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Research Article / Araştırma Makalesi

GENOTYPING OF QUORUM SENSING AND BIOFILM VIRULENCE FACTORS GENES IN LOCAL ISOLATES OF ACINETOBACTER BAUMANNII

YEREL ACİNETOBACTER BAUMANNİİ İZOLATLARINDA ÇOĞUNLUĞU ALGILAMA VE BİYOFİLM İLE İLİŞKİLİ VİRÜLANS GENLERİNİN GENOTİPLENDİRİLMESİ

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ABSTRACT

Objective: *The current investigation focused on Acinetobacter baumannii (A. baumanni), due to its growing significance as a hospital infection-causing pathogen and its resistance to several medications.*
Material and Method: *Sixty-five isolates of A. baumannii were isolated from wound samples of patients admitted to different hospitals in Baghdad between January and April of 2023. Two types of methods were used in the detection of biofilm formation: the first one was Congo red agar method and the second one was microtiter plate method. Genotypic detection of various virulence factors associated with A. baumannii was performed using monoplex, multiplex, and ERIC-PCR.*
Result and Discussion: *To use the PCR method to examine virulence genes like biofilm and quorum sensing (QS). The genes responsible for biofilm formation were identified by the PCR method, followed by ompA 63/65 (96.92%) and bap 48/65 (73.84%), whereas the genes responsible for chemical signals were found to be rhlI 43/65 (66.15%), LasI 58/65 (89.23%), LasR 56/65 (86.15%), and rhlR was 39/65 (60%) after quorum sensing (QS) system genes. ERIC-DNA fingerprinting's phylogenetic analysis illustrated the variety of all isolates by utilizing the Dice coefficient and the UPGM of phylogenetic analysis. Based on statistical analysis, the ERIC-PCR genotyping method correlation coefficient with the study virulence genes, antibiotic sensitivity test, and other variables of virulence was significant at $p < 0.05$.*
Keywords: *Acinetobacter baumannii, biofilm formation, multidrug resistance, virulence factors*

ÖZ

Amaç: *Mevcut araştırma, hastane enfeksiyonuna neden olan patojen olarak artan önemi ve çeşitli ilaçlara karşı direnci nedeniyle Acinetobacter baumannii (A. baumanni) üzerine odaklanmıştır.*
Gereç ve Yöntem: *Ocak-Nisan 2023 tarihleri arasında Bağdat'taki farklı hastanelere başvuran hastaların yara örneklerinden altmış beş A. baumannii izole edilmiştir. Biyofilm oluşumunun tespitinde iki tür yöntem kullanılmıştır: Birincis Kongo kırmızısı agar yöntemi ve ikincisi mikrotitre plaka yöntemi. A. baumannii' ye ait ait çeşitli virülans faktörlerinin genotipik tespiti monopleks, multipleks ve ERIC-PCR kullanılarak gerçekleştirilmiştir.*

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Sonuç ve Tartışma: *PCR yöntemiyle biyofilm ve çoğunluğu algılama (quorum sensing; QS) ilişkili virülans genleri incelenmiştir. Biyofilm oluşumundan sorumlu genlerden ompA 63/65 (%96.92) ve bap 48/65 (%73.84) oranında tespit edilmiştir. Kimyasal sinyal mekanizmalarından sorumlu çoğunluğu algılama genlerinde ise rhlI 43/65 (%66.15), LasI 58/65 (%89.23), LasR 56/65 (%86.15) ve rhlR 39/65 (%60) bulunmuştur. ERIC-DNA parmak izi analizi, Dice katsayısı ve UPGMA filogenetik analiz yöntemiyle izolatların çeşitliliğini göstermiştir. İncelenen türler iki ayrı kümeye (A ve B) ayrılmıştır. İstatistiksel analizlere göre ERIC-PCR genotiplendirme yönteminin virülans genleri, antibiyotik duyarlılık testi ve diğer virülans değişkenleriyle korelasyon katsayısı anlamlı bulunmuştur ($p < 0.05$).*
Anahtar Kelimeler: *Acinetobacter baumannii, biyofim oluşumu, çoklu ilaç direnci, virülans faktörleri*

INTRODUCTION

Infectious disease remain a major cause of death, especially in developing countries, as evidenced by the advent of new infectious diseases and more multidrug-resistant strains of microbial pathogens [1]. As a consequence of its exceptional capability to cause infection outbreaks and develop resistance to almost all currently used antibiotics, including carbapenems, *A. baumannii* is a pathogen of greater clinical importance and a public cause of healthcare-associated infections [2]. *A. baumannii* is distinguished by its remarkable ease of acquiring resistance to antibiotics, its ability to disseminate, and its ability to live on the majority of ecological surfaces [3]. Many *A. baumannii* healthcare-associated infections that have been restricted globally during the past 15 years are resistant to practically every family of contemporary antibiotics [4]. The last concern mostly pertains to gram-negative bacteria, particularly *A. baumannii*, whose multidrug resistance is a major factor in the failure of therapy of infectious disease [5]. The discovery of virulence components crucial to *A. baumannii* pathogenicity has been aided in recent years by combinatorial techniques combining genomic, phenotypic, and infection model investigations [6]. Multidrug resistance and desiccation tolerance have enabled *Acinetobacter* to emerge as one of the main healthcare-associated infections. The regulation of virulence genes, antibiotic resistance, and biofilm formation in *A. baumannii* is facilitated by quorum sensing (AHL molecules), a mechanism that also regulates other virulence factors such as swarming motility, and pellicle formation, entry into the stationary phase, sporulation, conjugal transfer of plasmid DNA, and transformation competence [7]. *A. baumannii* is one of the greatest common healthcare-associated infection outstanding to its capacity to survive in hospital environments, withstand a variety of antimicrobial drugs, and colonize vulnerable individuals receiving broad-spectrum antibiotic treatment [8]. Numerous healthcare-associated infections, such as urinary tract infections, bacteremia, surgical site infections, secondary meningitis, and healthcare-associated infections or ventilator-associated pneumonia, are caused by the opportunistic pathogen *A. baumannii*, particularly in patients admitted to the intensive care unit [9]. Previously thought to be a low-virulence bacterium, *Acinetobacter* species can occasionally be highly pathogenic and cause invasive diseases, as evidenced by the prevalence of fulminant community-acquired *Acinetobacter* species [10]. Because of its ability to create biofilms, *A. baumannii* is a sneaky, newly discovered bacterial pathogen that can cause serious healthcare-associated infection [11]. *A. baumannii* is one of the most prevalent bacteria that contaminates medical equipment through biofilm formation [12]. However, little research has been conducted on the ability of *A. baumannii* to produce biofilms, even though it plays a significant role in the pathophysiology and development of antibiotic resistance. Only a colorimetric assessment of biofilm mass related to colistin resistance has been used to analyze biofilm-forming capabilities [13]. Compared to their planktonic counterparts, biofilms are responsible for up to 80% of infections and are challenging to treat because of their significant antibiotic resistance [14]. The basic structural units of a biofilm are called microcolonies, which are detached populations of bacterial cells fixed into EPS. Depending on the type of bacteria, microcolonies can be rod-like or mushroom-shaped and contain 10–25% cells and 79–90%, the EPS matrix. Most bacteria grow in a free-living planktonic form (motile), but others can display a variety of phenotypes that differ in physiological features involving metabolic changes and structure. Biofilm formation is thought to be a three-step process involving several genes [15]. The bacterial cell-to-cell communication

mechanism known as quorum sensing (QS) requires the synthesis, identification, and reaction of extracellular signaling molecules known as autoinducers (AIs). Bioluminescence, sporulation, competence, antibiotic synthesis, biofilm development, and virulence factor secretion are some of the processes that QS regulates [16]. Almost all Gram-negative quorum-sensing systems have four characteristics. First, acyl-homoserine lactones (AHLs) or other compounds derived from S-adenosylmethionine (SAM) are the autoinducers in these systems. These molecules may readily cross the bacterial barrier. Second, the autoinducers attach to certain receptors in the cytoplasm or inner membrane. Third, quorum sensing often modifies dozens or even hundreds of genes that support various biological processes. Fourth, a feed-forward loop that is believed to enable population-wide synchronized gene expression is created when autoinducer-driven quorum sensing activation leads to increased autoinducer synthesis. This process is called autoinduction [17]. By generating N-3-hydroxydodecanoyl-L-HSL 3 hydroxy-C12-HSL, quorum sensing regulates the improvement of virulence factors such as biofilm formation and swarming motility in *A. baumannii*. These genes include *Las I*, *LasR, RhlR*, and *RhlI* [18]. The study aims to investigate the genotypic features of virulence genes, biofilm, quorum sensing, and genetic relatedness using ERIC-PCR genotyping of local clinical isolates of *A. baumannii*.

MATERIAL AND METHOD

Sample Collection

Throughout the four months, from January to April of 2023. Sixty-five different clinical samples were collected from inpatients with wound damage infections and injured soldiers. Swabs were taken from various anatomical sites (e.g., bone, Joints, connective tissues) with wound infections (osteomyelitis and burn infection). The swabs were collected from a military operation at the Al-Kindi Teaching Hospital, Medical City, Baghdad, Imam Ali, Sader City, Ibn Albalady, and Al-Noaman Hospital, among other facilities in Baghdad, including (in and out) patients and the burn unit from different hospitals in Baghdad, of various ages ranging from 22 to 54 years. The number of samples in men was 57 (87.69%), while, number of samples in women resulting from injuries to soldiers was 8 (12.30%). Samples were collected under medical and specialized staff and sterile conditions.

Identification of *A. baumannii*

Following a 24-hour incubation period, the samples were inoculated onto McConkey agar, CHROMO agar, and blood agar. Colony features were analyzed on the plates. Isolates were identified using Gram and capsule stain, catalase, oxidase, and biochemical tests, and the final identification was performed using the Vitek 2 system [19].

Antibiotic Susceptibility Test

The antimicrobial sensitivity test for several antibiotics, AK: Amikacin, was conducted using the VITEK-2 system. AUG: Augmentin (amoxicillin/clavulanic acid). AM: Ampicillin. Aztreonam, or AZT. CZ: Cefazolin, SAM: Ampicillin/sulbactam. The CPM is cefepime. CTX, or cefotaxime. CAZ ceftazidime. The CRO is ceftriaxone. CIP, ciprofloxacin. DXT, doxycycline. GM: Gentamicin. IMI, imipenem. The LEV was levofloxacin. MEM is meropenem. NIT, or nitrofurantoin PRL: Piperacillin. PTZ: Piperacillin/tazobactam. T: Tetracycline. Clavulanate/ticarcillin (TIM). TM: Tobramycin. TS: Using the AST-N207 card, trimethoprim/sulfamethoxazole was determined to be resistant (R), susceptible (S), and intermediate (I) for *A. baumannii* isolates, according to Clinical and Laboratory Standards Institute (CLSI, 2024).

Biofilm Production

Congo-red agar method (CRA) described method for screening biofilm formation isolates [20], while microtiter plate method is considered the gold standard method for biofilm detection. All isolates from fresh agar plates were inoculated in 10 ml of trypticase soy broth with 1% glucose w/v. Broths were incubated at 37ºC for 24 hours. The culture was then diluted 1:100 with fresh medium and inoculated into individual wells of a sterile 96-well, flat-bottom polystyrene tissue culture plate.

Negative control wells contained inoculated sterile broth. The plates were incubated at 37ºC for 24hours. After incubation content of each well was removed by gentle tapping. The wells were washed with Deionized water once. This removed free-floating bacteria. Biofilm formed by bacteria adherent to the wells was stained by (0.1%) w/v crystal violet for 15 min. Excess stain was removed by using Deionized water, and the plates were kept for drying. Optical density (OD) of stained adherent biofilm was obtained by using a micro ELISA auto reader (Human reader ELISA HS) at a wavelength of 630 nm strong, moderate, and weak, as shown by their respective means (≥ 0.453, 0.425-0.400, and < 0.345) [21,22].

Extraction and Estimation of DNA Concentration and Purity

A. baumannii genomic DNA was isolated using the Geneaid GBB100, a Korean device. As extracted according to the firm, DNA instructions. DNA purity and concentration were estimated using a NanoDrop device. To assess the optical density (O.D.) at 260 and 280 nm wavelengths, one microliter of each DNA sample was administered in a nanodrop. DNA purity was determined using the following formula: DNA purity = O.D. 260 / O.D. 280. Purity in the range of 1.7 to 2.0 is often considered acceptable [23].

Detection of Genes by PCR

Genes Selection

QS system genes and genes linked to biofilm formation were examined separately in this study by [24,25]. The gene primers used are listed in (Table 1). As advised by the supplier, these primers (IDT DNA, USA; Alpha DNA, Canada) were lyophilized, and stored in a deep freezer until required, after being diluted in sterile deionized distilled water to a final concentration of 100 picomole/µl.

Table 1. Sequence of primers with product size and reference

Primer Name	Sequence of Primers (5'-----3')	Product Size (bp)	Reference
OmpA	F:GCTACTATGCTTGTTGCTGCT R:CGCTTCTTGACCAGGTTGAAC	1023	[25]
Bap	F: ATGCCTGAGATACAAATTAT R: GTCAATCGTAAAGGTAACG	1449	
LasI	F: GTGTTCAAGGAGCGCAAAGG R: AACGGCTGAGTTCCCAGATG	238	[24]
LasR	F: TCGAACATCCGGTCAGCAAA R: GTTCACATTGGCTTCCGAGC	128	
RhlI	F: CCGTTGCGAACGAAATAGCG R:CAGTTCGACCATCCGCAAAC	308	
RhlR	F:TCGCTCCAGACCACCATTTC R: GACGGAGGCTTTTTGCTGTG	284	

ERIC PCR Reaction Condition

ERIC-PCR was performed using a GoTaq Green Master Mix PCR Kit. There were 2µl of forward and reverse primers and 12.5µl of 2X PCR Master Mix in the final 25µl reaction mixture, F-ATGTAAGCTCCTGGGGATTCAC and R-AAGTAAGTGACTGGGGTGAGCG [26], 3.5µl of template DNA, and 25µl of nuclease-free water to finish the volume. Denaturing the DNA for seven minutes at 94°C, followed by 30s at 94°C, two minutes at 48°C, and four minutes at 72°C, using the next thermal cycling program used to amplify the DNA. The Amplification was completed after a final extension at 72°C for 15 min. and a soak at 4°C for 5 min. The PCR results were electrophoresed for 80 minutes on a 1.5% agarose gel in 1X TBE buffer, and then colored with 5µl/100ml of red-safe dye [23].

Statistical Analysis

The data output of the correlation analysis was considered statistically significant if the P values were less than 0.05. The discriminatory index was calculated by comparing various typing techniques

using Simpson's index of diversity [discriminatory index (D)], which is based on the likelihood that two unrelated isolates from the test population are identified in different typing groups.

$$D = 1 - \frac{1}{N-1} \cdot \sum_{j=1}^{s} nj(nj-1)$$

where NJ is the number of strains belonging to the jth type, s is the total number of reported types, and N is the total number of isolates in the sample population. The diversity score on the Simpson Index was between 0.0 and 1.0.

RESULT AND DISCUSSION

Isolation and Identification of *A. baumannii*

A. baumannii was isolated from wound infections in only 65 of the 145 clinical samples. The species was confirmed by colony morphology, Gram staining, oxidase and catalase tests, biochemical testing, and the Vitek 2 system.

VITEK-2 System for Identification of *A. baumannii*

A. baumanni was finally identified using this approach. Using Identification Gram-Negative Bacteria (ID-GNB) cards, the system could identify bacteria more quickly, efficiently, and away from contaminants that might hinder pathogen identification. According to these findings, *A. baumanni* was present in 65 of the isolates. The following is how the manufacturer explained the outcomes: Excellent identification is defined as 96% to 100%, very good identification as 93% to 95%, good identification as 89% to 92%, and acceptable identification as 85% to 88%.

Biofilm Formation

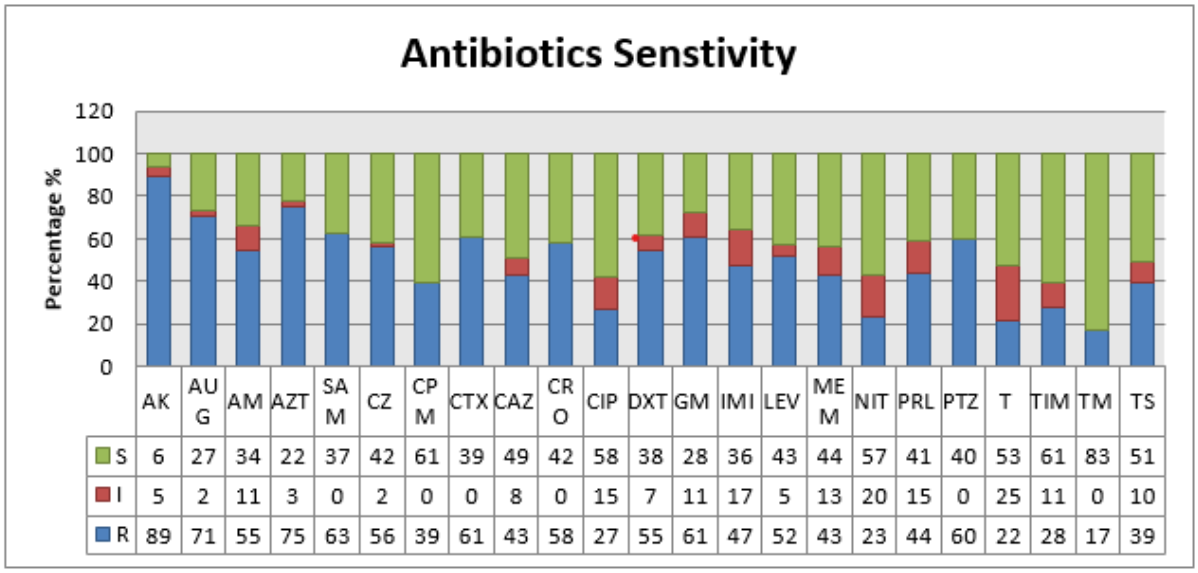
According to the results, 47 of 65 (72.30%) isolates of *A. baumannii* produced biofilms on Congo red agar medium. Black colonies with dry, crystalline consistency were indicative of a successful outcome. Weak slime producers often stayed pink, with sporadic darkening of the colony cores, although some isolates produced biofilms on a medium that was weak or nonexistent. While biofilm formation by microtiter plate method, according to the O.D. in an ELISA reader was found in 48 (73.84%) of the 65 clinical isolates of *A. baumannii*, produced strong adherence, 11/65 (16.92%) produced moderate production, and 6/65 (9.23%) produced weak production [22], as shown in (Table 2).

Table 2. Biofilm results in ELISA reader according to O.D

Biofilm Formation	No. of Isolates	Range of O.D
Strong produce	48	≥ 0.453
Moderate produce	11	0.400-0.425
No produce biofilm	6	≤ 0.345

Antibiotic Susceptibility Testing

All 65 *A. baumannii* isolates were tested for antibiotic susceptibility using a VITEK-2 system card. Twenty-three antibiotics were evaluated for susceptibility. The antibiotic sensitivity results revealed that clinical isolates of *A. baumannii* from a variety of sources had a high level of resistance. Cefotaxime 59/65 had the highest level of antibiotic resistance (90.76%), whereas Ciprofloxacin 14/65 had the lowest (21.53%). Furthermore, several isolates indicated intermediate resistance to some antibiotics; the full specifics of the drug susceptibility data are displayed in (Figure 1).



	AK	AUG	AM	AZT	SAM	CZ	CPM	CTX	CAZ	CRO	CIP	DXT	GM	IMI	LEV	MEM	NIT	PRL	PTZ	T	TIM	TM	TS
S	6	27	34	22	37	42	61	39	49	42	58	38	28	36	43	44	57	41	40	53	61	83	51
I	5	2	11	3	0	2	0	0	8	0	15	7	11	17	5	13	20	15	0	25	11	0	10
R	89	71	55	75	63	56	39	61	43	58	27	55	61	47	52	43	23	44	60	22	28	17	39

Figure 1. Antibiotics sensitivity test results

Over the past few decades, the development of antibiotic resistance in bacteria has become a significant global public health concern. The Centers for Disease Control and Prevention (CDC) reports that in the US alone, more than 2.8 million antimicrobial infections and more than 35,000 fatalities occur annually. If the issue of antibiotic resistance is not addressed by 2050, public health is expected to suffer greatly, with an estimated 10 million deaths per year [27]. Numerous physiological and metabolic mechanisms can lead to antibiotic resistance. Lack of fundamental knowledge about the processes behind the spread and emergence of resistance to antimicrobial agents is one of the primary causes of the lack of advancement in the control and prevention of the development of resistance. The improper use of already sluggish development and available antibiotics and new ones are the main causes of this catastrophe. The healthcare system will be threatened by the widespread emergence of antibiotic resistance, and diseases will soon become difficult to cure in the post-antibiotic era. In addition to endangering public health, the rise of antibiotic resistance threatens the national security and financial stability of healthcare systems [28]. Extended-spectrum β-lactamases (ESBLs) are commonly associated with gram-negative bacteria, especially *A. baumannii*. The opportunistic pathogen *A. baumannii* is frequently encountered in medical settings, such as hospitals. Carbapenemases are a family of powerful Metallo Beta Lactamases (MBLs) enzymes that cause antibiotic resistance. *A. baumannii* has been shown to include four different types of these enzymes: IMP-like, NDM-type, SIM-1, and VIM-like carbapenemases. Furthermore, genes encoding metallo-beta-lactamases (MBLs), such as blaSIM, blaIM, and blaVIM, have been found in isolates of *A. baumannii* that carry these enzymes, according to genetic research. Genes such as SHV-type,TEM-type, and CTX-M-type encode ESBLs, which provide phenotypic resistance to penicillin and third-generation cephalosporins [29,30]. The CDC states that multidrug-resistant (MDR) and *A. baumannii* are related to approximately 500 fatalities annually and 7,300 infections. As a pathogen that causes sepsis, wound infections, and hospital-acquired pneumonia, this bacterium is especially noteworthy. When the World Health Organization (WHO) released its overall priority list of antibiotic-resistant bacteria in 2017, *A. baumannii* the highest priority category. The main reason for this categorization is the lack of effective treatment options that are either currently available or developed to treat disease caused by these bacteria [31]. The emergence of multi-drug, pan-drug, and prolonged drug-resistance strains of *A. baumannii* bacteria that are resistant to almost all antibiotics makes it difficult to treat *A. baumannii* infections. The opportunistic pathogen *A. baumannii* causes a variety of diseases and mostly affects critically ill patients in intensive care units (ICUs). Chronic wound infections have often been linked to *A. baumannii*, especially in burn victims and those who have experienced military trauma. Bloodstream infections, ventilator-associated pneumonia, wound infections, skin infections, urinary tract infections (UTIs), and secondary meningitis are hospital-

acquired diseases caused by *A. baumannii* [32].

Genotyping of *A. baumanni* Using PCR

Two distinct amplification techniques, Uniplex-Multiplex PCR and ERIC-PCR typing methods, were used to genotype *A. baumannii* isolates. The genes of 65 *A. baumannii* had their genes amplified using PCR to identify virulence factors, including biofilm formation and quorum sensing (QS). As shown in (Figure 2) and (Figure 3), PCR revealed genes involved in biofilm formation, followed by *ompA* 63/65 (96.92%) and *bap* 48/65 (73.84%). In contrast, the Quorum sensing (QS) system genes responsible for chemical signals were *LasI* 58/65 (89.23%), *LasR* 56/65 (86.15%), *rhlI* 43/65 (66.15%), and *rhlR* 39/65 (60%). The quorum sensing and biofilm gene genotyping findings are shown in Supplemental (A).

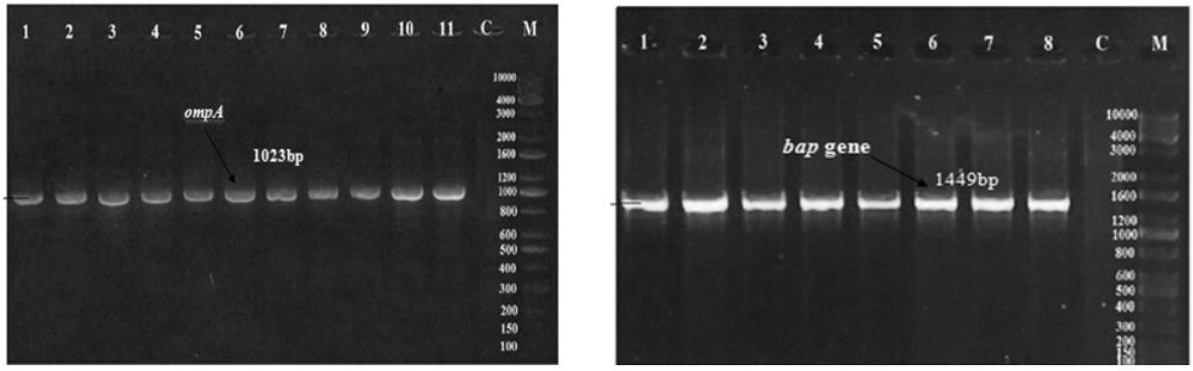


Figure 2. Monoplex PCR was used to detect the *bap* and *ompA* genes in isolates of *A. baumannii* (Lane M: 100 bp DNA ladder; Lane C: Negative control). Agarose gel (1.5%) was used for detection, dyed with Red Safe dye, and seen on a UV transilluminator documentation system for one hour at 5 V/cm

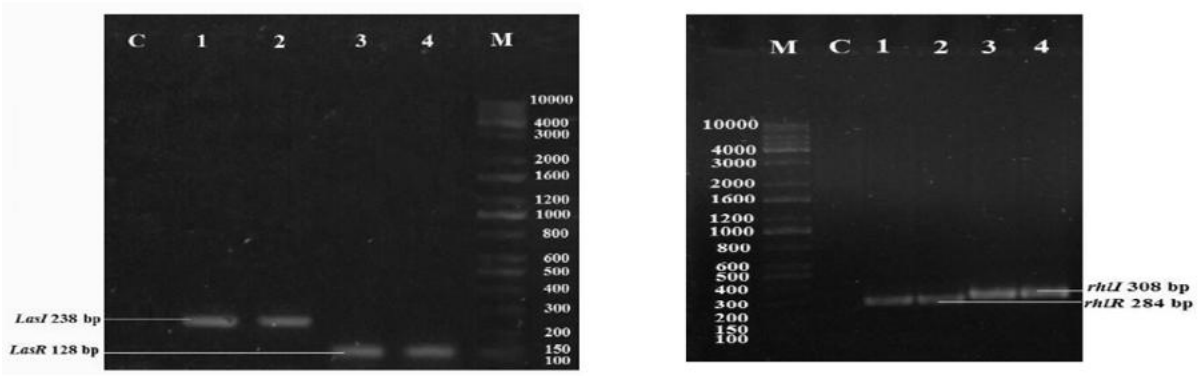


Figure 3. Multiplex PCR was used to detect the *rhLl*, *rhLR*, *LasI*, and *LasR* genes in isolates of *A. baumannii* (Lane M: 100 bp DNA ladder; Lane C: Negative control). Agarose gel (1.5%) was used for detection, dyed with Red Safe dye, and seen on a UV transiluminator documentation system for one hour at 5 V/cm

Genotyping of *Acinetobacter baumanni* by ERIC-PCR

ERIC-PCR was the second technique used to genotype *A. baumannii*. 65 isolates (100%) exhibited amplification bands, based on the results of clinical isolates of *A. baumannii* amplified using ERIC. One to ten bands were the most common. The range of ERIC-PCR findings was 100_10,000 bp. Additionally, according to statistics, the low frequency band was 10,000 bp, while the high frequent band was between 300 and 900 bp. As shown in (Figure 4).

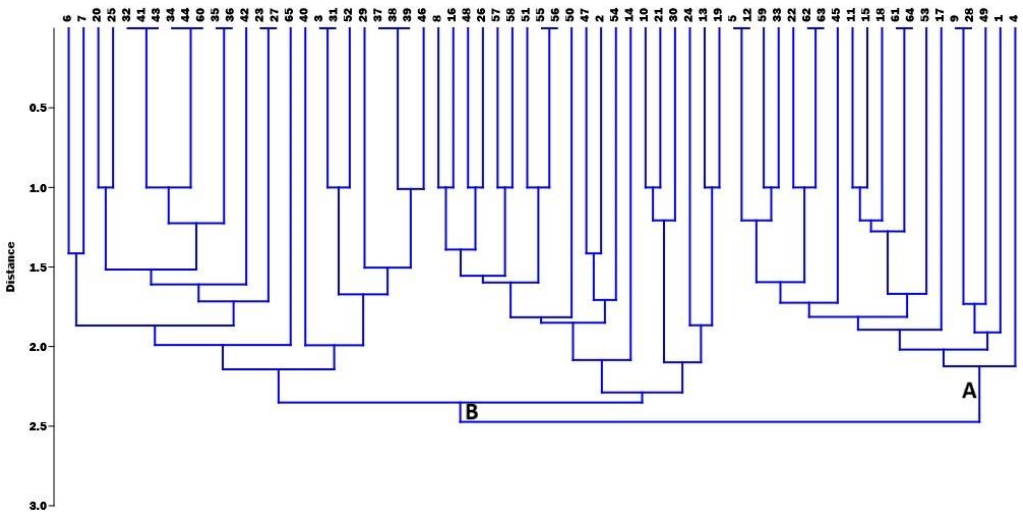


Figure 4. Enterobacterial Repetitive Intergenic Consensus (ERIC) genotyping of *Acinetobacter baumannii* isolates revealed genetic similarities in a dendrogram created using the Dice coefficient and the UPGMA clustering algorithm

The ERIC dendrogram of *A. baumannii* revealed two primary clusters, A and B. It was evident from the 93% similarity threshold that the isolates examined by species were divided into two distinct groups. Based on statistical the correlation coefficient of the ERIC-PCR genotyping method with the study's antibiotic sensitivity test, virulence genes, and other virulence parameters was significant, $p < 0.05$ (two-tailed), as shown in (Figure 5).

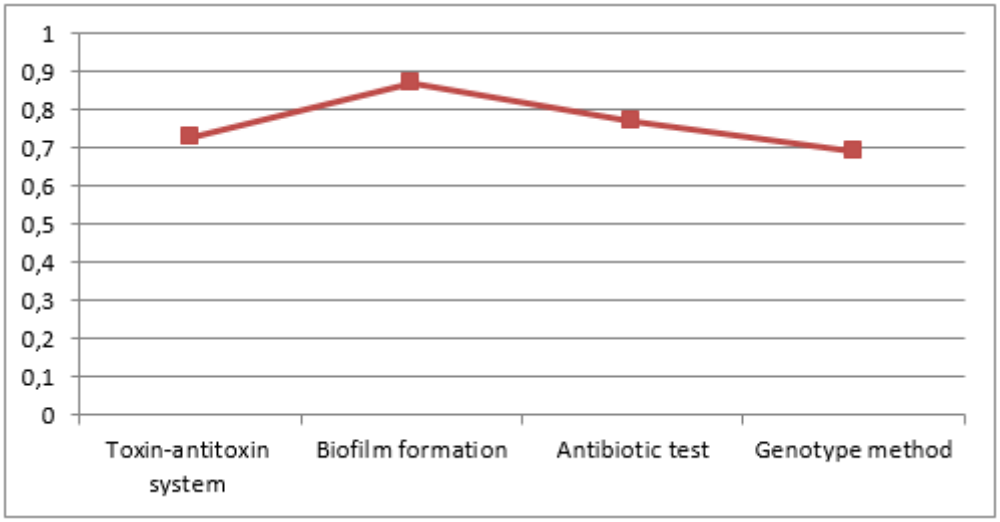


Figure 5. The relationship between the Toxin-antitoxin system, biofilm formation, antibiotics test, and genotype method by ERIC-PCR according to Simpson index diversity (SID)

A. baumannii may develop biofilms on both non-living (abiotic) and living (biotic) surfaces in addition to being resistant to many drugs. The effectiveness of existing therapies for wound infections is threatened by their capacity to colonize skin epithelial cells. It has been shown that the development

of biofilms increases the bacterial population's chances of surviving in hospital environments, resulting in a higher incidence of healthcare-associated infections [33]. Bacterial clumps on surfaces, known as biofilms, are a significant colonization mechanism that leads to the development of several diseases and is challenging to remove. Resistance to antimicrobial drugs is up to 1000 times greater than that of planktonic cells. It is believed that the existence of an additional polymeric substrate layer that might provide less drug penetration, and a physical barrier, is the cause of this enhanced resistance. Even when several antibiotics are used, this results in the persistence of biofilms on surfaces that are challenging to cure [34]. Urinary tract infections (UTIs) caused by *A. baumannii* are uncommon; they only occur 1.6% of the time in intensive care unit (ICU) settings and are mostly linked to colonization or catheter-related infections. Nonetheless, the frequency of *A. baumannii*-caused UTIs has been steadily increasing [35]. Endotracheal tubes, intravascular catheters, ventricular catheters, and urine catheters are examples of medical devices that frequently provide an opening for opportunistic microorganisms to colonize [36]. In people who are otherwise healthy and not in a medical facility, *A. baumannii* frequently causes UTIs [37]. However, it has also been noted that *A. baumannii* may infect the skin, nose, and throat of healthy people. Additionally, the dirty hands of medical staff members are a common way for *A. baumannii* to spread throughout the hospital environment. Research is still being conducted to learn more about the colonization, infection, and systemic transmission of *A. baumannii*, even if the precise processes underlying the pathogenesis of these infections are still unknown. To prevent the spread of *A. baumannii*, strict personal hygiene and cleanliness are essential because the bacteria can remain in patients' bodies for weeks after colonization. Several possible processes have been proposed that, highlight their different roles in infection, colonization, and the spread of epidemics. Adhesion to host cells, resistance to disinfectants, antibiotics, desiccation, biofilm formation, quorum sensing, inducing induction, and cytotoxicity are some of these processes. Further research is necessary to completely understand the complex the interaction that occur between *A. baumannii* and its host during infection [38]. Bacterial adhesion to epithelial cells is the crucial first stage of colonization and infection. The ability of *A. baumannii* to assault epithelial cells was shown in a study by Choi et al. (2008), [39], even if the specific host cell type may affect the extent of invasion. Research has indicated that respiratory tract epithelial cells are more vulnerable to *A. baumannii* invasion than other types of epithelial cells. This suggests that *A. baumannii* could prefer respiratory epithelial cells for invasion. Outer membrane proteins (Omp) have a major impact on the pathogenicity of several gram-negative bacteria, including some strains of *Acinetobacter*, particularly *A. baumannii*. Outer membrane protein A (OmpA, 38 kDa) is one of these proteins that has been found in several *Acinetobacter* strains; upon infection, *A. baumannii* Cell death results from interaction of OmpA with eukaryotic cells and subsequent translocation into the nucleus [38]. Additionally, it has been discovered that *A. baumannii* OmpA causes early apoptosis and postpones necrosis in dendritic cells. In *A. baumanni*, OmpA also plays an important role in serum resistance, biofilm formation, and surface motility [40]. Omp33-36, another outer membrane protein, has been identified as being essential to *A. baumannii* pathogenicity. By triggering caspases and altering autophagy, host cells undergo apoptosis [41]. Furthermore, adhesins and fimbriae (pili) on the cell surface are responsible for *A. baumannii* adhesion to both inanimate surfaces and host cells [42]. Microorganisms attached to surfaces and encased in a hydrated polymeric matrix form a complex material known as a biofilm. This matrix forms three-dimensional structures and is composed of proteins, nucleic acids, and polysaccharides [33]. *A. baumannii*, a bacterium frequently seen in hospital settings, is a constant source of infection because it can create biofilms that allow it to stay in the environment and shield it from disinfectants, *A. baumannii*, a bacterium frequently seen in hospital settings, is a constant source of infection. Furthermore, ability of *A. baumannii* to produce biofilms contributes to its antibiotic resistance. Biofilms are essential for pathogen survival and the emergence of antibiotic resistance in medical settings because of the ability *A. baumannii* to attach to both living and non-living surfaces [43]. The propensity of *A. baumannii* to form biofilms is influenced by several variables, such as the presence of DNA and polysaccharides, surface features such as pili, quorum sensing, omp, and the availability of nutrients. The CSU/BABCDE, chaperone-usher assembly system, which encodes pili in *A. baumannii*, is governed by a two-component regulatory system involving the *bfmR*, and *bfmS* genes. Together, these elements influence the development and control of biofilms in *A. baumannii*, which affects the resistance and persistence of bacteria to antibiotics [44,45]. According

to transposon mutagenesis, pili synthesis, biofilm formation, and bacterial adhesion depend on *csuA* and *csuE* [46]. In a second investigation, the same researchers found that the lack of CSU gene expression was caused by inactivation of the bfmR response regulator. As a result, *A. baumannii* completely stopped producing pili and formed biofilms. However, a mutation in the bfmS gene, only slightly hampered biofilm formation, suggesting that it plays a less significant role in the process. It was also discovered that the creation of biofilms in *A. baumannii* was linked to another protein called biofilm-associated protein (*Bap*). When compared to the wild-type strain, the biofilm thickness was significantly reduced by more than 50% as a result of the Bap gene mutation [47].

In summary, patients with wound injuries had a higher percentage of *A. baumannii* isolates, particularly male patients, than female patients. The most effective antibiotic against *A. baumannii* isolates was ciprofloxacin; nevertheless, when testing antibiotic susceptibility, most clinical isolates showed multidrug resistance, especially to cefotaxime. It's time to switch to other antibiotics as the majority of first-line antibiotics lose their therapeutic efficacy. In the laboratory, the majority of *A. baumannii* isolates developed biofilm and other virulence factors at varying percentages. The *ompA* gene was the most prevalent among local *A. baumannii* isolates, followed by *LasI* and *LasR*. *rhlR* was the least prevalent gene. Most *A. baumannii* clinical isolates exhibited a significant degree of genetic similarity in their ERIC-PCR results. Clusters A and B are of two types. Based on statistical analysis, the correlation coefficient of the ERIC-PCR genotyping method with the study's antibiotic sensitivity test, virulence genes, and additional virulence variables was significant.

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AUTHOR CONTRIBUTIONS

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CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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