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Antibiogram and biofilm-forming capacity of *Acinetobacter baumannii* isolated from Anbar hospitals

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***Abstract*---**In hospitals, *Acinetobacter baumannii* is considered one of the major pathogens due to its antibiotic and antiseptic resistance capacity thus it persists in a stressed environment and leads to nosocomial infections. Its persistence is influenced by biofilm development. This study looked at 60 *A. baumannii* isolates from four Iraqi hospitals. A significant distribution of biofilm-forming characteristics was identified among *A. baumannii* isolates obtained from several hospitals in this research. They determined the antibiotic sensitivity pattern of *A. baumannii* towards 13 different antibiotics (Imipenem, Meropenem, Colistin Sulfate, Doripenem, Ceftazidime, Cefepime, Cefiderocol, Amikacin, Levofloxacin, Tobramycin, Ciprofloxacin, Gentamicin, and Piperacillin). The frequency of multidrug resistance (MDR) was found to be 58.3 % in the tested strains, while the frequency of extensively drug resistance (XDR) was found to be 31.6 %. 100% of the clinical isolates could form biofilms, with 6.6 % having weak biofilm formation ability, 86.6 % having moderate biofilm formation ability, and 6.6 %having high biofilm formation capacity. The presence of the virulence genes *csuE*, *csgA*, and *cvaC* in the bacteria studied was detected by PCR. The *16 Sr RNA* gene was used to identify all *A. baumannii* isolates, and the prevalence of the *csuE*, *csgA*, and *cvaC* genes was 100%, 85%, and 30%, respectively, among the isolated strains. We examine the biofilm development in *A. baumannii*, as well as the links between biofilm formation, pathogenicity, and antimicrobial resistance. In conclusion, *A. baumannii* has been shown to be resistant to practically all presently available medicines, making it a "superbug" in hospitals, particularly in intensive care units (ICU).

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1. Introduction

A. baumannii is a G-ve common bacterium that is one of the top seven most pathogenic organisms in the healthcare system [1]. Due to several virulence characteristics, such as the high prevalence of resistance of drug and the development of biofilm, it usually results in nosocomial pathogens [2]. Antimicrobial resistance is a frequent characteristic of *A. baumannii* strains. Many strains isolates are resistant to a wide variety of therapeutically useful antibiotics, including *β*-lactams (including carbapenems), aminoglycosides, fluoroquinolones, and Lipopeptides, and eventually become MDR or XDR pathogens. [3]. This makes it extremely challenging to provide appropriate treatment choices for *A. baumannii* strains.

MDR *A. baumannii* isolates are a major source of infection in hospitals across the world, especially in Europe, America, and some other parts of the world [4,5]. MDR strains have been found in the majority of persons in ICU, and they've been associated to greater patient death and isolated survival rates in hospitals [6]. The formation and growth of MDR bacteria is hypothesized to be aided by horizontal transfer of antibiotic resistance genes. Highly resistant isolate infections, such as MDR, XDR (extensively drug-resistant), and PDR (pan drug-resistant), can be difficult to treat [7]. Because of the limited treatment options available, the rise of carbapenem resistant *A. baumannii* (CRAB) has become a severe public health problem during the last decade [8].

A biofilm is a collection of microorganisms that are contained in a matrix. This matrix works as a collective collaboration that protects the microbe and improves its resistance to a range of drugs and stressful situations [2]. The capacity to form biofilms is critical for *A. baumannii's* survival [9]. It also makes it easy for this bacterium to spread across hospital settings, contaminating a variety of biotic and abiotic surfaces such as cerebrospinal fluid shunts, and vascular catheters, Foley catheters [10,11]. Biomedical researchers are very interested in the association between biofilm production and antibiotic resistance. Over the last two decades, the nature of this relationship has been disputed and changeable. Some research suggested that *Acinetobacter* spp. abilities to form biofilms was determined by acquired resistance elements [3,7]. Low dosages of some antibiotics, for example, might cause biofilm development. Furthermore, MDR/XDR *A. baumannii* strains demonstrated a better potential to build biofilm than susceptible bacteria [9,12]. *A. baumannii's* ability to form biofilm on both biotic and abiotic surfaces has been demonstrated, and it plays a key role in nosocomial and recurring infections. [13]. The outer membrane protein A (OmpA), biofilm-associated protein (Bap), chaperon – usher pilus (*csu*), betalactamas blaPER, blaNDM, and the *16SrRNA* gene, which are utilized to identify the species, are all involved in A. baumannii biofilm production [14]. The presence of virulence factors (VF) in *A. baumannii* leads to disease [15]. Some of the most important VF of A. baumannii strains are curli fibers (*csgA*), colicin V production (*cvaC*), and siderophores such aerobactin (*iutA*), and cytotoxic necrotizing factor (*cnf*) [15,16]. Major epidemiological themes include characterization of latent virulence genes, antibiotic resistance, and molecular detection of this bacterium's carbapenems on abiotic and biotic surfaces in hospital settings. The aim was to determine antibiotic resistance, detect biofilm formation, and genetically identify virulence genes in *A. baumannii* isolated from hospital environments and clinical specimens in Anbar hospitals.

2. Materials and methods

2.1Collection of bacterial species

Between January 2021 to June 2021, this research was carried out at Iraqi government hospitals in Anbar. A total of 2450 bacterial isolates were obtained, with 1880 environmental isolates collected from various hospital settings such as waiting room doors, door knobs, patient beds, pillows, mattresses, walls, laboratory tables, basins, floors, and toilets of hospital patients. Patients' urine (n = 115), sputum (n = 41), burns (n = 119), blood (n = 93), wounds (n = 126), and otitis (n = 48) were among the 570 clinical isolates studied. The samples were taken in a sterile, leak-proof container that was labeled appropriately and processed right away.

2.2 Identification of Isolates and Sample Processing

Nutritional agar, nutrient broth, blood agar, MacConkey agar, Acinetobacter CHROMagar (CHA) medium, and brain heart infusion (BHI) medium were used to semi-quantitatively cultivate all bacterial isolates. The cultural media was provided by India's HiMedia Laboratories. Normal microbiological procedures such as colony morphology, in addition, number of biochemical tests (fermentative, catalase, oxidase, and motility) were used to confirm *Acinetobacter* sp. [13]. The VITEK® 2 technology was used to identify the isolates in order to confirm and diagnose *Acinetobacter baumannii.*

2.3 Antimicrobial susceptibility testing

Antibiotic susceptibility was tested on Muller Hinton agar (MHA) using the simplified disc [17] diffusion technique, according to the Clinical and Laboratory Standards Institute (CLSI) 2021 criteria [18]. Antibiotic disks were employed in this experiment; Doripenem (10 µg), Meropenem (10 µg), Imipenem (10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Cefiderocol (30 µg), Colistin Sulfate (10 µg) , Amikacin (30 µg), Levofloxacin (5 µg), Tobramycin (10 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg), and Piperacillin (100 µg). If a strain proved resistant to at least one agent from three or more antimicrobial classes, it was classified as multidrug-resistant (MDR) [19]. The lot number, manufacturer, expiration date, and correct storage condition of antibiotic discs were all evaluated using CLSI standards. To avoid contamination from a remote location, an aseptic environment was maintained throughout the collecting and processing of the specimens. By incubating a sample in the medium for 24 hours at 37°C, the culture and biochemical media were ensured.

2.4 Biofilm formation Assay

Biofilm development was assessed using crystal violet staining, as described in earlier works [20,21]. Next, bacterial suspension was injected into 96-well plates and incubated for up to 72 hours at 37°C. The plates were then gently washed three times with phosphate-buffered saline (PBS) and stained for 15 minutes with 1% crystal violet. The stains were solubilized with 95% ethanol after three items of washing with PBS, and the OD_{630} of the supernatant in each well was measured. The data was examined using the criteria outlined previously [12].

2.5. DNA Extraction and Virulence Genes Detection Using PCR

The *16S rRNA* gene was utilized to confirm all *A. baumannii* isolates, and virulence genes (*csuE*, *csgA*, and *cvaC*) were discovered by extracting bacterial DNA from each isolate using the Geneaid kit process. For the identification of (*csuE*, *csgA*, and *cvaC*) genes, A temperature gradient thermal cycler was used to do PCR amplification using a specific forward and reverse primer. The primer was then diluted in nuclease-free water to a concentrated action of 10 pm (Table 1). The PCR was done in a total volume of 20 µL, with 2 µL of template DNA, 1 µL of each primer, 4 µL of the master mixture, and 12 µL of nuclease-free water and Taq-polymerase enzyme. For *csuE*, *csgA*, and *cvaC* the annealing temperature was 58°C, 51°C, and 57°C respectively. (Table 1) illustrates the PCR condition.

Table1: Primer sequences and conditions for PCR amplification of species-specific virulence genes in *A. baumannii*

Virulence factors	Target genes	Primer names	Sequence (5′ to 3′)	Annealing T (°C)	Amplicon (bp)
	16srRNA	*16srRNA-* *16srRNA-*	CAGCTCGTGTCGTGAGATGT CGTAAGGGCCATGATGACTT	58	150
pili system	*csuE*	*csuE* -f *csuE* -r	CCAGACTGCCGAATTTTAGC TATTTCCCGTTTGCGACTTC	56	187
curli fibers	*csgA*	*csgA* -f *csgA* -r	ACTCTGACTTGACTATTACC AGATGCAGTCTGGTCAAC	51	200
colicin V	*cvaC*	*cvaC* -f *cvaC* -r	TTCACCGAGAAGCCAAACTT GCCACTGGCAGGATAAAGA	57	175

2.6 Statistical Analysis

SPSS, version 28, Armonk, New York, was used to conduct statistical analyses of the data. The frequencies calculations were performed for all parameters.

3. Results:

3.1. Pattern of Growth.

Of 2450 samples tested, 827 (33.75%) showed strong growth (i.e., >105 CFU/mL); of these, 60 (7.25%) tested positive for *A. baumannii*. Clinical samples showed the most significant increase (445), while environmental samples showed the least (382). Similarly, environmental samples had the lowest quantities of *A. baumannii* (28) whereas clinical samples, such as urine, blood, wounds, burns, sputum, and stool samples, had the highest amounts (32). The VITEK® 2 system was used to identify the isolates collected from MacConkey agar. On MacConkey agar, *A. baumannii* showed as tiny, pale, lactose non fermenter colonies [13].

3.2. Distribution of MDR & XDR A. baumannii.

The most *A. baumannii* isolates were detected in 32 (53.3%) clinical patients, and burn samples were found to be greater than those found in the patients. Burns accounted for 13 (40.6 %), wounds and urine for 6 (18.75 %), blood and sputum for 3 (9.375 %), and stool for one (3.12 %). The prevalence of MDR *A. baumannii* was higher in clinical specimens. The antibiotic susceptibility profiles of *A. baumannii* isolates are shown in Table 2. MDR isolates were discovered in 35 (58.3%) of the 60 *A. baumannii* isolates, whereas XDR isolates were found in 19 (31.6%).

Table 2: Distribution of MDR & XDR *A. baumannii* in the resources

Attributes	Sample types	A. baumannii isolates, *n* (%)	MDR, *n* (%)	XDR, *n* (%)
Environmental Samples.	door knobs	7 (11.6%)	5 (71.4%)	2 (28.5%)
	floors	6 (10%)	3 (50%)	2 (33.3%)
	pillows	5 (8.3%)	2 (40%)	2 (40%)
	beds	6 (10%)	3 (50%)	2 (33.3%)
	tables	4 (6.6%)	3 (75%)	1 (25%)
Clinical specimens	Urine	6 (18.75 %)	4 (66.6%)	2 (33.3%)
	Blood	3 (9.375 %)	2 (66.6%)	1 (33.3%)
	sputum	3 (9.375 %)	2 (66.6%)	1 (33.3%)
	Burn	13 (40.6 %)	7 (53.8%)	4 (30.7%)
	Wound	6 (18.75 %)	3 (50 %)	2 (33.3%)
	stool	1(3.12 %)	1(100%)	0(0.00%)

3.3. Susceptibility to Antimicrobial Agents.

Patterns of antibiotic susceptibility of *A. baumannii* isolates revealed that Amikacin, meropenem, and imipenem were shown to be the most effective antibiotics in vitro, with sensitivities of 44(73.3 %), 41(68.3 %), and 36(60 %), respectively. Colistin Sulfate (CS) resistance was found in the majority of *A. baumannii* isolates 58 (96.6 %). Table 3, shows the antibiotic susceptibility profiles of *A. baumannii* isolates distributed between sensitive(S), intermediate(I), and resistant(R) [22].

(Table 3). *A. baumannii* antibiotic susceptibility pattern

Antibiotics	Antibiotic class	Sensitive, *n* (%)	Intermediat *n* (%)	Resistant, *n* (%)
Doripenem		20(33.3%)	9(15%)	31(51.6%)
Meropenem	Carbapenems	41(68.3%)	0(0%)	19(31.6%)
Imipenem		36(60%)	0(0%)	24(40%)
Ceftazidime		9(15%)	2(3.3%)	49(81.6%)
Cefepime	Cephalosporins	6(10%)	2(3%)	52(86.6%)
Cefiderocol		0(0%)	3(5%)	57(95%)

Gentamicin		28(46.6%)	1(1.6%)	31(51.6%)
Amikacin	Aminoglycosides	44(73.3%)	0(0%)	16(26.6%)
Tobramycin		24(40%)	1(1.6%)	35(58.3%)
Ciprofloxacin	Fluoroquinolones	13(21.6%)	4(6.6%)	43(71.6%)
Levofloxacin		21(35%)	2(3.3%)	37(61.6%)
Piperacillin	Penicillins	6(10%)	3(5%)	51(85%)
Colistin Sulfate	Lipopeptides	0(0%)	2(3.3%)	58(96.6%)

3.4. Biofilm formation

One of *A. baumannii's* most important virulence-related traits is its ability to build biofilms. 100% of the 60 isolates tested were capable of forming biofilms. 6.6% have a low ability to produce biofilms, 86.6 % have a moderate ability to form biofilms, and 6.6% have a strong ability to form biofilms. Table 4 reveals that *A. baumannii* isolates have the potential to produce biofilms. The biofilm-related gene *csuE* was found in all 60 *A. baumannii* isolates. The capacity to create biofilms (classified as weak, moderate, or strong) varied across the isolates, with the majority (n = 52/60) creating moderate biofilms, a minority (n = 4/60) forming weak biofilms, and only a small minority (n = 4/60) forming strong biofilms (figure 1). The ICU provided two of the four samples that generated high biofilms; nevertheless, no link was observed between the isolates' biofilm-forming potential and the ward where they were collected. Only 2 (or 7%) of the 28 *A. baumannii* isolates obtained from various settings such as ICUs showed high biofilm-forming capability, while 24 (or 85.7%) had intermediate biofilm-forming capacity.

(Table 4). The ability of *A. baumannii* isolates to form biofilms and their sources

Isolates code	Source	OD_{630}	Biofilm intensity	Isolates code	Source	OD_{630}	Biofilm intensity
A1	1	0.153	moderate	A31	3	0.1545	moderate
A2	4	0.153	moderate	A32	5	0.08505	weak
A3	3	0.1515	moderate	A33	1	0.149	moderate
A4	4	0.1945	moderate	A34	1	0.134	moderate
A5	1	0.1385	moderate	A35	1	0.2305	strong
A6	2	0.1205	moderate	A36	2	0.14	moderate
A7	2	0.152	moderate	A37	4	0.1815	moderate
A8	1	0.2025	moderate	A38	1	0.177	moderate
A9	4	0.195	moderate	A39	1	0.176	moderate
A10	1	0.126	moderate	A40	1	0.155	moderate
A11	1	0.1615	moderate	A41	1	0.1665	moderate
A12	4	0.155	moderate	A42	2	0.156	moderate
A13	3	0.148	moderate	A43	4	0.3445	strong
A14	1	0.236	strong	A44	6	0.1705	moderate
A15	4	0.201	moderate	A45	7	0.1545	moderate
A16	1	0.197	moderate	A46	2	0.13	moderate
A17	4	0.148	moderate	A47	1	0.076	weak
A18	1	0.2135	moderate	A48	4	0.137	moderate
A19	2	0.186	moderate	A49	1	0.09	weak

A20	4	0.192	moderate	A50	1	0.1285	moderate
A21	1	0.1875	moderate	A51	1	0.11	moderate
A22	5	0.20895	moderate	A52	6	0.109	weak
A23	3	0.291	strong	A53	1	0.1645	moderate
A24	1	0.205	moderate	A54	1	0.189	moderate
A25	1	0.19	moderate	A55	5	0.132	moderate
A26	1	0.1215	moderate	A56	3	0.094	moderate
A27	1	0.1785	moderate	A57	1	0.135	moderate
A28	4	0.1925	moderate	A58	4	0.1475	moderate
A29	3	0.18	moderate	A59	4	0.17	moderate
A30	6	0.148	moderate	A60	1	0.1475	moderate
NC		0.0551		NC		0.0551	

NC= Negative Control 1=(E.S.) 2=Wound 3=Urine 4= Burn 5=Blood 6=Sputum 7= Stool

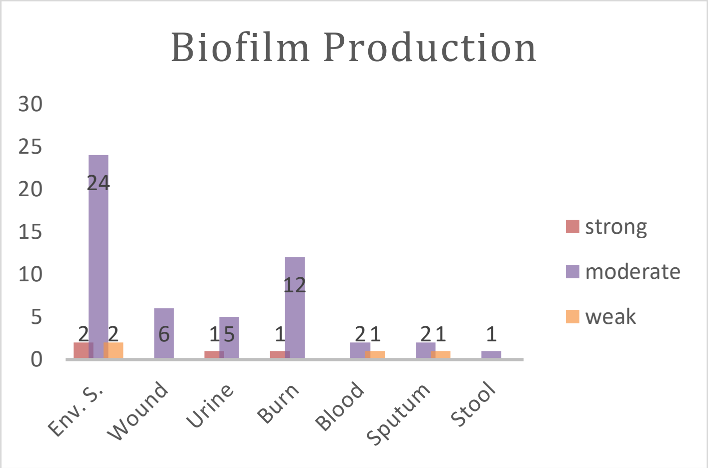
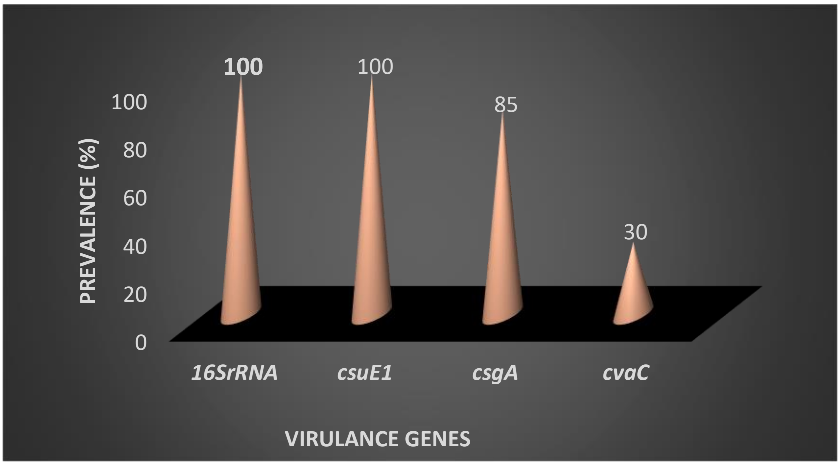


Figure 1: number of *A. baumannii* isolates and their capacity of Biofilm forming

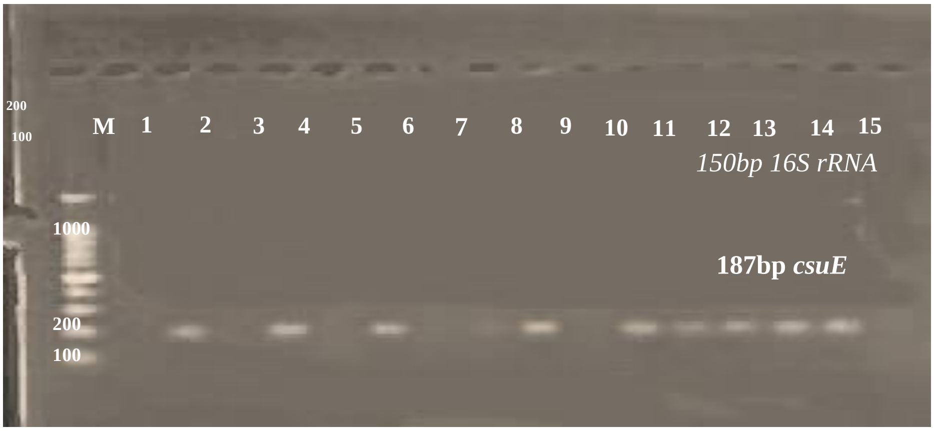
3.5. Virulence Genes of A. baumannii

After *16SrRNA* gene confirmation, the virulence gene *csuE* was found in all 60 (100%) *A. baumannii* isolates as shown in figure 2, whereas the genes *csgA* and *cvaC* were found in 51 (85%) and 18 (30%), respectively. Figures 3,4,5 and 6 show PCR synthesis of the *16SrRNA, csuE, csgA*, and *cvaC* genes, respectively.

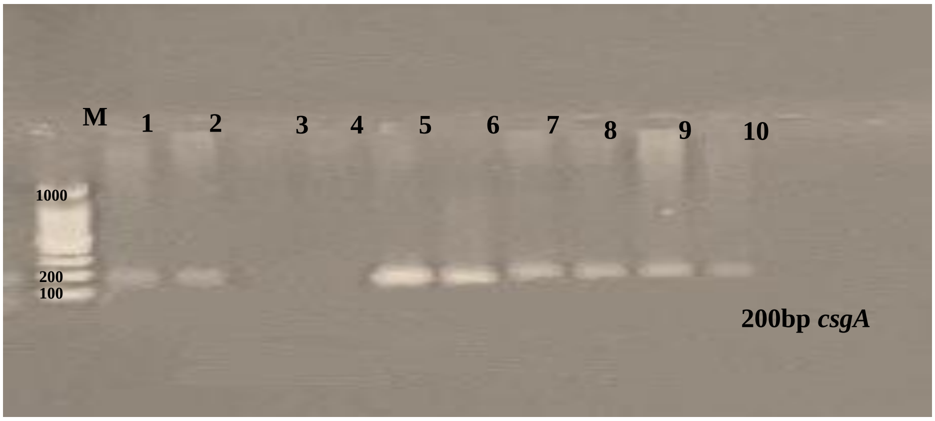


(Figure 2): Prevalence of virulence genes among all confirmed *A. baumannii* isolates

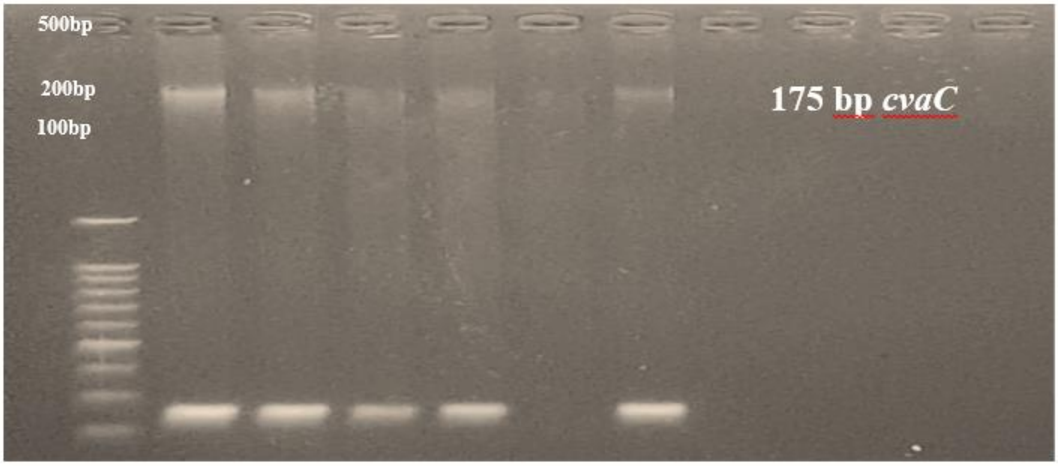
(Figure 3). Gel electrophoresis of amplified PCR result for identification of *A. baumannii 16srRNA* gene (150bp) on 1% agarose (70 min at 90 volt), M: marker DNA ladder 100bp.



(Figure 4). Amplification of *csuE* gene of *A. baumannii* by PCR with product 187bp. Lane (M) DNA marker (100 bp), all Lane (2,4,6,9,7,8,9,11,12,13,14 and 15) represent positive isolates for *csuE* gene, and Lanes (1,3,5,7,8 and 10) represents negative isolates for *csuE* gene.



(Figure 5). Amplification of *csgA* gene of *A. baumannii* by PCR with product 200bp. Lane (M) DNA marker (100 bp), all Lane (1,2,5,6,7,8,9 and 10) represent positive isolates for *csgA* gene, and Lanes (3 and 4) represents negative isolates for *csgA* gene.



(Figure 6). Amplification of *cvaC* gene of *A. baumannii* by PCR with product 175bp. The lane (M) DNA marker (100 bp), all Lane (1,2,3,4, and 6) represent positive isolates for *cvaC* gene, and Lane (5) represents negative isolates for *cvaC* gene.

4. Discussion

A. baumannii is an opportunistic pathogen that may infect the skin, mouth, respiratory tract, conjunctiva, urinary tract, and gastrointestinal tract. Because of this pathogen's capacity to live in the environment for a long period, nosocomial infections are usually transferred directly from healthcare staff or via environmental surfaces to patients. *A. baumannii* colonization has been recorded often in intensive care units and surgical wards, where the bulk of nosocomial pathogens have occurred [23]. *A. baumannii* isolates were shown to be resistant to medicines widely used to treat *A. baumannii* in this investigation. Furthermore, 31.6% of isolates were XDR, whereas 58.3% were MDR. Antibiogram testing found that the isolates were resistant to over 90% of antibiotics and were not susceptible to colistin, with all 60 isolates being resistant to the antibiotic. Because there are no new treatments or alternatives to existing drugs for this illness, as well as no vaccination, the only approach to eradicate the bacteria's consequences is to restrict its spread [24]. In current study, 96.6 % of *A. baumannii* were colistin-resistant, compared to zero % [25], 6 % [26], and 7.6 % [27] in previous studies. Despite the fact that colistin resistance was discovered in our investigation, this medicine remains the most effective and best treatment choice for this illness. The study found that 44 (73.3%), 41 (68.3%), and 36 (60%) of the 60 clinical strains were sensitive to Amikacin, Meropenem, and Imipenem, respectively. In contrast, previous studies showed high resistance to Imipenem and Meropenem, but none were resistant to colistin [24]. Despite having greater resistance rates to ciprofloxacin, ceftazidime, cefepime, cefiderocol, norfloxacin, and levofloxacin, the isolates exhibited a higher resistance rate to ceftazidime, cefepime (table 3). Although the fact that biofilm development increases antibiotic resistance, combination treatment may be useful in treating biofilm-related diseases. The stability and effectiveness of colistin, tigecycline, and levofloxacin as lock solutions against biofilm-embedded *A. baumannii* strains were tested in vitro, either alone or in combination with clarithromycin and/or heparin [27,28].

The capacity of *A. baumannii* to produce biofilms in a four-step process, namely, attachment of bacteria to the surface, creation of micro-colonies, maturation of biofilms, and ultimately dissociation leading to additional colonization, is a significant virulence factor. The capacity to generate biofilm is one of the most critical aspects in *A. baumannii* pathogenicity among many virulence factors [29]. The current study found that 100% of isolates could form biofilms (to varying degrees), which was correlated with the presence of the *csuE* gene in 100% of our isolates, indicating that this gene plays an important role in biofilm production and produces strains that are more resistant to antimicrobial agents when they have a biofilm layer Monfared [23] discovered that only 72.9 % of isolates could form biofilms, which was lower than our results. In a research done in Hamadan [8] nearly all isolates were able to produce biofilms. Furthermore, in a study conducted in India, all isolates were able to generate biofilms [29]. The frequency of genes linked to biofilm development was comparable to prior studies [8, 30, 31]. The *csuE* gene is found in the majority of *Acinetobacter* isolates [23].

The production of biofilm in *A. baumannii* is mediated by cell-to-cell adhesion through curli fibers, which confers virulence and pathogenicity. Large differences in the ability of clinical *A. baumannii* isolates to produce biofilms have previously been documented [32,33], and the findings of our investigation confirmed this. Despite the fact that biofilm is identified as a role in *A. baumannii* pathogenicity and its ability to remain in the harsh hospital environment, a recent research found that epidemic or outbreak *A. baumannii* isolates had much lower biofilm forming capacity than sporadic isolates [33]. As a result, the authors concluded that biofilm formation may not be a significant factor in *A. baumannii* epidemic spread [33].

As a result, the goal of this work is to molecularly characterize the *csgA* gene and determine its frequency in *A. baumannii* strains. To combat the drug-resistant strains of *A. baumannii*, preventing the formation of biofilms would be an alternative strategy. In our research discovered that the *csgA* gene is present in around 85% of people. Earlier investigations show that pathogenic *csgA* from *A. baumannii* present in 63.63 % [34] and 66.7% [35]. According to another study, the *csgA* gene is found with about 70% [36]. According to Daryanavard *et al.*, the prevalence of the same is around 55 % [36]. Another study in Iran found that 12.39 % have the *csgA* gene, which is lower than our findings [37]. In contrast, a research found no evidence of *csgA* in multidrug-resistant strains, highlighting the importance of additional genes involved in the same process [38]. As a result, it's clear that the expression of the *csgA* gene linked to biofilm formation might fluctuate, necessitating frequent monitoring to better investigate its powerful virulence roles. In a few of our *A. baumannii* clinical infections, the *cvaC* virulence gene was discovered. Our data revealed that the *cvaC* gene was discovered in 18 (30%) of isolates, which is higher than finding of AL-Kazmi *et al.* [35] just 9.5%. According to previous studies, the total prevalence of the *cvaC* virulence gene is 3.4% [16], which is lower than our findings [35]. Momtaz *et al.* [39] found that the prevalence of the *cvaC* virulence gene was 21.48 % in Iran, which was slightly comparable to our findings.

The primary features should be studied in order to provide relevant and practical strategies that can be used as a strategic plan for infection control committees in hospitals, particularly in ICUs, to effectively control infection. Physicians should utilize this knowledge to develop better medications, prevent antibiotic resistance, lower medical expenditures, and lower death rates. The focus of this research was to look at a variety of factors, including the ability to form biofilms, the frequency of biofilm-related genes, the synthesis of toxins, and the involvement of the *csuE*, *csgA*, and *cvaC* genes in cell adhesion and pili formation, respectively. Biofilm development and antimicrobial drug resistance were also investigated.

5. Conclusion

In our research, the virulence genes *csuE* and *csgA* were found to be the most frequently. This study also discovered antibiotic resistance indicators in *A. baumannii* from hospital settings. The use of antibiotics based on the results of disc diffusion studies can help to reduce the prevalence of resistance [40]. Biofilm production is connected to certain resistance gene determinants, making agreement on the links between biofilm formation and environmental triggers, antibiotic resistance, virulence mechanisms, and the contributions of bacterial isolates' origins challenging. As a conclusion, future study should concentrate on the influence of bacterial sources, environmental variables, antibiotic resistance, and virulence determinants on the development of biofilms.

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