Iran, for which isolates were reported as resistant to ampicillin/sulbactam 59% and piperacillin/tazobactam 40% [23]. Our isolates for fluoroquinolones were 93.75% resistant in the case of ciprofloxacin, which was comparable to 95.24% in Tehran [24]. Nonetheless, this is greater than 59.25% resistance to ciprofloxacin in Al Sulaymaniyah City, Iraq [25]. Our isolates showed 93.75 % resistance to ceftazidime, cefepime and 100 % to cefotaxime concerning cephalosporins. The same result was reported in Tehran, with resistance to 100% ceftazidime, cefepime, and cefotaxime [24, 26]. According to our findings, a higher percentage of antibiotic resistance has been reported as compared in Hilla / Iraq where the known percentage resistant to cefepime is 60.8%, 70.2% to cefotaxime and 70.2% to ceftazidime [27]. In terms of carbapenems, our isolates showed resistance to both imipenem and meropenem at a rate of 93.75%. This result was comparable to the 91.3% imipenem and 93.4% meropenem rates from Sulaymaniyah/Iraq [28] but much higher than the 73.4% imipenem and 75% meropenem rates from Kurdistan/Iraq [29]. In our study, a resistance of 62.5% of the isolates was encountered for trimethoprim/sulfamethoxazole, which is comparable to the 59.6% reported in China [30] but lower than the 89.1% reported in Kurdistan/Iraq [29] and higher than the 50% and 46.5% reported in Duhok / Iraq [31] and Libya [32] respectively. Interestingly, in this study, all isolates were found to show colistin susceptibility in intermediate (100%). This is different from the findings in Iraq where all of the isolates were fully sensitive to colistin [33]. The increased resistant to these antibiotics among the different studies could be due to fake, low-quality drugs, misuse, and overuse. Many developing countries still lack AMR action plans. The problem is made worse by untrained drug sellers, self-medication and poor treatment compliance. Use of antibiotics in agriculture and industry creates a selective pressure that favors survival and spread of resistance [34]. Unlike free-floating (planktonic) bacteria, biofilms are

communities of microorganisms that stick to living or non-living surfaces and are embedded within a protective extracellular polymeric substance (EPS) matrix [35]. *A. baumannii* can attach to and multiply as biofilms on body tissues like mucosal surfaces and medical devices like catheters. Its contribution to hospital-acquired infections is greatly increased by this capability [36]. In our study, none of the *16 A. baumannii* isolates formed strong biofilms. Most of them (75%) produced only weak biofilms, while the remaining 25% produced biofilms of moderate strength. In Erbil / Iraq, a similar pattern was reported, with no strong biofilm producers detected. Among the 17 clinical isolates, 18.75% did not form biofilms, while 37.5% exhibited weak and 43.75% moderate biofilm formation [22]. In Baghdad, Iraq, the situation was also comparable: no strong biofilm producers were found, while 15% of isolates did not form biofilms, 54% were weak producers, and 31% were moderate producers [37]. The well-known and highly stable outer membrane protein *ompA* has been found to have a critical role in helping *A. baumannii* adhesion and biofilm formation [38]. *OmpA* helps the bacteria attach to human epithelial cells. At the same time, the *epsA* gene produces exopolysaccharides (EPS) that let the cells stick to each other and to surfaces, giving them protection from the host's immune system. Together, *ompA* and *epsA* promote biofilm formation [39]. In our study, the *ompA* gene was present in all isolates. This finding aligns with research from Iran, where *ompA* was also detected in all 27 strains examined [40]. The *epsA* gene was found in 31.25% of our isolates. Similarly, in Baghdad, *epsA* was detected in 37% of samples (19 out of 65), and *ompA* was reported in 90% of isolates [41]. In Shiraz, Iran, *ompA* was found in 97% of isolates, which is very close to our findings. However, that study detected that *epsA* was found in 88% of isolates, which is a much higher rate than what we found [42]. Conversely, a different study found lower detection rates, with *ompA* present in 57.89% of isolates and *epsA* in only 10.52% [43]. Our study showed no significant correlation between strength of biofilm formation and the presence of *epsA* or *ompA* genes. Research in Iran confirmed similar findings, where no significant relationship between *ompA* gene and strength of biofilm formation was observed. However, that study reported a significant association of *epsA* and strength of biofilm formation, although we did not observe any association with *epsA* in our study [42]. On the other hand, our result differs from a recent study in Sulaymaniyah, Iraq, which found that *ompA* was more frequent in strong biofilm producer strains [44]. Our study found no relationship between strength of biofilm producer and resistance grouping. In Southern Poland, researchers conducted a study that revealed the same results. There was no correlation between type of biofilm production and resistance groups (MDR, XDR, and E-XDR) [45]. Conversely, a study in Sulaymaniyah/ Iraq presented a significant relationship between the resistance patterns and the strong biofilm production [44].

Conclusion

This study showed that directly culturing environmental samples on MacConkey agar was not enough to recover bacteria effectively. Instead, pre-incubating the samples overnight in Brain Heart Infusion (BHI) broth before subculturing onto MacConkey agar gave much better results. The results were troubling *Acinetobacter baumannii* taken from different surfaces of the hospitals showed very high levels of antibiotic resistance, higher than what has been found in previous studies. Each isolate was capable of biofilm formation, enhancing their survival and pathogenic potential. All of the isolates showed intermediate susceptibility to colistin, which still offers some hope for treatment. Overall, the results underline how urgently hospitals need stronger cleaning practices and infection control measures to protect patients from the threat of *A. baumannii* and other nosocomial infections.

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