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Evaluation of the Correlation between Biofilm Formation and Drug Resistance in Clinical Isolates of *Acinetobacter baumannii*

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Author's contribution

The sole author designed, analyzed and interpreted and prepared the manuscript.

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ABSTRACT

Aims: The aim of this study was to determine correlation between biofilm formation and drug resistance in clinical isolates of *Acinetobacter baumannii.*
Study Design: Bacteriological study.
Place and Duration of Study: Laboratory of Microbiology of BilecikSeyhEdebali University, in Turkey, between April 2019 and November 2019.
Methodology: Antibiotic susceptibility of the strains were determined using Kirby-Bauer disc diffusion method in accordance with the principles of Clinical and Laboratory Standards Institute (CLSI). Biofilm presence in *A. baumannii*was identified by the quantitative method. the isolates were incubated in nutrient agar and was prepared from fresh cultures in tubes containing glucose-Luria-Bertani (LB) medium. The *A. baumannii*(ATCC 19606) type strain was used for comparisons.
Results: In this study was determined the relationship between the biofilm production capacity of the *A. baumannii*bacteria and its antimicrobial resistance. According to the results obtained from our study, the highest resistance rate (%) was found ceftazidime and piperacillin (95 %) while the highest sensitivity was found colistin (96.6 %) and tigecycline (86.6 %) of the total 60 *Acinetobacter baumannii* isolates. In addition, the presence of biofilm in the bacteria was defined by quantitative method using microplate. In this study, biofilm was positive in 54 (90 %) isolates and it has been

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found 51 (85%) of the biofilm positive isolates to be resistant to piperacillin, ceftazidime, cefotaxime and meropenem.
Conclusion: As a result, there is a positive relationship between biofilm formation and antibiotic resistance in thesebacteria.

Keywords: A. baumannii; virulence; biofilm; antimicrobial resistance.

1. INTRODUCTION

Acinetobacter species are gram-negative, nonfermentative, immobile, aerobic microorganisms. These bacteria are classified within the *Moraxellaceae*family of *Pseudomonadales*order. *A. baumannii*, *Acinetobacter heamolyticus*and *Acinetobacter calcoeceticus*are important clinical species [1]. *A. baumannii*is an important nosocomial pathogen that has been proven to be responsible for various human infections with high morbidity and mortality. *Acinetobacter* ranks first among hospital infectious agents together with *Staphylococcus* and *Pseudomonas* species. *A. baumannii*with MDR can create infection in humans with the effect of weak virulence factors. However, they can also cause serious diseases such as hospital-acquired pneumonia, bacteremia, meningitis, urinary tract infections, kidney, heart and liver failure when host defencesare weakened [2,3,4]. *A. baumannii*is responsible for a significant portion of hospital infections worldwide and can often cause serious nosocomial infections in the ICU. This opportunistic pathogen can colonize patients in various clinics and lead to seriousinfections,septic shock and death [5,6]. *Acinetobacter* species can be found in animal and human flora in nature [7]. A. *baumannii*also cause health-threatening infections such as ventilator-related pneumonia, nervous system infections, skin and bone infections and urinary system and wound infections [8].

Microorganisms that are located on the surface and move freely can adhere to the surface. The ability of the bacteria to locate on specific surfaces when they find a suitable environment is called colonization [9]. Bacteria are able to survive by clinging to a surface and it is stated that these microorganisms and thus biofilms form a phenotype. These mucoid structures which are also called live layers, unlike planctonic organisms, have been named biofilm [10,11]. A biofilm is also defined as a group of microorganisms that can live inside a polymeric and gel-like layer produced by the bacteria colonies after adhering to a surface. This layer is known to be a polysaccharide-based network structure, called an extracellular polymeric structure, exopolysaccharide (EPS), and produced by the bacterial cells [12,13]. The main part of the biofilm is constituted of microorganisms and extracellular substances and the main EPS section has been reported to be (50-90 %) organic carbon. EPS has also been reported to contain a high proportion of water together with hydrophilic and hydrophobic parts [14]. Bacteria that adhere to the surface proliferate here and form microcolonies and then the biofilm layer. The extracellular matrix consisting of polysaccharide, protein, DNA and water enables the cells forming the biofilmtoad her eat this location. EPS production is necessary for the organism to adhere to the surface [15]. The biofilm layer can protect the microorganisms from nutritional deficiency, pH changes, toxins, and some disinfectants and antibiotics. The microorganisms can also proliferate on live tissue surfaces [16]. The layer has been observed to form in the patient's body and on materials such as prostheses, coronary stents and peritoneal dialysis catheters [10]. These formations can result in infections by acting as a focus of infection with the help of planktonic cells released from the microbial population when host defences are inadequate [17].

Biofilms are held responsible for dental and peridental diseases, and chronic infections such as chronic sinusitis and otitis media. The Centers for Disease Control and Prevention has recently reported that a biofilm formation rate of at least (65%) was present in infections seen in humans [18]. Cell motility, bacterial exopolysaccharide synthesis, flagella and pili play a role in the adherence of bacteria to surfaces [19]. The presence of plasmids in *A. baumannii* has also been reported to be associated with antibiotic resistance and these plasmids can be transferred to other pathogenic bacteria [20].

A. baumannii also make antibiotic treatment more difficult with their ability to gain resistance to antibiotics. These bacteria that are resistant to most of all known antibiotics have been found

and can survive in the hospital environment for a long time with this resistance profile [21]. This bacteria has a high capacity to develop resistance. The development of resistance in these bacteria is generally through the decrease and inactivation of the antimicrobial effect, weakening of the targets, and weakening of cellular functions through various enzymes. MDR and pandrug resistance concepts commonly used in recent years are clinically controversial [22,23]. A positive relationship has been found between biofilm formation and antibiotic resistance, and the antibiotic resistance rate was observed to be higher among biofilm- producing isolates in the *A. baumannii* study by they [24]. It is difficult to control hospital infections due to the role of patients and staff in accommodating and transferring drug-resistant bacteria. Paying attention to the body cleaning of the healthcare staffandd is infection of the hospital environment is extremely important [25]. Treatment opportunities are decreasing in number and becoming more difficult to find in both the general population and in patients hospitalized in ICU due to the antibiotic resistance developing in *A. baumannii*. We aimed to investigate the relationship between the gradually increasing antibiotic resistance and biofilm formation in clinical isolates of *A. baumannii* in this study.

2. MATERIALS AND METHODS

In this study, samples from patients with followed up in various clinics in Bilecikprovincein Turkey between April 2019 and November 2019 were investigated. These clinical samples taken from patients sent to the microbiology laboratory in an appropriate sterile container and were planted in sheep blood agar and MacConkey agar media and incubated at 37ºC for 18-24 hours. These samples were evaluated according to colony morphology, gram stain and biochemical test results. Gram-negative, non-hemolytic, oxidase-negative, catalase-positive colonies considered to be *A. baumannii* were taken into account. In addition to classic microbiological methods, The isolated strains were determined with API (Analytic Profile Index Test Strips) 20NE (bioMérieux, France) identification system. The multi test system (API) was developed primarily for the identification of Gram-negative Enteric Bacteria in clinical laboratories. 60 *A. baumannii* isolates have been identified from these clinical samples. These strains were stored in glycerol medium and were cultivated in nutrient agar for further analysis. Antibiotic susceptibility of the strains were determined using Kirby-Bauer disc diffusion method in accordance with the principles of Clinical and Laboratory Standards Institute (CLSI). For this purpose, 4 mm thick Mueller-Hinton agar medium was prepared in 9 cm diameter petri dishes. The colonies taken from a recently grown culture were inoculated into the Mueller-Hinton agar (Oxoid, UK) fluid medium and incubated for 4-5 hours. After incubation, the bacterium suspension adjusted to a value equivalent to 0.5 (~ 1.5 × 108 CFU/ml) Mc Farland standard turbidity was placed homogenously to the previously prepared agar medium. The medium surface was then left to dry. Then, antibiotic discs were taken with sterile forceps and placed on the medium surface 15 mm from the edges and 25-30 mm apart. After waiting for the antimicrobial factor to diffuse for 20 minutes, the plates were incubated at 35-37º C for 18-24 hours in an incubator. The inhibition zone diameters around these discs were measured with a millimeter ruler and compared with the zone table recommended by CLSI guidelines against a total of 19 antibiotics [26]. Resistance rates for colistin (CT), tigecycline (TGC), netilmicin (NET), gentamycine (CN), amikacin (AK), trimethroprim- sulfamethoxazole (SXT), tobramycin (TOB), ampicillin/Sulbactam (SAM), cefoperazone-Sulbactam (CES), piperacillin/Tazobactam (TPZ), chloramphenicol (C), ticarcillin/Clavulanic acid (TIM), imipenem (IPM), meropenem (MEM), ciprofloxacin (CIP), cefepime (FEP), cefotaxime (CTX), ceftazidime (CAZ), piperacillin (PRL) were investigated in a total of 60 *A. baumannii* isolates in thisstudy. Biofilm presence in *A. baumannii* was identified by the quantitative method. After the isolates were incubated in nutrient agar, 2 cc suspension was prepared from fresh cultures in tubes containing (1 %) glucose-Luria-Bertani (LB) medium with 0.5 McFarland (~ 1.5 × 108 CFU/ml) turbidity. Then, 200 microliters from the prepared suspension was dispensed to a 96-well microplate and the microplate was incubated in an aerobic environment at 37°C for 24 hours. After incubation, the microplate was washed three times with 0.2ml phosphate buffer edsaline(PBS;pH7.4)anddriedatroomtemperature .Then,200 microliters of (0.1 %) crystalline violet (Sigma-Aldrich, St. Louis, MO) solution was dispensed to all wells and kept at room temperature for 15 minutes. Biofilm formation could be macroscopically observed on the walls of the wells. A total of 200 microliters of (95%) ethanol was then added to the wells to dissolve the material. They were then analyzed in a spectrophotometer device using a wavelength of 570 nm (Microplate Reader; Molecular

Devices®). The biofilm experiments were performed three times for each strain and the mean absorbance value was identified.

Odc was identified by calculating the mean value and the standard deviation of the negative controls. Each experiment was performed three times and the mean optical density (OD) was calculated. Biofilm results were identified by taking the OD and Odc values into account and the biofilm results of *A. baumannii* strains at a wavelength of 570 nanometers after 24 hours of incubation were investigated.

The results obtained by analyzing the microplate wells in a spectrophotometer device were classified as weak positive (+), strong positive (++), stronger positive (+++) and negative (-) (Table 1) [27,28,29].

The statistical significance of the comparison of the biofilm formations and antibiotic susceptibilities of the strains included in the study was assessed with Fisher's exact chi-square test by using the statistical package for social science (SPSS) 21.0 software. p <0.05 was considered significant for statistical evaluation.

3. RESULTS

The distribution of the isolated *A. baumannii* isolates by clinical sample was as follows: 38 tracheal aspirates, 10 blood, 6 urine, 2 wound and 4 other samples (sputum, CSF, abscess, bronchoalveolar lavagefluid).Biofilm presence was identified following 24 hours of incubation using the crystal violet stain. The *A. baumannii*(ATCC 19606) type strain was used for comparisons. LB medium with (1 %) glucose where no bacterial culture was added was used for negative control. Strains with higher values than negative control were considered to be positive for biofilm formation. Values of OD ≤ 0.318 were accepted as negative,0.318 < OD ≤ 0.636 as weak positive, 0.636 ≤ OD < 1.272 as strong positive and 1.272 ≤ OD as stronger positive during biofilm determination (Table 1).

Number and percentages (%) of the biofilm results at a wavelength of 570 nanometers in a total of 60 *A. baumannii*isolates were identified. Accordingly, 37 (61.66 %) isolates were stronger positive (+++), 13 (21.67 %), strong positive (++), 4 (6.67 %) weak positive (+), and 6 (10 %) negative (Table 2).

The 60 A. baumannii isolates in the study were positive for biofilm in 54 (90 %) and negative in 6 (10 %). The highest biofilm positive strain value was in the tracheal aspirate samples at 34 (56.6 %). Biofilm was positive in 9 (15 %) blood samples, 5 (8.3 %) urine samples, 2 (3.3 %) wound samples and 4 (6.6 %) of the other samples (sputum, CSF, abscess, bronchoalveolar lavage fluid (Table 3).

Table 1. OD values used in the evaluation of biofilm measurements

OD	OD	biofilm value
OD ≤ ODc	OD ≤ 0.318	negative
ODc < OD ≤ 2 x ODc	0.318 < OD ≤ 0.636	(+) weakpositive
2x ODc ≤ OD<4x ODc	0.636 ≤ OD < 1.272	(++) strong positive
4x ODc ≤ OD	1.272 ≤ OD	(+++) stronger positive

OD: The mean value of the 3 microplate well measurements on the spectrophotometer. ODc: Mean OD of negative control + standard deviation of 3 x negative control

Table 2. Number and percentage (%) of biofilm results of *A. baumannii* strains

	Number of biofilm results and percentage (%) rates			
biofilm formation capacity	negative	+	++	+++
Number	6	4	13	37
%	10	6.67	21.67	61.66

Table 3. The distribution of biofilm presence according to clinical samples

Clinical Examples total		Tracheal Aspirate	Blood	Urine	Wound	Other
Total	60	38	10	6	2	4
Biofilm positive	54	34	9	5	2	4

Other samples: Sputum, CSF, Abscess, Bronchoalveolar lavage fluid

Table 4. The comparison of antibiotic resistance according to biofilm formation

Antibiotic	Biofilm	Antibiotic resistance						95% confidence interval	
		P	N	x^2	p	Odds	Odds Ratio	under	high
CT	P	1	53	3.68	0.192[1]	0.02	0.10	0.01	1.75
	N	1	5			0.20			
TGC	P	6	48	2.31	0.178[1]	0.13	0.26	0.04	1.67
	N	2	4			0.50			
NET	P	23	31	0.19	0.508[1]	0.74	1.48	0.25	8.81
	N	2	4			0.50			
CN	P	25	29	0.03	0.598[1]	0.86	0.86	0.16	4.66
	N	3	3			1.00			
AK	P	35	19	2.26	0.145[1]	1.84	3.68	0.62	22.00
	N	2	4			0.50			
SXT	P	37	17	2.94	0.106[1]	2.18	4.36	0.73	26.12
	N	2	4			0.50			
TOB	P	40	14	1.54	0.216[1]	2.86	2.86	0.52	15.83
	N	3	3			1.00			
SAM	P	47	7	10.40	0.008[1]	6.71	13.42	2.06	87.47
	N	2	4			0.50			
CES	P	48	6	6.41	0.038[1]	8.00	8.00	1.31	48.95
	N	3	3			1.00			
TPZ	P	50	4	9.51	0.017[1]	12.50	12.50	1.88	83.31
	N	3	3			1.00			
C	P	49	5	7.76	0.027[1]	9.80	9.80	1.55	62.08
	N	3	3			1.00			
TIM	P	50	4	4.03	0.105[1]	12.50	6.25	0.86	45.24
	N	4	2			2.00			
IPM	P	50	4	0.61	0.421[1]	12.50	2.50	0.23	26.91
	N	5	1			5.00			
MEM	P	51	3	5.45	0.074[1]	17.00	8.50	1.09	66.58
	N	4	2			2.00			
CIP	P	52	2	15.15	0.005[1]	26.00	26.00	3.08	219.75
	N	3	3			1.00			

Antibiotic	Biofilm	Antibiotic resistance						95% confidence interval	
		P	N	x^2	p	Odds	Odds Ratio	under	high
FEP	P	50	4	0.48	0.649[1]	12.50	0	0	
	N	6	0						
CTX	P	51	3	1.07	0.351[1]	17.00	3.40	0.30	39.1
	N	5	1			5.00			
CAZ	P	51	3	0.35	0.725[1]	17.00	0	0	
	N	6	0						
PRL	P	51	3	0.35	0.725[1]	17.00	0	0	
	N	6	0						
Total	P	767	259	16.37	0.000	2.96	2.23	1.50	3.32
	N	65	49			1.33			

P: positive, N: negative

The resistance of *A. baumannii*to certain antibiotics was also identified. The highest resistance rate was found for Ceftazidime and Piperacillin with 57 (95 %) isolates. Resistance was seen in 56 (93.4 %) isolatesforCefotaximeandCefepime;55(91.7%)for Ciprofloxacin,MeropenemandImipenem;54(90%) for Ticarcillin/Clavulanic acid; 52 (86.7 %) for Chloramphenicol, Piperacillin/Tazobactam; 51 (85 %) for Cefoperazone-Sulbactam; and 49 (81.7 %) for Ampicillin/Sulbactam. Besides, resistance was identified in 43(71.7%)isolatesforTobramycin,39(68.4%)forTri methroprim-sulfamethoxazole, 37(61.7%) for Amikacin,28(46.7%)forGentamycin,25(41.7%)for Netilmicin,8(13.4%)forTigecycline,and2(3.4%) for Colistin. The highest antimicrobial sensitivity was found for Tigecycline at (96.6 %) for Colistin at(86.6%). Biofilm was positive in 54 (90 %) of the 60 *A. baumannii*isolates. Piperacillin, Ceftazidime, Cefotaxime and Meropenem resistance was present in 51 biofilm-positive isolates and Cefepime, Ciprofloxacin, Imipenem and Ticarcillin/Clavulanic acid resistance in 50 biofilm-positive isolates. Besides, resistance to Chloramphenicol and Piperacillin/Tazobactam was found in 49, Cefoperazone-Sulbactam in 48, Ampicillin/Sulbactam in 47, Tobramycin in 40, Trimethroprim-sulfamethoxazole in 37, Amikacin in 35, Gentamycin in 25, Netilmicin in 23, Tigecycline in 6, and Colistin in 1 biofilm-positive isolate(Table 4).

Antibiotic resistance against SAM, CES, TPZ, C and CIP antibiotics showed a statistically significant difference compared to being biofilm positive ($p<0,05$) and in biofilm positive strains, antibiotic resistance was found to be significantly higher against SAM, CES, TPZ, C and CIP antibiotics. When the total numbers of antibiotic resistance were examined, it was found that there was a statistically significant difference according to the biofilm positive state ($p <0.05$). As a result, the rate of antibiotic resistance of biofilm positive in CT drug compared to antibiotic resistance of Biofilm negative is 9%. Antibiotic resistance of those positive for biofilm positive in CT drug is very low. Since the ratio is very low, X^2 value is not significant. In the SAM drug sample, there are 0.50 and 6.71 rates, respectively. Antibiotic resistance of biofilm positivein SAM drug is 13 times higher than those of biofilm negative. Antibiotic resistance of those who are positive for biofilm positivein SAM drug is very high. Since the ratio is very low, X^2 value is not significant (Table 4).

4. DISCUSSION

*A.baumannii*is opportunistic pathogenic microorganism and among the most commonly isolated infectious gram-negative bacteria. *A. baumannii*also causes serious infections in subjects with a failed or inadequate immune system and can especially cause severe infections in patients in ICU. *Acinetobacter* infections are mostly found in organ systems with a high water content (respiratory system, peritoneal fluid, urinary tract) [30]. Staying for extended periods in the hospital and care facilities creates a very suitable environment for infections caused by multi-drug resistant *A. baumannii* [31]. It has been shown that an amorphous exopolysaccharide is present around the cells and this structure is responsible for biofilm formation. Biofilm formation of certain microorganisms on abiotic surfaces was reported to facilitate their survival but the studies on biofilm formation in *A. baumannii*are insufficient [32]. Microorganisms could possibly be employing a gene transfer mechanism such as conjugation and transformation as many of them are present together in a biofilm [33]. *A. baumannii*can adhere to bronchial epithelial cells with the biofilm it produces. Biofilm formation also suggests epithelial cell compatibility [34]. In another study, they found the rate of biofilm (80 %) [35]. Biofilm-producing bacteria can colonize the respiratory system of the patient for longer than those that do not produce biofilm and a longer duration of colonization increases the colonization pressure. This can result in the development of multi-drug resistant *A. baumannii*in the patient. Studies on ventilator-related pneumonia have indicated that respiratory tract colonization and biofilm and pneumonia development have a microbial connection [36,37]. Biofilm-producing bacteria can colonize the patient's respiratory tract for a long period of time and create a risk of pneumonia. It has al so been shown that *A. baumannii*can be present on both abiotic and biotic surfaces. However, the structure called biofilm was reported to contribute to the final effect, together with S. aureus and Candida albicans [38].They reported that they successfully treated 16 ventilator-associated pneumonia or blood circulation infections together with the combination of colistin and rifampicin [39]. In this study, the distribution of isolated *A. baumannii*isolates according to the clinical samples was tracheal aspirate (63.3 %), blood (16.6 %), urine (10 %), wound (3.3 %) and other (6.6 %) (sputum, CSF, abscess,

bronchoalveolar lavage fluid) in our study. They studied trachea (23 %), pus (21 %), urine (17 %), burn (17 %) and wound (10 %) samples [30]. These results are similar to the their results. They reported that isolates were most commonly found in the trachea (25 %), followed by urine (19 %) [40]. Most isolates were from the trachea in our study. Among *A. baumannii*strains, drug resistance is common and resistance leads to higher morbidity and mortality besides limiting treatment options [6]. They reported that researchs has shown a high resistance to common antibiotics in A. baumannii infections in many countries and tigecycline and colistin are expected to become widespread if precautions are not taken [41].We detected resistance of *A. baumannii*bacteria to certain antibiotics in this study. The highestsensitivity rate was for colistin with (96.6 %), tigecycline with (86.6 %) and netilmicin with (58.3 %). A resistance rate of over (90 %) was found for piperacillin, ceftazidime, cefotaxime, cefepime, ciprofloxacin, meropenem, imipenem and ticarcillin/clavulanic acid. They found an alarming increase in colistin resistance over time (between 2010 and 2014) in some gram-negative bacteria (*A. baumannii, P. aeruginosa and K. pneumoniae*) and emphasized that this was a serious problem [42]. The most effective antibiotics in *A.baumannii*strains were colistin (99.5 %) and amikacin (21 %) [43]. In their study, colistin showed the highest sensitivity rate [4]. They found that all isolates were resistant to ciprofloxacin and imipenem (100 %) and piperacillin (99 %) [44]. They found a resistance rate of (20.9 %) for amikacin, (29.9%) fornetilmicin, (36.3%) formeropenem, (40.5%) forimipenem, (57.2%)forciprofloxacin,(66.4%) for piperacillin-tazobactam, (69.4 %) for ceftazidime, (69.7 %) for ampicillin-sulbactam, (71.1 %) for gentamycin, (84.6 %) for ceftriaxone and 84.6 % for aztreonam in 402 *A. baumannii*isolates. These rates are somewhat lower than our study results [45]. There is a parallel between our studies and our research. He found no increase in the resistance rate after biofilm formation. There was an inverse relationship between meropenem resistance and biofilm production in 116 isolates. These results are not compatible with ours [46].

*A. baumannii*strains were most commonly isolated from the respiratory tract samples (39 %) followed by blood samples (23%) in their study. The strain resistance rates were colistin 3 %, tobramycin 8 %, tigecycline 15 %, piperacillin-tazobactam (93%) and ciprofloxacin 92 %. These results are similar to ours but our tobramycin resistance rate was (28.3 %) [47]. *A. baumannii*isolates were reported to be resistant to imipenem (92%) and gentamicin (84 %) in their study [40]. We found a similar rate for imipenem resistance but the gentamycin resistance rate was higher. They found the resistance rate for ceftazidime and ceftriaxone as (99 %) and (97 %), respectively, in their study. Such a high resistance rate for third- generation cefalosporins may be associated with their very common use in the general population and the hospital [48].They found the highest resistance rates as (100 %) for colistin and (94%) for tigecycline in their study [49]. While they found resistance rates of (99 %) for colistin, (53%) for tigecycline and (85 %) for netilmicin, the highest resistant rates were to ampicillin/sulbactam and piperacillin-tazobactam [50]. In this study, biofilm was found positive in 54 (90%) of our 60 *A. baumannii*isolates. Resistance to Piperacillin, Ceftazidime, Cefotaxime and Meropenem was detected in 51 (85%) biofilm-positive isolates and to Cefepime, Ciprofloxacin, Imipenem and Ticarcillin/Clavulanic acid in 50(83%) biofilm-positive isolates. They found 100 % resistance to Amoxicillin, Ceftriaxone, Ceftazidime, Cefuroxime and Aztreonam in biofilm-forming *Acinetobacter* species. This results is parallel to ours [51]. They showed that the presence of biofilm in addition to the gelatinase and hemagglutination characteristics may play a role in the pathogenesis of *A. baumannii*bacteria. Besides, they found a biofilm production rate of (74 %) in *A. baumannii*isolates [52].They found a biofilm formation rate of (52.9%) in 17 *A. baumannii*isolates and the biofilm was strong in one, moderately strong in five and weak in two in the study they conducted in 2006 [53]. They reported a biofilm rate of (62.5%) and multi-drug resistance rate of (90.3%) in 72 isolates [54]. They reported high resistance rate to imipenemand piperacillin-tazobactam (89.7%), followed by piperacillin (87.1%), amikacin (79.4 %), aztreonam (74.3%) and ciprofloxacin (76.9%) inbiofilm-producing isolates in their study. They also found *Acinetobacter* isolates to produce a weak film in 12 (16%), moderately strong film in 9 (12%), and strong film in 30 (40 %)isolatesin the tests they performed with the tissue culture plate method [55]. Biofilm formation is the virulence factor of *A. baumannii* and may be associated with long-term hospitalization. Biofilm formation capacity may affect antibiotic sensitivity in clinical isolates. A study has shown (77%) higher biofilm formation capacity compared to standard *A. baumannii* (ATCC 19606) in 100 clinical isolates [56].

In another study, multi-drug resistance was observed in both biofilm positive and negative strains. Biofilms production was detected in (61.7 %) of the isolates. Isolates that were not burned (59.5%) were found to produce more biofilm than burned strains (40.5%). Burned strains produced significantly higher amounts of ESBL, but biofilm production was reported not to be associated with antibiotic resistance or ESBL production [57]. They reported that 24 (48 %) of their 49 isolates formed higher amounts of biofilm than the standard *A. baumannii*strain. Antibiotic resistance was similar between isolates forming and not forming biofilm. A total of 38 isolates (77 %) were collected from the patients hospitalized in the ICU and the strains were found to resistant to carbapenem. Besides, resistance to aminoglycosides and tigecycline was found at higher rates in isolates not forming biofilm than biofilm-forming ones [58]. The (48%) biofilm formation rate observed in 24 *A. baumannii*isolates was higher than for the standard *A. baumannii*strain (ATCC 19606). Besides, the colonization duration for biofilm-producing isolates was found to be longer than for the isolates not producing biofilm [58]. The biofilm formation rate of *A. baumannii*clinical isolates (48 %) was found to be higher than in *A. baumannii*ATCC 19606. Besides, factors such as long-term hospitalization in ICU and intensive antibiotic treatment were shown to carry a high risk of bacterial colonization [59]. The presence of a biofilm is thought to be an important feature in the development of *A. baumannii*infections. They observed a biofilm formation rate of (63 %) in 92 clinical isolates in their study. Although limited in number, the results demonstrated that the biofilm also plays a role in the pathogenesis of some environmental *A. baumannii*infections [60]. Biofilm formation is known to have an effect on antibacterial resistance and the ability to survive for extended periods in the external environment in *A. baumannii*. Biofilm-forming bacteria have been shown to have morphological, metabolic and physiological differences [61].The elimination of the biofilms formed by the bacterial population is quite important in decreasing the gene transfer rate. The development of strains MDR could also be decreased in this manner [62].

5. CONCLUSION

*A. baumannii*bacteria increase their colonization and persistence in the clinical environment through biofilm production together with their resistance against unfavorable conditions. In the results of the research, it is understood that the formation of biofilm in different ratios plays an important role in drug resistance. Therefore, further research on the role of biofilms and their role in creating life-threatening infections should bedone.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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