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Detection of biofilm-related genes and antibiotic resistance in *Acinetobacter baumannii* isolated from clinical specimens

SHNA OTHMAN KAKA SAADULLA[1], SIRWAN MUHSIN MUHAMMED[2,♥]

[1]Department of Nursing, Sulaimani Polytechnic University. Wrme Street 327/76, Chwarchra, Sulaimani Kurdistan Region, F.R. Iraq
[2]Department of Biology, College of Sciences, University of Sulaimani. Kirkuk Road, Sulaimani, Kurdistan Region, F.R. Iraq. Tel.: +96-47729969810, ♥email: sirwan.muhammed@univsul.edu.iq

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Abstract. *Saadulla SOK, Muhammed SM. 2023. Detection of biofilm-related genes and antibiotic resistance in* Acinetobacter baumannii *isolated from clinical specimens. Biodiversitas 24: 1809-1816.* A total of 53 *Acinetobacter baumannii* clinical isolates were collected from patients treated at Shar Hospital in Sulaimani city to determine antibiotic susceptibility patterns and their association between biofilm formation and the distribution of biofilm-related genes. Identification of suspected *A. baumannii* was carried out by conventional and molecular approaches, including 16S rRNA and bla_{oxa}-51 gene amplification. In addition, disk diffusion and microtiter plate methods determined antibiotic susceptibility and biofilm-forming capability, respectively. Out of 53 *A. baumannii* clinical isolates 42 (79.24%), were Multidrug-Resistant (MDR), of which 3 (5.66%) were resistant to colistin. Overall, 33 (62.26%) of the clinical isolates were strong biofilm producers, 6 (11.32%) were moderate or weak biofilm producers, and 8 (15.09%) were non-biofilm-forming isolates. The frequency of biofilm-related genes *ompA*, *bap*, *csuE*, *blaPER-1*, and *cpaA* among *A. baumannii* strains was 92.45%, 79.24%, 73.58%, 9.43%, and 7.54%, respectively. The high frequency of multi-drug resistant strains, and their ability to form biofilms, is a potential threat to effective therapy and may increase morbidity and mortality in infected patients. Colistin appeared to be the most effective antibiotic for treating MDR *A. baumannii* infections.

Keywords: *Acinetobacter baumannii*, biofilm formation, biofilm-related genes, multidrug-resistant, virulence factors

Abbreviations: MDR: Multidrug-Resistant; XDR: Extensively Drug-Resistance

INTRODUCTION

Acinetobacter baumannii is a Gram-negative, nonmotile, obligate, aerobic coccobacillus that harbors several virulence factors (Farouk et al. 2020). Due to the high burden of Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR) isolates, *A. baumannii* has been identified as one of the most common serious pathogens. Recent studies have reported a successful relationship between biofilm production and antibiotic resistance patterns in *A. baumannii* isolates (Qi et al. 2016). The ability of *A. baumannii* to colonize and produce biofilms on abiotic surfaces is considered a major contributing factor in several types of infections, including urinary tract infections, skin and soft tissue infections, endocarditis, intra-abdominal abscess, and surgical site infections (Donelli and Vuotto 2014). Bacterial cells inside the biofilm are highly coordinated and undergo phenotypic switching to build a community resistant to the harsh external environment. Moreover, this type of phenotypic switching increases the evolution of antibiotic resistance. Antibiotic-resistant strains of *A. baumannii* have been shown to form strong biofilms that support their survival and distribution (Shenkutie et al. 2020). *A. baumannii* grows readily in a wide range of pH and temperature environments and on a variety of substrates. Moreover, this strain is known to thrive in both dry and humid environments and inanimate objects. That included infected medical equipment such as ventilators, catheters, respirometers, pillows, and bed mattresses. *A. baumannii* is widespread in water and soil, and some strains have been isolated from animals (Tiwari et al. 2015).

Acinetobacter baumannii is classified as one of the ESKAPE organisms (i.e., *A. baumannii*, *Enterococcus faecium*, *Enterobacter* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*). These organisms pose a global threat to human health and present a therapeutic challenge due to emerging and increasing drug resistance. In 2018, carbapenem-resistant *A. baumannii* (CRAB) was designated by the World Health Organization (WHO) as a primary concern and a key target for antibiotic research and development. Carbapenem was chosen as a drug-resistance marker since carbapenem resistance is typically linked to a broad range of co-resistance across other antibiotic classes (Kyriakidis et al. 2021). Furthermore, an increased prevalence of carbapenem-resistant *A. baumannii* isolates has been identified in Northern and Eastern Europe and the Levant countries of the Arab League (i.e., Iraq, Jordan, Lebanon, Palestinian territories, and Syria) (Rizk et al. 2021). Carbapenems were previously the standard treatment for MDR *A. baumannii* infections. However, their widespread use in recent years has increased carbapenem resistance (Piperaki et al. 2019). Furthermore, the introduction of many new antibiotics and the concomitant high level of

antibiotic use globally has led to the development of numerous strategies by bacterial pathogens that inactivate the action of antibiotics (Sultan et al. 2018). The mechanisms of antibiotic resistance can be classified into three groups: i) resistance is acquired by a reduction in membrane permeability or an increase in the efflux of the antibiotic, thereby preventing access to the target; ii) bacteria protect the antibiotic target through genetic mutation or post-translational modification; and iii) antibiotics are inactivated directly through hydrolysis or modification (Blair et al. 2015).

Several key factors are involved in the biofilm formation of *A. baumannii*. Those including the Outer membrane protein A (OmpA), the Biofilm-associated protein (Bap), and the Chaperone-usher (Csu) pathway for pili formation, which may provide a structural basis for *A. baumannii* biofilm formation (Thummeepak et al. 2016). OmpA, the main outer membrane protein of *A. baumannii*, is also involved in cell invasion (Moubareck and Halat 2020) and plays an important role in the attachment and invasion of epithelial cells via interactions with fibronectin (Zeighami et al. 2019). Increased antibiotic resistance is one of the most important characteristics of bacteria that develop biofilm strategies. Therefore, it has been shown that biofilms can tolerate as much as 100 to 1000 times higher antibiotic concentrations than planktonic bacteria (Macià et al. 2014). Furthermore, to our knowledge, only a few studies have investigated the association between biofilm formation and the prevalence of biofilm-related genes as a source of *A. baumannii* virulence in the Sulaimani City-Kurdistan region. Therefore, we aimed to determine the susceptibility patterns of antibiotics and the association between biofilm formation and the distribution of biofilm-related genes (i.e., *ompA, bap, cpaA, blaPER-1*, and *csuE*) in various *A. baumannii* isolates obtained from clinical specimens obtained from this area.

MATERIALS AND METHODS

Study site and population

A total of 53 *A. baumannii* clinical isolates were isolated from various clinical sources (including 37 from urine, wounds, blood, burns, central venous catheters, and pharynx and tracheal aspirates, and 16 from hospital environments, such as critical burn units, adult and pediatric wards, operating rooms, toilets, and bathrooms) from patients admitted to Shar Hospital in Sulaimani during five months from November 2021 to January 2022.

Culture-based identification *of A. baumannii*

A single colony from a bacterial culture of the suspected *A. baumannii* isolates was transferred for initial identification based on standard morphological features, Gram staining, and biochemical test reactions, such as catalase and oxidase tests typically used to identify suspected colonies.

Identification of *A. baumannii* by VITEK® 2 System

The VITEK® 2 system was operated according to the manufacturer's instructions. VITEK® 2 ID-Gram Negative bacilli cards (BioMérieux, France) were utilized for all samples identified as *A. baumannii* by conventional methods.

DNA extraction

Genomic DNA was extracted from an overnight culture of *A. baumannii* clinical isolates (with the Genomic DNA Mini Kit Geneaid, Taiwan) according to the manufacturer's recommended protocols for Gram-negative bacteria.

Identification of *A. baumannii* using the 16S rRNA and the *bla*$_{oxa}$-51 genes

Extracted DNA from the clinical samples was amplified by Polymerase Chain Reaction (PCR) using 16S rRNA gene and *bla*$_{oxa}$-51 gene targeting primers (Table 1). PCR was performed on the T100 Thermal Cycler (Bio-Rad, USA) with the cycling conditions described in Table 2. For additional confirmation, the relevant genes were sent out for purification (QIAquick PCR Purification kit - QIAGEN) and sequencing (Applied Biosystems 3500 Genetic Analyzer / Macrogene genome center, South Korea). *A. baumannii* ATCC 19606 was used as the reference strain.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was carried out by disk diffusion, according to the Clinical and Laboratory Standards Institute (CLSI) (Weinstein and Lewis 2020). All isolates were tested against a panel of 7 antibiotics (HiMedia, India), including antibiotic agents from 7 different classes, using antibiotic disks on Mueller-Hinton agar. The following antibiotics were tested: ampicillin/sulbactam (10 µg), ceftriaxone (30 µg), imipenem (10 µg), amikacin (30 µg), colistin (25 µg), ciprofloxacin (5 µg), and gentamicin (10 µg). The clinical isolates were classified as either sensitive, intermediate susceptible, or resistant, according to CLSI breakpoints for *A. baumannii*. European guidelines for colistin disk susceptibility testing have been published by the British Society for Antimicrobial Chemotherapy (BSAC), using 25 µg colistin disks (Andrews 2007). MDR *A. baumannii* was defined in this analysis as resistance to three or more antibiotic classes (Gandra et al. 2019), while *A. baumannii* isolates with resistance to at least one agent in all but two or fewer antimicrobial categories were considered to be XDR. (Zeighami et al. 2019).

Table 1. Primers used in this study for amplification of 16S rRNA gene and *bla*$_{oxa}$-51 gene

Primer/ direction	Sequence (5′ → 3′)	Reference
16S rRNA (F)	GACGTACTCGCAGAATAAGC	Japoni-Nejad
16S rRNA (R)	TTAGTCTTGCGACCGTACTC	et al. (2013)
bla$_{oxa}$-51 (F)	TAATGCTTTGATCGGCCTTG	Zhang et al.
bla$_{oxa}$-51 (R)	TGGATTGCACTTCATCTTGG	(2017)

Quantitative biofilm formation assay

The biofilm formation assay was performed according to the procedure described by Babapour et al. (2016). First, *A. baumannii* was inoculated in 5 mL of Trypticase Soy Broth (TSB) and grown to the stationary phase. Next, the culture was diluted by 1:100. Subsequently, 200 µL of diluted culture was pipetted into eight wells of a 96-well flat-bottom polystyrene microtiter plate. Incubation was carried out at 37ºC for 24 hours. Cultures were subsequently aspirated, and the wells were washed three times with phosphate-buffered saline (pH 7.2). The plate was then air dried for 10 minutes at room temperature and stained by adding 200 µL of 0.1% crystal violet for 15 min. After removing the stain, 200 µL of 95% ethanol was added to each stained well and maintained for 10 to 15 min at room temperature. The optical density of the wells was measured at 596 nm using a microplate auto reader. Sterile TSB was used as a negative control (blank). Next, to compensate for background absorbance, the OD reading value of the blank was deducted from the test values. The results were divided into four categories according to their optical densities: i) strong biofilm producer ($4 \times ODc < ODT$); ii) medium biofilm producer ($2 \times ODc < ODT \leq 4 \times ODc$); iii) weak biofilm producer ($ODc < ODT \leq 2 \times ODc$); and iv) non-biofilm producer (ODT=ODc) (Gharrah et al. 2017). All experiments were performed in triplicate.

Detection of biofilm-related genes

Polymerase Chain Reaction (PCR) assays for the detection of *blaPER*-1, *bap*, *csuE*, *ompA*, and *cpaA* genes were performed with a set of primers, as shown in (Table 3). PCR assays were performed using PCR Red Master Mix (OnePCR Ultra) (and GeneDireX (Bio-Helix) in a Veriti® 96-well thermal cycler (Applied Biosystems, USA). The PCR mixture consisted of 1.0 µL of 10 pmole forward primer, 1.0 µL of 10 pmole reverse primer (Sinaclone, Iran), and 5 µL of PCR Master Mix. After adding 2 µL of genomic DNA, the final volume was brought to 20 µL with double distilled water (ddH_2O). Finally, the thermal cycler was programmed for the amplification of the genes. Conditions for the PCR assays are described in (Table 4). The PCR-amplified products were separated using 1.5% agarose gel electrophoresis (Cleaver, Scientific, Ltd, UK) in 10x TBE buffer at 80V for 60 min, then stained with 0.5 µg/mL ethidium bromide. Images were acquired using the Gel Doc XR+ (Bio-Rad, USA) imaging system.

Table 2. PCR conditions for the amplification of 16S rRNA gene and blaoxa-51 gene

Genes	Temperature (ºC) / time					
	Cycling conditions					
	Initial denaturation	**Denaturation**	**Annealing**	**Extension**	**Final extension**	**No. cycles**
16S rRNA	94ºC / 5 min.	94ºC / 1 min.	57ºC /1 min.	72ºC/ 1 min.	72ºC/ 7 min.	30
bla_{oxa}-51	95ºC / 2 min.	95ºC / 10 sec.	60ºC/ 34 sec.	72ºC / 30 sec.	72ºC/ 5 min.	45

Note: *The PCR condition was carried out following the references given for each primer

Table 3. The primers used in this study for the detection of biofilm-related genes

Primers	Primer sequence (5'-3')	Product size (bp)	References
blaPER-1	F: ATGAATGTCATTATAAAAGC R: AATTTGGGCTTAGGGCAGAA	925	Mazraeh et al. (2021)
bap	F TGCTGACAGTGACGTAGAACCACA R TGCAACTAGTGGAATAGCAGCCCA	184	Sadr et al. (2021)
csuE	F: ATGCATGTTCTCTGGACTGATGTTGAC R: CGACTTGTACCGTGACCGTATCTTGATAAG	976	Zeighami et al. (2019)
ompA	F: ATTTACCAGGATGGGCCGTG R: GCGCCACAACCAAGCAATTA	182	Priyadharsini et al. (2018)
cpaA	F: CTG CTT TAG GAA AAT GGG R: CGCCTTCAATCATTCTAAG	669	Fallah et al. (2017)

Table 4. PCR conditions for the amplification of *blaPER*-1, *bap*, *csuE*, *ompA*, and *cpaA* genes

Genes	Temperature (ºC) / time					
	Cycling conditions					
	Initial denaturation	**Denaturation**	**Annealing**	**Extension**	**Final extension**	**No. of cycles**
blaPER-1	95ºC / 3min.	95ºC / 30 sec.	50.5ºC / 1 min.	72ºC/ 1 min.	72ºC/ 5 min.	35
bap	95ºC / 5 min.	95ºC / 1 min.	54ºC/ 30 sec.	72ºC / 1 min.	72ºC/ 5 min.	35
csuE	94ºC / 5 min.	94ºC / 1 min.	60ºC/ 1 min.	72ºC / 1 min.	72ºC/ 10 min.	30
ompA	95ºC / 10min.	95ºC / 15 sec.	60ºC/ 1 min.	72ºC / 1 min.	72ºC/ 5 min.	40
cpaA	95ºC / 3 min.	95ºC / 30 sec.	63ºC/ 30 sec.	72ºC / 1 min.	68ºC/ 5 min.	35

Note: *The PCR condition was carried out following the references given for each primer

RESULTS AND DISCUSSION

Isolation and identification

In the present study, 53 samples of *A. baumannii* were isolated from different wards at Shar Hospital (50.94%) and the Burn and Plastic Surgery Hospital (49.05%). Overall, 37 isolates were obtained from the patient's clinical specimens as follows: Endotracheal Aspirate (ETA) (26.41%), burns (20.75%), wounds (9.43%), urine (5.66%), blood (3.77%), and 1.88% each for Bronchoalveolar Lavage (BAL) and central venous line (cv-line). In addition, 16 isolates (30.18%) were acquired from the hospital environment. Most clinical isolates were obtained from patients in intensive care units (24.52%), while other isolates were obtained from wards such as the burn, internal, surgery, and pediatric wards. The age of the patients ranged from 4 to 63 years. In general, 39.62% (n=21/37) and 30.18% (n=16/37) of *A. baumannii* isolates were obtained from male and female patients, respectively. The recovered colonies were faint pink in color, non-lactose fermenters on MacConkey agar, with a negative oxidase test, catalase-positive, and pleomorphic Gram-negative bacilli; these colonies were identified to the species level as *A. baumannii* using the VITEK® 2 system, 16S rRNA, and bla_{oxa}-51 gene. Overall, in 100% of the isolates, the 16S rRNA and bla_{oxa}-51 genes were detected, and the bacterial species was confirmed as *A. Baumannii* (Figure 1).

Antibiotic susceptibility testing

Among the 53 test isolates, resistance to ceftriaxone (81.13%) was the most common; followed by resistance to ciprofloxacin (79.24%), imipenem (75.47%), amikacin (73.58%), gentamycin (67.92%), ampicillin/sulbactam (37.73%), and colistin (5.66%), as shown in (Figure 2). Overall, (88.67%) of the isolates were resistant to at least one of the tested antibiotics. None of the bacterial isolates was resistant to all seven antibiotics. A total of 42 (79.24%) *A. baumannii* isolates were resistant to three or more antimicrobial agents, and these isolates were considered to be MDR.

Distribution of biofilm-related genes

Among biofilm-related genes, *ompA* (92.45%) was the most frequent gene identified, followed by *bap* (79.24%), *csuE* (73.58%), *blaPER-1* (9.43%), and *cpaA* (7.54%), (Table 5). However, the biofilm-related genes were undetected in 3.77% of *A. baumannii* isolates. We also found that 11.32%, 64.15%, 3.77%, and 16.98% of isolates carried 4, 3, 2, and biofilm-related genes, respectively.

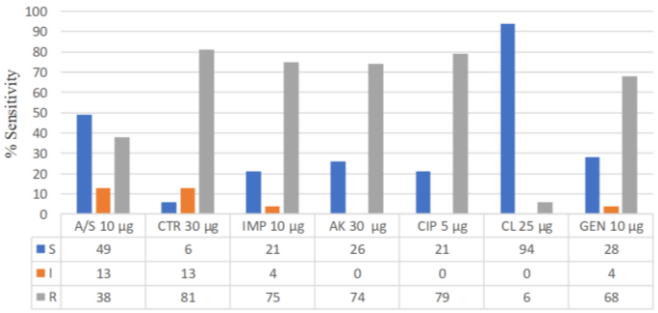
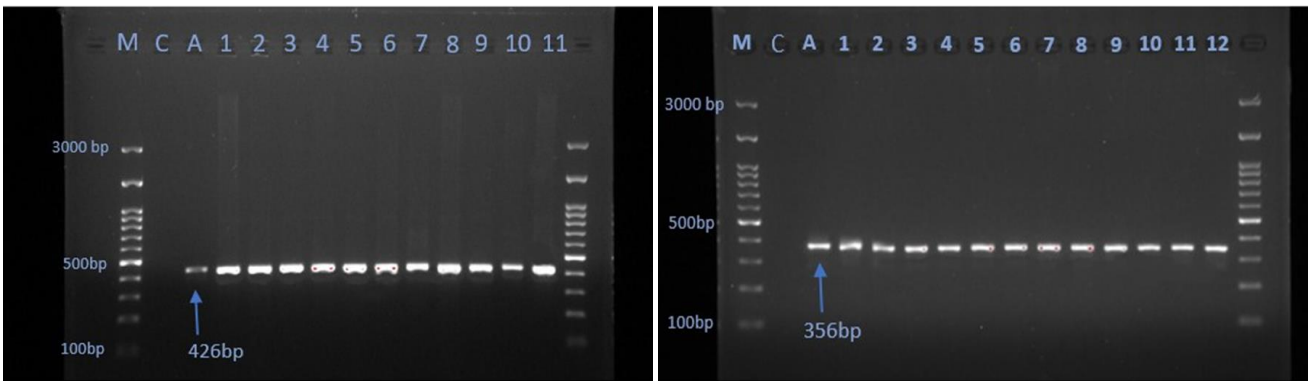


Figure 2. Antibiotic susceptibility test by the diffusion method. R: Resistant; S: Sensitive; I: Intermediate. A/S: Ampicillin/Sulbactam; CTR: Ceftriaxone; IPM: Imipenem; AK: Amikacin; CL: Colistin; CIP: Ciprofloxacin; GEN: Gentamicin

Table 5. Frequency of biofilm-related genes among *A. baumannii* isolates

Biofilm related genes	No. of isolates	(%) of isolates
ompA	49	92.45
bap	42	79.24
csuE	39	73.58
blaPER-1	5	9.43
cpaA	4	7.54

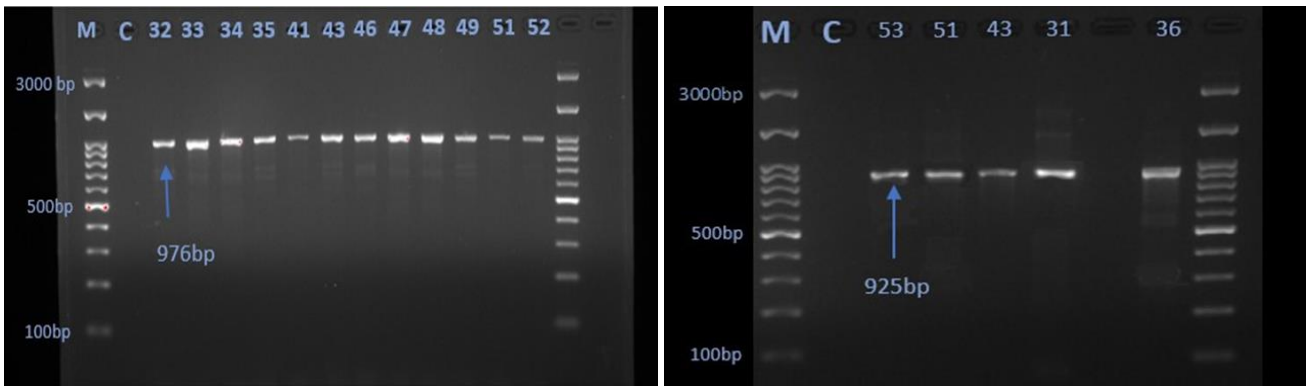


A **B**

Figure 1. The image of PCR product gel electrophoresis. **A.** M: DNA ladder 100bp. (A) *A. baumannii* ATCC:19606. C: negative control. PCR amplification of 16S rRNA gene fragment in clinical *A. baumannii* isolates (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11). **B.** M: DNA ladder 100bp. (3) *A. baumannii* ATCC:19606. C: negative control. Amplified fragments of the bla_{oxa}-*51*-like gene in clinical *A. baumannii* isolated in the present study (1,2,3,4,5,6,7,8,9,10,11, and 12)

Table 6. Biofilm-related gene expression and biofilm intensity in clinical isolates of *A. baumannii*

Biofilm intensity	Biofilm-related genes					
	ompA	***bap***	***csuE***	***blaPER-1***	***cpaA***	**MDR**
Strong (n=33)	33	32	30	3	4	25
Moderate (n=6)	4	3	3	1	-	4
Weak (n=6)	5	5	4	-	-	5
Non-biofilm (n=8)	7	2	2	1	-	8
Total (n=53)	49	42	39	5	4	42
% Biofilm-related genes/biofilm former	89	85	78	8	8	79.24



A **B**

Figure 3. The gel electrophoresis image of PCR product (biofilm-related genes). **A.** M: DNA ladder 100bp; C: negative control. Amplified fragments of *csuE* gene fragment in clinical *A. baumannii* isolate (32, 33, 34, 35, 41,43,46,47,48, 49,51, and 52). **B.** M: DNA ladder 100bp. C: negative control. (53,51 43,31and 36) PCR amplification of *blaPER-1* gene fragment of *A. baumannii* isolates

Relationship between biofilm formation and biofilm-related genes

Among the 53 test strains, a total of 47 isolates were biofilm formers, of which 33 (62.26%) were strong biofilm formers, 6 (11.32%) were moderate weak biofilm formers, and 8 (15.09%) were non-biofilm forming isolates. After analyzing the association between biofilm formation and biofilm-related genes, the results showed that *ompA*, *bap*, and *csuE* genes were found in 89% (42/47), 85% (40/47), and 78% (37/47) of the biofilm producers, respectively. As shown in Table 6, the strains carrying *ompA*, *bap*, and *csuE* genes tended to form stronger biofilms than the isolates lacking these genes. Moreover, the present study found a significant relationship between antimicrobial resistance patterns and strong biofilm production, with the results showing that 75.75% of strong biofilm-forming isolates were MDR.

Discussion

MDR *A. baumannii* infections are reported to have shown dramatic growth worldwide, and outbreaks have posed a severe burden on the public healthcare system. Like many other geographical areas, our region (Kurdistan) has been confronted with this growing problem (Pourhajibagher et al. 2016). However, limited data are available on *A. baumannii* susceptibility, biofilm generation, and the frequency of biofilm-related genes. Therefore, the present study aimed to determine antibiotic susceptibility patterns and the association between biofilm formation and the distribution of biofilm-related genes. We identified 53 isolates of *A. baumannii* collected from various wards at Shar Hospital and from the Burn and Plastic Surgery Hospital in Sulaimani, and our findings were compared to those from previous research conducted in Iraq (Hassan and Khider 2019; Al-Kadmy et al. 2020; Shali et al. 2022) and Iran (Fallah et al. 2017). The bla_{oxa}-51-like gene was found in all isolates, and its presence has been suggested as a species marker for *A. baumannii* (Zander et al. 2013). The prevalence of MDR isolates was 79.24%, similar to the 74% (Pourhajibagher et al. 2016) and 83.9% (Monfared et al. 2019) identified in 118 clinical isolates. Other investigations from hospitals in Sulaimani and Erbil, Iraq, have revealed rates of MDR isolates of 37% and 18.92%, respectively (Hassan and Khider 2019; Shali et al. 2022). Possible explanations for the increased incidence of MDR *A. baumannii* isolates include excessive antibiotic drug use, insufficient duration of antibiotic therapy, and incorrect detergent and disinfectant use, all of which have recently led to increased mutagenesis in bacteria. Furthermore, cross-border interactions among countries with the highest number of MDR cases, such as Iraq, Iran, and Turkey, may explain the increasing prevalence of MDR *A. baumannii* isolates. In Turkey, 96% of *A. baumannii* strains were classified as MDR (Cicek et al. 2014), while in Iran, 83.9% of 118 *A. baumannii* clinical isolates were classified as MDR (Monfared et al. 2019).

The present study revealed that antibiotic resistance rates in *A. baumannii* clinical isolates were very high,

notably against ceftriaxone (81.13%). However, this resistance rate was lower than in earlier studies conducted in Iraq, when 90% resistance was reported for this ceftriaxone (Al-Kadmy et al. 2018). Ampicillin/sulbactam was the most active antimicrobial against most *A. baumannii* isolates (with a susceptibility rate of 62.3%). Our study investigated that Ampicillin/sulbactam was resistant to 37.7% of the isolates. However, a lower percentage was reported by Shali et al. (2022) at 18.92%. Moreover, based on our data, amikacin resistance is consistent with that reported by (Ayan et al. 2003) (74%), who observed that degree of resistance in 52 clinical isolates. On the other hand, the gentamycin resistance rate determined in our study was higher than the 59.6% reported by Qi et al. (2016) but lower than the 100% reported by Al-Kadmy et al. (2018). Imipenem, a carbapenem antibiotic, exhibits broad-spectrum antibacterial activity against aerobic, anaerobic, Gram-positive, and Gram-negative. Many previous studies have shown that imipenem was highly effective against biofilm formation (Qi et al. 2016); however, resistance to imipenem and ciprofloxacin in the present study was higher than that reported by Shenkutie et al. (2020) (51.9% and 51.9%) and lower than the findings reported by Fallah et al. (2017) (i.e., 92% and 91%, respectively, resistance to imipenem and ciprofloxacin). Colistin is usually effective against MDR and XDR Gram-negative bacteria, particularly *A. baumannii* (Pormohammad et al. 2020). However, recent reports of resistance to colistin as the last line of treatment have raised further concerns in the field of antibiotic-resistant pathogens (Khoshnood et al. 2020). In the present study, resistance to colistin was found to be 5.66%, which is consistent with Fallah et al. (2017), Khoshnood et al. (2020), and Monfared et al. (2019); however, Al-Kadmy et al. (2020) and Mazraeh et al. (2021) noted that 76% of the 121 *A. baumannii* isolates and 12.8% of the 70 isolates studied, respectively, were resistant to colistin.

The capacity of *A. baumannii* to generate biofilms promotes its survival under harsh conditions while at the same time raising the risk of healthcare-associated illnesses (Shenkutie et al. 2020). The present study found that 84.9% of clinical *A. baumannii* isolates formed biofilms to various degrees. This observation is compatible with the results reported by Hassan and Khider (2019). Based on their capacity for biofilm formation, the isolates were categorized as either non-biofilm, weak, moderate, or strong biofilm producers, with 33 (62.26%) of the isolates classified as strong biofilm producers. Moreover, these isolates demonstrated an increased capacity for initial attachment to microtiter wells. Similar outcomes (63%) were reported in an earlier investigation by Qi et al. (2016). Therefore, 6 (11.32%) of these isolates formed moderate and weak biofilms, whereas 8 (15.09%) were non-biofilm-forming isolates. Relative outcomes have already been reported (Yang et al. 2019; Khoshnood et al. 2020), in which a link was found between antimicrobial resistance patterns and high biofilm development. For example, an increased number of antibiotic-resistant *A. baumannii* isolates could form biofilms compared to susceptible isolates. Overall, 72% of strong biofilm formers were resistant to at least four to five distinct antibiotics; similar results have previously been reported (Al-Shamiri et al. 2021). The biofilm development of clinical isolates of *A. baumannii* with multi-drug resistance is influenced by the distribution of virulence genes (*bap*, *blaPER1*, *ompA*, *csuE*, and *cpaA*). In the present study, PCR was carried out to confirm the presence of biofilm-related genes. We discovered a significant link between antibiotic resistance, biofilm formation, and the relevant genes involved. Some related genes, such as *ompA*, *bap*, and *csuE*, were shown to have increased biofilm-forming capabilities. The OmpA, a significant component of Outer Membrane Proteins (OMPs), is a critical virulence factor that promotes bacterial biofilm formation, eukaryotic cell infection, antibiotic resistance, and inflammatory responses (Nie et al. 2020). In the present study, *OmpA* was the most common gene (92.45%) identified in test isolates, followed by *bap* (79.24%), with nearly identical results of 91.6% and 79.2% reported by Yang et al. (2019).

According to a recent study, *bap* plays a role in biofilm development and maintenance, boosting biofilm thickness and biovolume on glass surfaces (Tiwari et al. 2018). The Bap surface protein enables *A. baumannii* to live and persist on abiotic materials and has been associated with nosocomial infection (Fallah et al. 2017). The biofilm data presented in the present study and other studies confirm that *bap* is an extremely important factor in biofilm development. A strong association was observed between the presence of the *bap* gene and biofilm formation by *A. baumannii* isolates, with 85% of biofilm formers carrying the *bap* gene. The *csuE* gene is a chaperone-usher assembly system member, which promotes attachment and biofilm development (Yang et al. 2019). It has been demonstrated that inactivating the *csuE* gene prevents the assembly of pilus subunits and biofilm formation (Zeighami et al. 2019). In the present study, bacteria carrying the *csuE* gene accounted for 73.58% of the test isolates. Similar results of 72.73% were reported by Elbehiry et al. (2021). Even though the prevalence of the *blaPER-1* gene was 9.43% in the test strains, that was lower than previous studies (18%) performed in Kuwait (Vali et al. 2015). In the present study only three strong biofilm-forming isolates were positive for PER-1-lactamases among thirteen n=30 strong biofilm formers. This observation is consistent with the findings of Bardbari et al. (2017), who found no relationship between PER-1-lactamase-positive isolates and biofilm producers. Rao et al. (2008) also proposed that blaPER-1 is more important for cell adhesion than biofilm development. Patients with *A. baumannii* infections have been found to have unusual coagulation-related proteins (Fallah et al. 2017). Therefore, zinc-dependent metalloendopeptidase *cpaA* (coagulation targeting metalloendopeptidase of *A. baumannii*) has been found to deregulate human blood coagulation in vitro. Therefore likely to contribute to *A. baumannii* virulence (Tilley et al. 2014). Our analysis found a lower rate (7.54%) of the *cpaA* gene than Fallah et al. (2017), who reported a rate of (36%). However, this gene was lacking from the *A. baumannii* strain ATCC 19606, similar to observations previously reported by (Kinsella et al. 2017).

In conclusion, the present study showed that sequencing the 16S rRNA and *bla*$_{oxa}$-51 genes is rapid and accurate identification method for *A. baumannii* isolates. The frequency of MDR *A. baumannii* isolates was elevated in the area investigated, compared with a previous study, and with a high prevalence of biofilm-forming genes. The ability to produce biofilms was associated with the presence of the *ompA* and *bap* genes in the *A. baumannii* isolates. The detection of biofilm-forming genes is an alarming finding, as well as a serious healthcare issue in our hospital. Furthermore, colistin resistance was observed in three isolates (5.66%). Therefore, developing novel and effective strategies is essential for preventing drug-resistant infections caused by biofilm-forming *A. baumannii*.

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