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Detection of carbapenemase in *acinetobacter baumannii* enrolled in the relationship between biofilm formation and antibiotic resistance

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Abstract

Background and objective: *Acinetobacter baumannii* is a significant pathogenic bacterium in the health system. The ability to resist antimicrobial drugs and biofilm formation gives the considerable capacity to *A. baumannii* for existing in a harsh environment, enabling this bacterium to cause hospital-acquired infection. Carbapenem is an important treatment option for severe nosocomial infection and patients infected by multidrug-resistant organisms. The main aim of this study is to detect carbapenemase in isolates, and its association with biofilm formation as well as antibiotic resistance.

Methods: Sixty *A. baumannii* isolates were obtained from several hospital districts in Erbil city. Identification and antimicrobial susceptibility test (AST) of isolates were performed by VITEKII compact system. Phenotypic identification of carbapenem by sCIM also biofilm-forming was detected by 96 well method. Additionally, three antimicrobial agents were used if they were successful in eliminating biofilm formation. .

Results: The majority of the isolates were from sputum, accounting 75% and antibiotic susceptibility showed that the isolates are resistant to the most available antibiotics, and significant of the isolates formed strong biofilm. The sensitivity of meropenem, ceftazidime, and ciprofloxacin were employed for ten isolates of *A. baumannii* after biofilm formation it was found that biofilm cells need more concentration of antibiotic than planktonic cells then phenotypic detection of carbapenem showed that the overall positive values were 30 (50.0%) for sCIM.

Conclusion: We revealed that most resistant isolates have a greater capacity for biofilm development than sensitivite isolates. Biofilm-producing strains of *A. baumannii* cannot be killed with the relatively similar concentration of antimicrobial drugs that are needed to kill planktonic cells.

Keywords: *Acinetobacter baumannii*; Carbapenemase; Biofilm development; Antibiotic susceptibility.

Introduction

Acinetobacter baumannii is an opportunistic gram-negative bacteria, and it is known as one of the top seven concern bacteria inthe human health system.[1] This bacterium can cause infection in an immune-deficient individual, commonly in those who have been admitted to hospital incredibly intensive care unitspatients.[2,3] *A. baumannii* is a member of the ESKAPE bacteria group that consists of "*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Enterobacter* species" that are highly responsible for nosocomial and antimicrobial-resistant infections. Nosocomial infections triggered by Gram-negative bacteria *A. baumannii* are accountable for 2– 10% of infections.[4] This bacterium can cause infections in some body parts like lungs (Pneumonia),

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soft tissue (Skin infection), and urinary tract (Urinary tract infections).[5,6] *A. baumannii* could stay alive for long times upon the sides and surfaces, sometimes for many years.[7] It commonly causes nosocomial infections because of many virulence factors, like biofilm formation and a high rateof antibiotic resistance.[8]

A. baumannii has a high ability to resist many antibiotics. A major of them that are isolated in diseased individuals are usually insusceptible to the large number of antimicrobials that are clinically effective, like aminoglycosides, tetracycline, fluoroquinolones, β-lactams, and trimethoprim-sulfamethoxazole, and as a final point develop multidrug-resistant (MDR).[9] This causes difficulty in adding suitable treatment for *A. baumannii* infections.[10]

Carbapenems are known as the last choice antibiotics for severe infections since of their enzyme resistance and effectiveness clinically. Conversely, bacteria could tolerate and escape from β-lactam antibiotics through the production of β-lactamase; in gram-negative bacteria, this is the most prevalent resistance mechanism toward β-lactam. However, most β-lactamases are unable to disrupt carbapenems.[11] Biofilm can be defined as the aggregation of many bacteria on the surfaces to form a matrix. This matrix functions as a supportive layer that protects the bacteria and raises its capability to resist demanding environments and a wide range of antimicrobials.[8] Biofilm forming capability in *A. baumannii* has a high role in the persistence of this bacterium.[12]

This bacterium can be transferred rapidly in hospital settings and contaminates many surfaces and devices such as vascular catheters, foley catheters, and cerebrospinal fluid shunts.[13,14] The connection between biofilm development and antibiotic resistance is essential and attractable to the medical investigator. The idea of this relationship over the past two decades has been varied and is debatable. Several experiments confirmed that acquired resistance factors by *Acinetobacter* spp. Detected its ability to produce biofilms.[9,15] For example, minor amounts of some antibiotics can induce biofilm formation. Additionally, the multi-drug resistant *A. baumannii* isolates presented a more excellent capability to produce biofilm than sensitive isolates.[12,16]

The current study aims phenotypic detection of carbapenem producing *A. baumannii* and its correlation to biofilm development and their antibiotic resistance profiles.

Methods

Sample collection

Sixty isolates of *A. baumannii* were taken from diseased individuals of different age groups from clinical specimens, including sputum, blood, and wound swab, which were collected from various medical parts and Intensive Care Unit (ICU) sections of hospitals in Erbil city for a period of 8 months from September 1, 2021, to March 30, 2022.

Identification and Antimicrobial Susceptibility Test (AST) of *A. baumannii*

A. baumannii isolates were cultured on Blood and MacConkey agar (BD BBL, USA). Isolates were diagnosed by performing ordinary bacteriological methods like; Gram staining and biochemical tests such as; catalase test, oxidase test, and coagulase test. Then, the identification of isolates was confirmed by VITEK II compact system (bioMérieux, France). The antibiotic sensitivity of isolated *A. baumannii* was performed by VITEK II compact system. Fifteen antibiotics were used for AST analysis (Piperacillin (PIP), Pep/Tazobactum (TZP), Cefepime (CFP), Ceftazidime (CAZ), Imipenem (IMP), Meropenem (MEM), Amikacin (AK), Gentamicin (CN), Netilmicin (NET), Tobramycin (TOB), Ciprofloxacin (CIP), Levofloxacin (LEV), Tigycycline (TGC), Tri/sulfamethoxazole (SXT), and Colistin (CST). VITEK II compact system–identification and

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sensitivity testing confirmation were made using GN card and AST N222.

Biofilm detection by microtiter plate method

The capacity of biofilms to form was determined using the crystal violet staining technique. After a 24-hrs incubation period, bacteria were diluted to an optical density of 600 (OD_{600}). Then, 180 µL of Luria-Bertani (LB) broth with 1% glucose (LabM, UK) and 20 µL of bacterial suspensions were put on 96-well polystyrene microtiter plates (Costar, Corning, NY, USA). The plates were incubated for 24 hrs at 37°C, washed three times with phosphate-buffered saline (PBS), and then stained with 200 µL of 0.1 percent crystal violet (Oxoid, UK) for 20 minutes. Consequently, the plates were rinsed three times with PBS. The plates were dissolved in 200 µL of 95% ethanol by gently stirring at room temperature for 20 minutes. At 570nm, the absorbance was determined. Three standard deviations (SD) above the average OD of the negative control (LB broth only) was defined as the cut-off OD (ODc). The classification rules were as follows: OD≤ODC indicates non-biofilm producers(-); ODC<OD≤2×ODC indicates weak biofilm producers (+); 2×ODC<OD≤4×ODC shows moderate biofilm producers (++), and OD>4×ODC suggests strong biofilm producers (+++).[17]

Determination of minimal inhibitory concentration (MIC), minimum bactericidal concentration (MBC), Determination of minimal biofilm inhibitory concentration (MBIC), and minimum biofilm eradication concentration (MBEC) techniques

The procedure was done as described previously [17] with few modifications. Briefly, three antimicrobials commonly used to treat *A. baumannii* infections were selected to determine MICs/MBCs and MBICs/ MBECs. Ten biofilm-forming isolates were chosen randomly; seven of them were strong biofilm former the rest were moderate biofilm former. The MICs for Ceftazidime, Ciprofloxacin, and Meropenem were detected by performing the broth microdilution technique in 96-well plates according to the CLSI 2021-M100 instructions.[18] The MIC was observed, then tenµL of the suspension in the wells that did not show bacterial growth were cultured on LB agar plates and incubated for 24 hrs. at 37°C. The lowest concentration of antibiotic that made no observed bacterial growth on plates was considered MBC. Biofilm was done in 96-well plates for 24 hrs., and then plates were washed by PBS to remove planktonic cells. Two hundred µL of LB broth containing serially diluted antimicrobials was added to each well. The plates were incubated for 24 hrs. at 37°C. The minimum antimicrobial concentration OD_{600}<0.1 was determined as the MBIC. After MBIC detection, PBS was used for washing the plates to remove planktonic cells and antibiotics. Two hundred microliter of LB broth was inoculated to every well, and the plates were incubated at 37°C for 24 hrs. The lowest concentration at which OD_{600}<0.1 was considered the MBEC.

Simplified carbapenem inactivation method (sCIM)

The test was accomplished as described by Jing, Zhou [19] The isolated bacterial cells were cultured on a TSA medium at 37°C for 24 hrs. *E. coli* ATCC25922 was used to detect the ability of *A. baumannii* to generate carbapenemase, and the absorbance was adjusted to 0.5 McFarland and diluted 1:10 in a PBS solution before culturing onanMHA plate. Later an Imipenem 10µg disk was smeared on one side with the bacteria that needed to be tested, and then the disks were added to the MHA plates. The MHA plates were incubated for 24hrs. at 37°C, that inhibition zone diameter was recorded. Zone diameter >26 mm was measured negatively, whereas inhibition zone 6–20 mm colonies within zone diameter <22 mm were positive. The indeterminate outcome

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was interpreted when the zone diameter was within 23–25 mm.

Statistical analysis

Statistical analysis was conducted using GraphPad Prism software (San Diego, CA, USA version 9.0). *Spearman's rank correlation analysis* revealed the association between antibiotic resistance and carbapenemase detection with the sCIM test. *P* value <0.05 was considered statistically significant for all tests.

Ethical Considerations

The present study was ethically approved by the ethic committee of Pharmacy college, Hawler Medical University Kurdistan region-Iraq. All patients were informed about the purpose of the study.

Results

Distribution of *A. baumannii*

Sixty *A. baumannii* isolates were diagnosed depending on colonies' features on different culture media and biochemical tests. For more confirmation of *A. baumannii* identification, all isolates were re identified by VITEK II compact system. The distribution of isolates among clinical specimens were revealed at different rates, which included sputum 45(75%), that represents the primarymain source of *A. baumannii*, the second-largest source was wound swab 11 (18.33%), and followed by blood samples 4 (6.67%) as shown in Figure 1.

Antimicrobial susceptibility profiles of *A. baumannii*

The AST tests for 14 antimicrobial agents were performed on all sixty isolates of *A. baumannii* and are revealed in Table 1.

All isolates 100% were resistant to each of Piperacillin, Pep/Tazobactum, Cefepime, Ceftazidime, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, and Levofloxacin, followed by Netilmicin and Tobramycin 52 (86.367%), and Tri/sulfamethoxazole 48 (80.00%), and the lowest resistant rate was detected in Tigycycline 11 (18.33%). All isolates revealed the sensitive pattern against Colistin antibiotics.

Comparison of MIC and MBIC

Ten distinct sequence types were examined for antimicrobial susceptibility against Meropenem, Ceftazidime, and Ciprofloxacin. These isolates were biofilm-producing, and the antimicrobial susceptibility profiles for biofilm compared to counter-plankton cells as described in Table 2.

Compared to planktonic cells, the MBICs of the microbes improved by a significant amount. It was shown that the concentration rise for Meropenem varied from eight to 512 µg/mL, for Ceftazidime from 16 to 1024 µg/mL, and for Ciprofloxacin from two to 64 µg/mL for isolate 2^A, respectively.

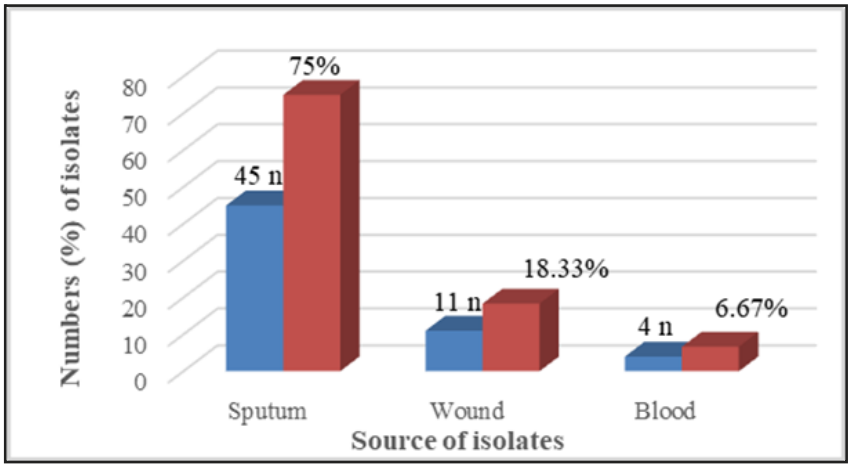

Figure 1 Prevalence of *A. baumannii* according to the source of isolates

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Table 1 Antibiotic susceptibility profiles of *A. baumannii* using VITEK II compact system AST card

Antibiotics	Antibiotic symbol	Resistant n. (%)	Intermediate n. (%)	Sensitive n. (%)
Piperacillin	PIP	60 (100.00)	0 (0.00)	0 (0.00)
Pep/Tazobactum	TZP	60 (100.00)	0 (0.00)	0 (0.00)
Cefepime	CFP	60 (100.00)	0 (0.00)	0 (0.00)
Ceftazidime	CAZ	60 (100.00)	0 (0.00)	0 (0.00)
Imipinem	IMP	60 (100.00)	0 (0.00)	0 (0.00)
Meropenem	MEM	60 (100.00)	0 (0.00)	0 (0.00)
Amikacin	AK	60 (100.00)	0 (0.00)	0 (0.00)
Gentamicin	CN	60 (100.00)	0 (0.00)	0 (0.00)
Netilmicin	NET	52 (86.67)	2 (3.33)	6 (10.00)
Tobramycin	TOB	52 (86.67)	1 (1.67)	7 (11.67)
Ciprofloxacin	CIP	60 (100.00)	0 (0.00)	0 (0.00)
Levofloxacin	LEV	60 (100.00)	0 (0.00)	0 (0.00)
Tigycycline	TGC	11 (18.33)	14 (23.33)	35 (58.34)
Tri/sulfamethoxazole	SXT	48 (80.00)	0 (0.0)	12 (20.00)
Colistin	CST	0 (0.0)	0 (0.0)	60 (100.0)

Table 2 Sensitivity to antimicrobials of specific sequencesof different kinds of *A. baumannii* planktonic and biofilm cells

	Meropenem		Ceftazidime		Ciprofloxacin	
***A. baumannii* isolates**	**MIC for Plank-tonic Cells (µg/mL)**	**MBIC for Biofilm Cells (µg /mL)**	**MIC for Planktonic Cells (µg /mL)**	**MBIC for Biofilm Cells (µg /mL)**	**MIC for Planktonic Cells (µg /mL)**	**MBIC for Biofilm Cells (µg /mL)**
2[A]	8	512	16	1024	2	64
6[A]	4	256	16	512	1	32
16[A]	64	1024	32	512	1	16
20[A]	16	256	32	256	2	64
28[A]	32	1024	32	1024	4	128
29[A]	32	512	32	512	4	64
48[A]	16	512	64	512	2	32
38[B]	16	1024	64	1024	8	128
40[B]	16	512	32	256	2	64
47[B]	32	512	32	512	2	32

Notes: [A:] Designates strong biofilm former, [B:] Designates moderate biofilm former.

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Comparison of MBC and MBEC

Tests for MBC and MBEC were done after finding the MIC and MBICs for 10 *A. baumannii* strains. MBECs for all ten isolates have gone up. Meropenem, Ceftazidime, and Ciprofloxacin had to be up to 64 times more concentrated to get rid of the *A. baumannii* biofilm than the planktonic cells (Table 3).

This is what happened when you compared MBECs and MBCs. The mean fold increase for Meropenem, Ceftazidime, and Ciprofloxacin was 21, 18, and 24 when you compared the two groups. In this study, the results show that biofilm-forming cells require a higher dose of antibiotics than planktonic cells in order to be eliminated completely.

The carbapenemase detection in *A. baumannii*

All sixty *A. baumannii* isolates were used for this experiment, (100%) revealed resistance to carbapenems on screening tests. Among the 60 *A. baumannii* isolates, sCIM demonstrated 30 (50.0%) positive results, 5(8.33%) showed intermediate results, and 25 (41.67%) of the isolates were recorded as a negative for carbapenemase, (Table 4).

Table 3 Correlation of MBICs and MBECs of strains of *A. baumannii*

	Meropenem		Ceftazidime		Ciprofloxacin	
***A. baumannii* isolates**	**MBC for Planktonic Cells (µg/mL)**	**MBEC for Biofilm Cells (µg /mL)**	**MBC for Planktonic Cells (µg /mL)**	**MBEC for Biofilm Cells (µg /mL)**	**MBC for Planktonic Cells (µg /mL)**	**MBEC for Biofilm Cells (µg /mL)**
2[A]	16	512	32	2048	4	256
6[A]	8	512	32	1024	4	64
16[A]	128	1024	64	1024	2	32
20[A]	32	256	64	512	4	128
28[A]	64	1024	64	1024	8	256
29[A]	64	512	64	1024	8	64
48[A]	32	512	128	1024	4	64
38[B]	32	1024	128	512	16	128
40[B]	32	512	64	512	4	128
47[B]	64	512	64	512	4	64

Notes: [A:] Designates strong biofilm former, [B:] Designates moderate biofilm former.

Table 4 Phenotypic method (sCIM) for detection of carbapenemase-producing *A. baumannii*

***A. baumannii* isolates**	**sCIM *n*. (%)**		
	P	**I**	**N**
Carbapenem resistant (Carbapenemase producers) (n=60)	30 (50.00)	5 (8.33)	25 (41.67)

*: sCIM: Modified carbapenem inactivation method; P: positive; N: negative; I: intermediate; n= number of samples.

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Biofilm-producingabilities of the *A. baumannii*

Using CV staining, biofilm production assessments were carried out on a microtiter plate. The OD_{600} values for the negative and positive controls were 0.099±0.012 and 0.488±0.085, respectively. Consequently, the ODc value was set at 0.138, and the classification breakpoints were the following: non-biofilm former: OD≤0.138, weak biofilm former: 0.315≥OD>0.138, moderate biofilm former: 0.490≥OD>0.315, and strong biofilm former: OD>0.490. Based on this, about 57 isolates from the total of 60 isolates of *A. baumannii* were capable of producing biofilm; only (n=3) 5.0% were non-biofilm former, (n=15) 25.0%, (n=19) 31.67%, and (n=23) 38.33% were weak, moderate, and strong biofilm producers, respectively (Table 5).

Association between antimicrobial resistance and carbapenemase detection methods

Spearman's rank correlation test found that antibiotic-resistant tended to show strong significant relation with carbapenemase test methods (*P* value = 0.0001; Table 6), suggesting a positive correlation between carbapenemase detection methods and antimicrobial resistance phenotype (r_s=0.917 and *P*-value = 0.0001).

Table 5 Screening of A. baumannii isolates from biofilm production by MTP method

Biofilm production status	No. (%) of biofilm-producing isolates
Strong	23 (38.33)
Moderate	19 (31.67)
Weak	15 (25.00)
None	3 (5.00)

Table 6 Correlation between carbapenemase detection and resistance to antimicrobial agents

Antimicrobial classes	Antimicrobial agents	Total antibiotic-resistant	sCIM + (n)	sCIM - (n)	*P* value
Penicillins	PIP	60	30	30	0.0001
	TZP	60	30	30	
Cephalosporin	CFP	60	30	30	
	CAZ	60	30	30	
Carbapenem	IMP	60	30	30	
	MEM	60	30	30	
Aminoglycoside	AK	60	30	30	
	CN	60	29	31	
	NET	52	24	28	
	TOB	52	25	23	
Quinolones	CIP	60	29	31	
	LEV	60	30	30	
Glycycline	TGC	11	4	7	
Trimethoprim	SXT	48	26	22	

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The relationship between antimicrobial sensitivity and the ability to produce biofilms

The ability of A. baumannii strains to develop biofilms (strong, moderate, and weak) was assessed among various antibiotic resistance belonging to different classes (Table 7). A substantially significant correlation appeared between antimicrobial classes and biofilm level (P = 0.002). For example, biofilm-forming isolates were concentrated in classes I, II, and III. Given that classes I, II, and III of antimicrobials are incredibly resistant, we examined the discrepancies in biofilm development between multidrug-resistant and susceptible isolates and discovered a significant correlation among them (P = 0.002).

Discussion

Acinetobacter baumannii is anopportunistic nosocomial bacteria. This bacterium can cause infection in a healthy individual. Because of the ability of this bacterium to resist a wide range of antibiotics, it is considered a significant problem and worry to the human health system, among other diseases. However, the presence of bacteria in high amounts in the hospital setting has been considered a typical misapprehension by several studies.[20] In the latest decades, hospital-acquired infections by *A. baumannii* have been raised. Curing of this bacteria is difficult, especially in multidrug-resistant and broad-spectrum beta-lactamases isolates, and is considered a common problem.[21] Carbapenems are a final option for treating the infection produced by multidrug-resistant gram-negative bacteria; carbapenem resistance is widespread in *A. baumannii*, so correct and soon discovery of Carbapenem positive *A. baumannii* is immediately required to accomplish effective treatment of those infections.[22] The antimicrobial susceptibility testing for 14 antimicrobials was done on all sixty *A. baumannii* isolates, and the results are summarized in Table 1.

The result showed that all isolates were insusceptible to the majority of antimicrobials the lower degree of insusceptibility was found in Netilmicin and Tobramycin at 86.367% followed by Tri/sulfamethoxazole at 80.00%, and Tigycycline 18.33%. The sensitivity pattern to Colistin antibiotic was identified in all isolates, our finding of this study agreed with the studies that were done in Erbil[23,24] both studies found most tested samples were sensitive to Colistin, and Polymyxin B. The distribution of samples in this study throughout clinical specimens demonstrated a range of these rates, with sputum 45 (75%) being the major source of

Table 7 Distribution of biofilm-producing capacities of A. baumannii isolates among different antibiotic-resistance classes

Antimicrobial classes	Biofilm formation (n.)			*P* value
	Strong (n=25)	Moderate (n=19)	Weak (n=15)	
Penicillins (Class I)	23	19	15	0.002
Cephalosporin (Class II)	23	19	15	
Carbapenem (Class III)	23	19	15	
Aminoglycoside (Class IV)	18	17	14	
Quinolones (Class V)	16	17	13	
Glycycline (Class VI)	5	1	5	
Trimethoprim (Class VII)	18	16	10	

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A. baumannii, wound swab 11 (18.33%) becoming the second-highest source, and blood samples being the third-largest source 4 (6.67%). In other investigations, from one hundred and four clinical *A. baumannii* strains from 18 different hospital units by Li, Ding[17] findings that most specimens were detected from sputum with less number of isolates were detected from body fluids like urine, blood. This work included 60 *A. baumannii* isolates, of which 100% demonstrated resistance to carbapenems in screening assays. sCIM detected carbapenemase activity in 50.0% of the 60 *A. baumannii* isolate. Association between the Carbapenem test (sCIM) and antibiotic resistance was performed, and we found a significant association *P* value was 0.0001. According to the study bykhuntayaporn Howard, Creighton,[25] the simplified Carbapenem detection method (sCIM) found 70.0%, which was higher than our finding. Biofilm is an essential mechanism for bacterial protection when infecting an individual; it protects the bacteria against harsh individuals and antibiotics.[26] In our study, 57 isolates among 60 isolates were biofilm former, and the rest were non-biofilm former. The majority 23 (38.33%) were strong biofilm former and 19 (31.67) were moderate, and 15 (25.00) were weak biofilm former similar results were found in a study in Baghdad by al mousawi.[27] who found most of the isolates were able to form biofilm and majority of them were strong.

Most antibiotic-resistant isolates formed a strong biofilm than the susceptible isolate, and the *P* value was significant (0.002), which agreed with the study of Sung,[28] who found that the majority of resistance isolates have a more extraordinary ability than susceptible ones. However, the study findings of Qi, Li[9] displayed a reverse outcome that the antimicrobial sensitive samples formed stronger biofilms than insusceptible sample. The MICs and MBICs for Meropenem, Ceftazidime, and Ciprofloxacin were determined against ten distinct sequence types, all of which formed biofilms, seven of them were strong biofilm former, and the rest were moderate biofilm former.

Table 2 summarizes the antibiotic resistance characteristics of biofilms against plankton cells. In comparison to planktonic cells, the MBICs increased significantly. For isolate, 2^A the concentration rise varied from 8-512 µg/mL for Meropenem, 16 to 1024 µg/mL for Ceftazidime, and 2 to 64 µg/mL for Ciprofloxacin. We assessed the MBCs and MBECs of ten *A. baumannii* isolates after determining the MICs and MBICs. The data indicated that all ten isolates had raised their MBEC counts. Compared to planktonic cells (Table 3). When MBCs and MBECs were compared, the average fold increase for Meropenem, Ceftazidime, and Ciprofloxacin was 21, 18, and 24. The study of Yang Eze, El Zowalaty[29] agreed with our findings, and they discovered that all strains' MBICs for biofilms improved dramatically compared to their MICs for planktonic cells. Additionally, the MBCs and MBECs of these isolates were analyzed. Without a doubt, the MBECs were significantly greater than the MBCs in all strains examined. Ciprofloxacin concentrations up to 4096-fold higher and Ceftazidime and Meropenem concentrations up to 1024-fold higher were required to remove biofilms compared to planktonic cells. Additionally, MIC/MBIC values revealed that isolates' resistance to this antibiotic was significantly boosted during biofilm development, irrespective of whether the isolates were initially sensitive or resistant and strains of *A. baumannii* capable of generating biofilms cannot be destroyed using a similar concentration of antimicrobials as planktonic cells.[30]

Conclusion

Acinetobacter baumannii is a well-known pathogen in medical parts settings, notably in ICU section. This is because it is resistant to a practically broad range of currently available antimicrobials,

especially carbapenem. Antimicrobial insusceptibility and biofilm development by this bacterium constitute a significant source of concern for hospitals in general. In the current study, biofilm production was compared between susceptible and insusceptible isolates. It was discovered that most insusceptible samples have a greater capacity for biofilm development than sensitivite samples and biofilm-forming cells need more concentration of antibiotics than planktonic cells to be eradicated.

Ethical Considerations

The current study was ethically approved by the ethic committee of Pharmacy college, Hawler Medical University/ Kurdistan region-Iraq. All patients were informed about the purpose of the study.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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