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Research Article

DETECTION OF SOME VIRULANCE GENES AND VARIATION OF *Acinetobacter Baumannii*

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Abstract: *Acinetobacter baumannii is gram-negative bacteria associated with hospital-acquired illnesses. The study involved molecular investigation of virulence genes of A. baumannii isolated from different sources using specific primers. Six genes were used for important virulence factors in this bacteria (ompA, plcN, csgA, lasB, iutA, fyuA), partially investigated using PCR, results showed the presence of these genes as 100% and 52.6% and 63.1% and 21% and 21% respectively, while the gene fyu A was not found in any A. baumanni isolate. The study also involved genetic variation in the DNA of these isolates using a universal primer (ERIC2). The results showed that 12 genotypes among 20 isolates of A.baumannii bacteria were revealed. The twelve isolates were divided into four groups, each of which contained genotype-identical isolates linked by bands with comparable molecular weights, as well as (8) single isolates that did not belong to any of the four groups.*

Keywords: *Acinetobacter baumannii, virulence genes, ERIC2, Genetic variation.*

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1. Introduction

Acinetobacter baumannii (*A.baumannii*) is a gram-negative opportunistic bacterial pathogen connected with infections acquired in hospitals, infections caused by this bacterium became increasingly problematic especially among patients in (ICU) and immunocompromised patients, because of its resistance to wide range groups of antimicrobial agents, it has significantly restricting treatment options. Being a major cause of infections, *A.baumannii* has many virulence factors that make it belong to (ESKAPE) group classified by the World Health Organization (WHO), which is placed this bacteria in the important priority list of microorganisms that urgently require the development of effective antibiotics. [1, 2].

High pathogenesis of *A.baumannii* is due to its possession of many effective virulence factors including adhesion, continuous presence on hard and dry surfaces, as well as their ability to obtain nutrients like iron, sticking to epithelial cells and then breaking them down, the ability of some strains

to produce the enzyme gelatinase and protease which destroy the host tissues, In addition, they can form biofilms that play an important role in the settlement process [3].

Acinetobacter genomes are adaptive and flexible, prone to acquiring antibiotic resistance determinants via horizontal gene transfer involving mobile genetic components. Microbial genotyping technologies are a significant tool for molecular epidemiology researches, especially in understanding pathogen transmission and population dynamics. Despite the prevalence of whole-genome data, multilocus sequence typing (MLST) remains the "gold standard" for molecular typing of bacteria [4, 5].

The bacterial genome of *A.baumannii* contains recurring sequences similar to the ERIC initiator sequence. This has enabled it to be used as a molecular biological tool for determining the genetic variation coding on DNA of *A.baumannii* isolates [6].

The study aimed to investigate some virulence genes in *A.baumannii* isolates as well as a study of genetic variation in the DNA and comparison of these isolates with the standard isolate of this bacteria.

2. Materials and Methods

2.1. Bacterial isolates

Nineteen bacterial isolates of *A.baumannii* were taken from previous work [7] isolated from different clinical cases from Mosul hospitals/ Iraq, which included specimens from urine wounds, sputum, burns, CSF, and samples from the hospital environment including samples from intensive care unit, surgical instruments and appliances, patient beds, emergency lobby, and baby incubators. As well, standard strains of *A. baumannii* 19606 were used.

2.2. Detection of virulence genes in *A. baumannii* bacteria

A.baumanniii DNA was extracted using the boiling method by [8]. To determine the presence or absence of some virulence factors genes in this bacteria as shown in table 1.

The sequences of specific primers used are shown in Table (2) according to each [13, 12]. These primers are prepared in the form of powder from the US Company (alpha DNA).

Table 1. PCR programs for the detection of virulence genes in *A. baumannii*

Genes	PCR program	PCR volume (25 µL)	Reference
ompA gene	95°C for 4 min 94°C for 60 sec 55°C for 60 sec 72°C for 60 sec (35 Cycles) 72°C for 5min		[9]
plcN and *lasB* gene	95°C for 4 min 95°C for 45 sec 60°C for 60 sec 72°C for 60 sec (35 Cycles) 72°C for 7min	- DNA 4 µL (50 ng) - Primers (1µL F. and 1µL R.) (10 picomol) - Premix (2x) 6.5 µL - Deionized water 12.5µL	[10]
csgA and *iutA gene*	95°C for 4 min 95°C for 50 sec 58°C for 60 sec 72°Cfor 45sec (30 Cycles) 72°C for 8 min		[3, 11]
fyuA gene	95°C for 12 min 95°C for 30 sec 63°C for 30 sec 68°Cfor 3min (25 Cycles) 72°C for 10 min		[12]

Table 2. The sequence of primer bases for virulence factor genes and their molecular sizes

The gene	Gene size bp	Primer sequence (5'-3')
ompA	578	GTTAAAGGCGACGTAGACG CCAGTGTTATCTGTGTGACC
plcN	466	GTTATCGCAACCAGCCCTAC AGGTCGAACACCTGGAACAC
lasB	300	GGAATGAACGAAGCGTTCTC GGTCCAGTAGTAGCGGTTGG
csgA	200	ACTCTGACTTGACTATTACC AGATGCAGTCTGGTCAAC
iutA	300	GGCTGGACATCATGGGAACTGG CGTCGGGAACGGGTAGAATCG
fyuA	880	TGATTAACCCCGCGACGGGAA CGCAGTAGGCACGATGTTGTA

2.3. Genetic variation of *A.baumannii* isolates

The genetic variation of *A.baumannii* isolates was determined by the Random Amplified Polymorphic DNA (RAPD) technique by using the nucleotide sequence ERIC-2 primer (ERIC2 AAGTAAGTGACTGGGGTGAGCG).

The reaction mixture was prepared with a final volume of 25 µl depending on [14], 4 µL DNA (50 µL), 2.5 µL primer (10 picomol), 12.5 µL Premix (2x) and 6µL deionized water.

The PCR program was used: 95°C for 5 min., 94°C for 60 sec., 51°C for 60 sec., 72°C for 5 min (35 Cycles), and 72°C for 10 min.

At the end of the program, 5 µl of the PCR reaction product was transferred to the electrophoresis on the prepared agarose gel at a concentration of 2%, DNA ladder was put in the first gel hole and the output of the standard isolation reaction in the hole (2) and the isolation product was added in the pits after that, and run the relay at 50 volts for an hour, the gel was then dyed with ethidium bromide stain for half an hour and then transferred to the UV-transluminator to observe the formed bands, the molecular sizes of the apparent DNA bands of each bacterial isolation were estimated based on the distance they traveled in the gel with the help of the DNA ladder used. The logarithmic curve of the molecular sizes of DNA bands was plotted volumetric on the y-axis, the logarithm of the distance traveled by each of the bacterial isolates understudy on the X-axis, and thus determine the values of the molecular sizes of the resulting bands.

The results were then transformed into a descriptive table containing the numbers of bands shown on the agarose gel, number (1) in the presence of the band and (0) in the absence of it, then data were analyzed using the program past 3 to find out the convergence and spacing between isolates *A.baumannii* by drawing the evolutionary tree.

3. Result and Discussion

3.1. Detection of some virulence genes in *A.baumannii* local isolates

The presence of virulence factor genes involving six genes under study (*ompA, plcN, csgA, lasB, iutA, fyuA)* was investigated in all our local isolates using PCR and specialized primers for each.

Molecular sizes of each gene were detected by electrophoresis of PCR product and compared with DNA marker to confirm the molecular size of each one.

The results show that our isolates have different genes of virulence factors. Table (3) shows the distribution of these genes between *A.baumannii* isolates for different types of samples.

Table 3. Distribution of genes of virulence factors of *A.baumannii* isolated from different samples

Samples types	Samples number	Virulence genes					
		fyuA	***iutA***	***csgA***	***lasB***	***plcN***	***ompA***
Burns	1	0	1	0	1	1	1
Wounds	10	0	1	1	8	6	10
Respiratory tract infections	3	0	0	0	2	2	3
Spinal cord fluid	1	0	0	0	1	0	1
Intensive care unit	2	0	1	1	0	1	2
Patient beds	1	0	1	1	0	0	1
Surgical instruments	1	0	0	1	0	0	1
Total number	19	0	4	4	12	10	19
percentage %		0	21	21	63.1	52.6	100

3.1.1 *ompA* gene

The *ompA* (outer membrane protein A) gene is responsible for the diagnosis of the outer shell protein and is responsible for the formation of the biofilm [8].

The results showed that all the isolates of the *ompA* gene at the molecular size were 578 base pairs, ie 100% as shown in Figure (1).

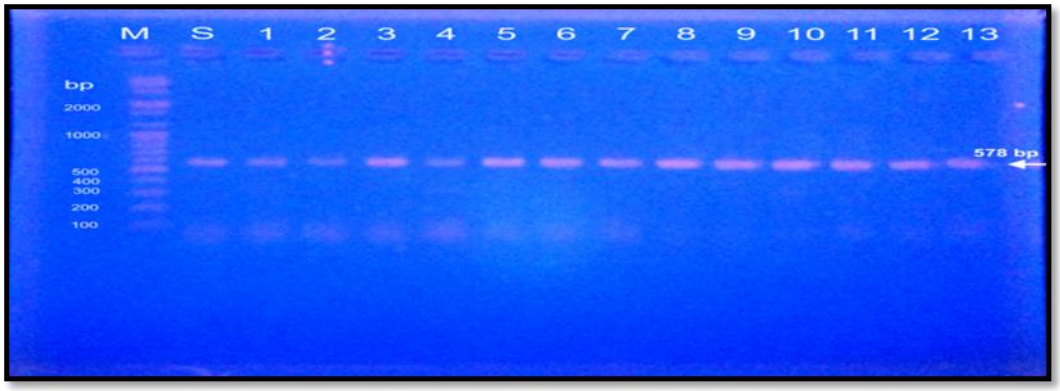

Figure 1. Electrophoresis of PCR product for *ompA* gene (578 base pairs) of *A.baumannii* isolates on 2% agarose gel at 50 volt for 60 minutes, M: DNA marker, S: Standard isolate

The results of our study are consistent with those of [15]. All of these isolates contained the *ompA* gene. The researchers revealed the gene in all sequences of *A.baumannii* isolates.

It was similar to the result of Ali and his group (2017) [9] in Saudi Arabia, where the number of isolates containing this gene was 30 isolates out of 32 isolates of the bacteria *A.baumannii*.

3.1.2 *plcN* gene

The *plcN* gene encodes the phospholipase enzyme responsible for host membrane analysis [16].

The results of the detection of the presence of *plcN* gene in our isolates were ten isolates, 52.6%, at a molecular size of 466 base pairs as shown in figure 2.

A. baumannii strains (isolated from clinical cases) which have this gene distributed as follows: one from burn sample, six (out of ten) from wounds, two from respiratory tract samples, whereas only one bacterial isolate had this gene was from an ICU and this may be due to moving this isolate carrying this gene to hospital environment in soon to take the sample, or the reason would be the transition of the genetic material by one of the known transition methods among these isolates like transformation.

The presence of this gene in our study was higher than that of the researcher Kareem and coworkers (2017) in Baghdad [9], where they obtained seven isolates out of thirty (23.3%) containing the *plcN* gene of *A. baumannii*, and claimed that the study was the first to investigate this gene in the *A.baumannii* isolates in Iraq.

This gene is an important virulence gene possessed by *A.baumannii* bacteria, which encodes the non-hemolytic Phospholipase C (PLC-N), which analyzes the host membrane by analyzing the membrane-forming phosopholipid [17, 16].

Stahl and his colleagues discovered the possibility of using *A. baumannii* to phosphotidylcholin – the main component of human cells – which makes up 50% of phospholipids in the body and 80% of lung phospholipids as a single carbon and energy source, and this explains the presence of this gene coding this enzyme in strains taken from the respiratory tract [18]. Also, except for the C.S.F sample, our strains were isolated from various human tissues.

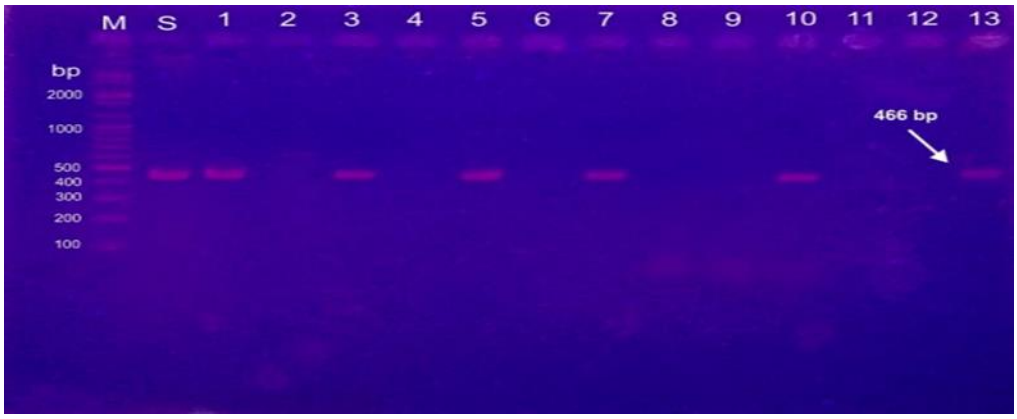

Figure 2. Electrophoresis of PCR product for *plc N* gene (466 base pairs) of *A.baumannii* isolates on 2% agarose gel at 50 volt for 60 minutes, M: DNA marker, S: Standard isolate.

3.1.3 *lasB* gene

The encoded *lasB* gene of the elastin enzyme responsible for breaking down host tissues through its analysis of elastin [19].

The results of our study showed the presence of this gene ranked second among the virulence genes in our local isolates at the molecular size of 300 base pairs as shown in fig. (3), as it had twelve isolates of the *lasB* gene, 63.1% of the total isolates *A.baumannii* obtained. This study was higher than that of researcher Kareem and his group (2017) where the proportion of this gene in isolates was 53.3% [10].

Containment of our local isolates for this high percentage of the *lasB* gene is evidence that they have high efficiency in the analysis of elastolytic activity by producing the enzyme elastase, which works to destroy the host tissue and thus release the nutrients necessary for the growth of bacteria, which

accelerates the attack of other tissues, moving the intracellular pathway and initiating the formation of biofilms in the host [20, 19].

The existence of this gene in such a high proportion may indicate the transmission of genetic via genetic transformation, in addition, plasmids and transposons can make this transfer easier.

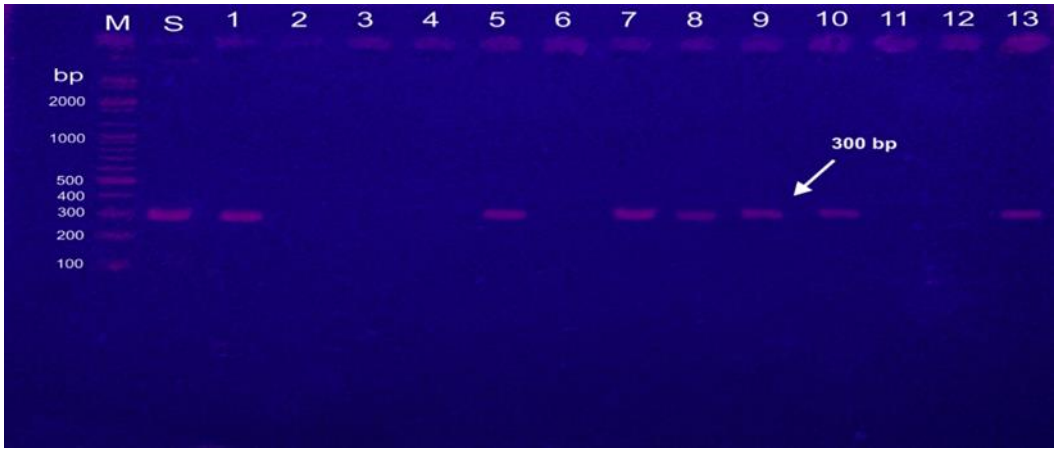

Figure 3. Electrophoresis of PCR product for *lasB* gene (300 base pairs) of *A.baumannii* isolates on 2% agarose gel at 50 volt for 60 minutes, M: DNA marker, S: Standard isolate.

3.1.4 *csg A* gene

The *csgA* gene that is responsible for the formation of curli fibers is also involved in the formation of the biofilm [5].

The *csgA* gene presents in only four (21%) of *A.baumannii* isolates at the molecular size of 200 base pairs as shown in the fig. (4). This gene was found in only one clinical isolate and three hospital environment isolates. This result might be due to the abiotic surface adhesion and curli fiber synthesis of bacteria which are under the control of this gene.

The number of *A.baumannii* isolates carrying this gene in the present study was lower than Al-Kadmy and coworkers 2018 [21], where the number of isolates carrying this gene was 14 isolates out of a total of 21 (66.6%), and less than the study of Darvishi (2016) where the proportion of this gene was 70% (14 out of 20) of *A. baumannii* isolates [3], while the percentage of *csg A* gene in our isolates was higher than that reported by Momtaz and his group (2015) where the gene was 12.3% by 15 isolates out of 21 isolates. *A.baumannii* [22].

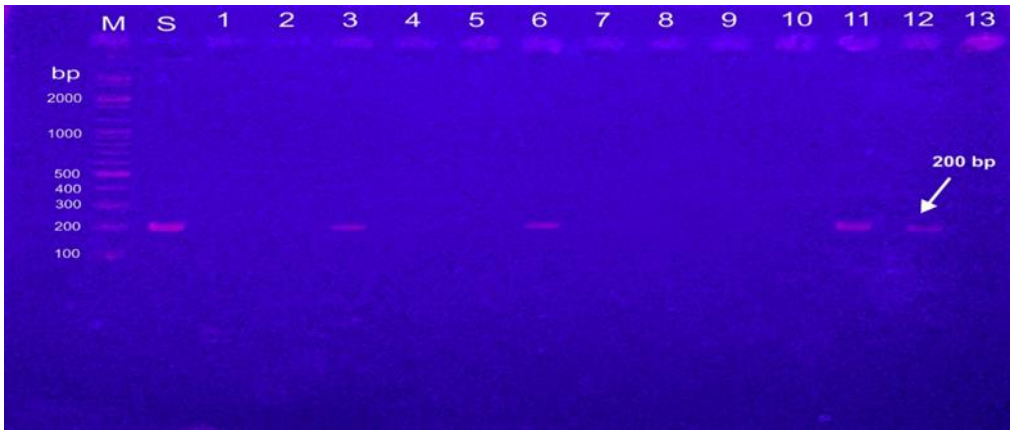

Figure 4. Electrophoresis of PCR product for *csgA* gene (200 base pairs) of *A.baumannii* isolates on 2% agarose gel at 50 volt for 60 minutes, M: DNA marker, S: Standard isolate

3.1.5 Presence of *iutA* and *fyuA* genes

Iron-taking genes include several genes that make up the system of Siderophores such as aerobactin (*iutA)*, and Yersinobactin (*fyu A*). These genes enable bacteria to survive and thrive in low-iron conditions. Iron–uptake systems are common in bacteria [11].

Results of the current study revealed the presence of *iutA* gene in 4 (21%) of the *A. baumannii* isolates at a molecular size of 300 basepairs; two of which were environmental strains as shown in figure (5).

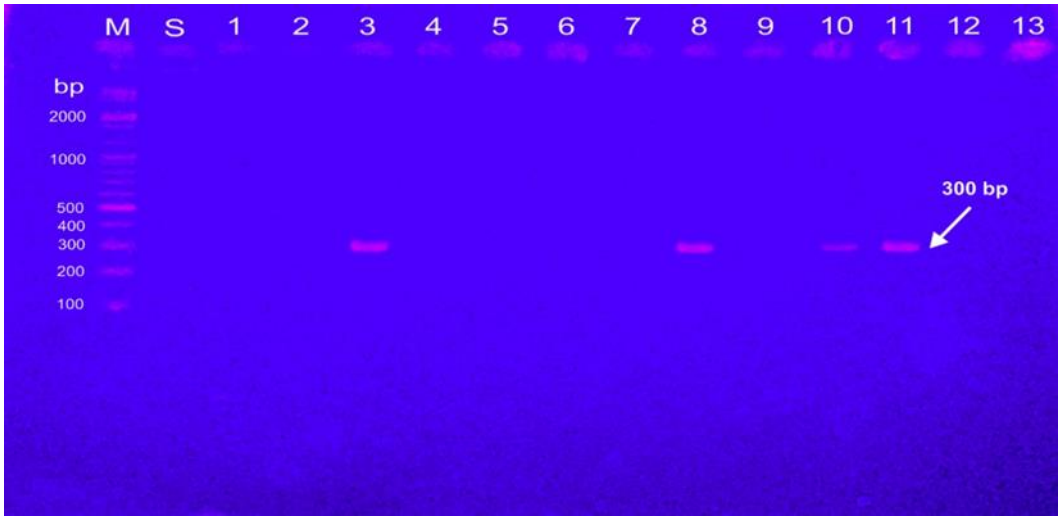

Figure 5. Electrophoresis of PCR product for *iut A* gene (300 base pairs) of *A.baumannii* isolates on 2% agarose gel at 50 volt for 60 minutes, M: DNA marker, S: Standard isolate

The results of our study were consistent with the study of Al-kadmy and coworkers (2018) and Momtaz and coworkers 2015 [21, 22] where the proportion of the gene in their isolation was 23.8% and 19%, respectively. It was higher than that of Mohajeri and coworkers 2016 [11], where the rate of genes in their isolates was 16% by 8 isolates out of 50. While Braun and Vidotto [12] could not discover this gene in their *A. baumannii* isolates recovered from UTI, they attributed this to the presence of other siderphore system genes besides *iut*, such as *iucA*, *iucB*, and *iucD*.

The *fyuA* gene, which encodes the Yersinobactin protein in the iron-taking system, was not found in any of our isolates. Because bacteria have several siderophores genes, our isolates may have diverse types of iron transporters. T Lack of *fyuA* gene was also reported by Mohajeri and coworkers (2016) and Braun and Vidotto 2004 [11, 12]. In contrast, Momtaz and coworkers (2015) were able to detect this gene in 41 out of 121(33%) isolates [22].

Genes that are responsible for encoding virulence factors of *A. baumannii* are very essential to identify especially in cases of outbreaks and epidemiological studies. Virulence factors play a role in the formation of biofilms, antibiotic resistance, and others which help the bacteria to survive, persist and cause diseases [17].

3.2. Genetic variation of *A.baumannii* isolates

Genetic variance studies of 20 *A. baumannii* isolates used in the present study 12 genotypes were found using RAPD DNA technique and the general ERIC-2 figure (6) and Table(4). Among these

isolates was the standard isolate of *A.baumannii* ATCC 19606 with molecular weights ranging from (1548-251) base pair.

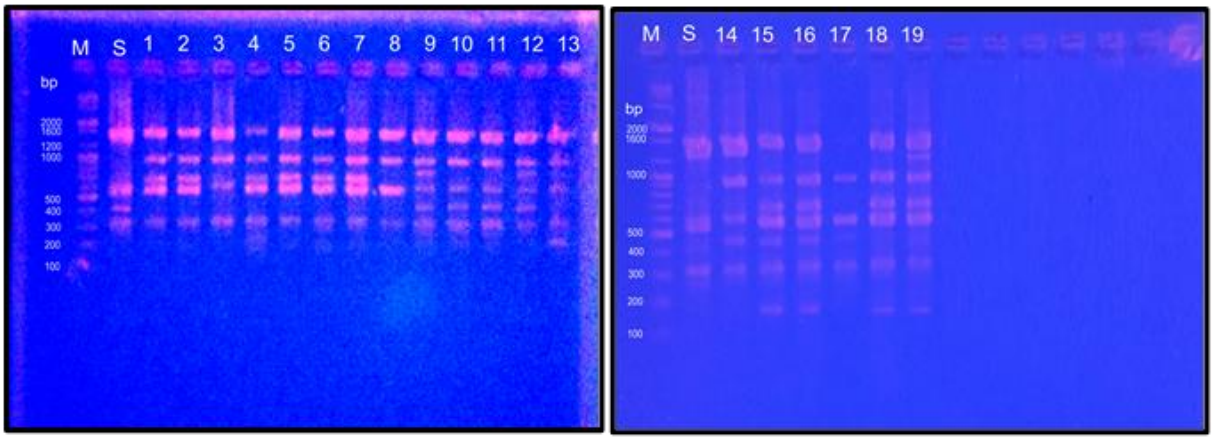

Figure 6. Electrophoresis of the PCR polymerase chain reaction (ERR-2) of *A.baumannii* isolates on a 2% agarose gel at a voltage of 50 volts for 60 minutes. M: DNA ladder, S: standard isolation

Twelve of these isolates were placed in four groups, each containing genotype-identical isolates through bands with similar molecular weights and (8) single isolates that did not belong to any of the four groups as shown in Table 5.

All isolates, including standard isolates, showed a band at a molecular weight of 309 base pairs (monoband). Similar results were obtained by He and coworkers in 2015 [23] as they demonstrated the presence of the molecular weight 300 bp bands in all *A.baumannii* strains, the presence of unique bands in one isolate called the unique band, was observed at the molecular weights (398,512,794,1202) base pairs in both standard, third, fourteenth and nineteenth isolates respectively.

The presence of a polymorphic band in eight molecular sizes included (251, 466, 501, 616, 630, 707, 977, 1548) base pair.

When comparing the genotype of the clinical isolates with hospital environmental isolates, We found that two of the environmental isolates were genotypically different, namely the third and seventeenth isolates, which contained individual bands of four and three bands respectively, These isolates were obtained from ICU beds. The third isolate had a unique band at a molecular weight of 562 base pairs.

Table 4. Genotypes of *A.baumannii* isolates

Number of isolates	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1	S	bundle Type	Molecular size of the bundle (bp)
6	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	Polymorphic band	251
20	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Mono band	309
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Unique band	398
8	1	0	0	1	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	0	Polymorphic band	446
18	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1		501
1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	Unique band	512
5	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		616
6	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	1	1	0	Polymorphic band	630
2	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0		707
1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Unique band	794
18	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	Polymorphic band	977
1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Unique band	1202
19	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	Polymorphic band	1548

Table 5. Recurrence of genotypes between *A.baumannii* isolates and the number of shared bands using ERIC-2

Groups	Isolates	No. of shared packages
Group 1	(1.2.4.5.6.7)	5 bands
Group 2	(9,11)	6 bands
Group 3	(10,12)	5 bands
Group 4	(15,16)	7 bands
Individual isolates	(3,8,13,14,17,18,19, S)	_____

Two of the environmental isolates showed genotype similarity with the clinical isolates 6 and 11, where the sixth isolate was a sample taken from the hospital equipment and belonged to the first group in Table (4) which included five bands, isolate No. 11 was taken from the bed sample and belongs to the second group, which included only two isolates containing six bands.

The new appearance of new genotypes of *A.baumannii* in different hospitals calls for effective sanitary methods to control the epidemic of these bacteria, such as washing hands and placing patients with isolation halls, and cleaning medical devices well, and Health awareness among staff and workers in the hospital is crucial to reduce the spread *A. baumannii* as much as possible [24].

The results obtained in Table (3) were analyzed using the Jaccard coefficient mediated by the past3 program to find the similarity between bacterial isolates by obtaining the evolutionary tree diagram As shown in figure (7); the similarity between the isolates, six main clusters were obtained to combine. The first cluster (A) contained isolate No. (3) obtained from ICU, and the second cluster (B) included both standard isolate *A. baumannii* 19606 and isolate (13) isolated from the respiratory tract where the similarity ratio was more than 60.

The third group (C) included isolates (14, 15, 16, 18, all of them were isolated from wounds plus isolate 19 which isolated from C.S.F) with 70% similarity.

The fourth cluster (D) had strains with No. 9, 10, 12 isolated from wounds and burns samples, in addition, to isolate No. 11 which is only isolated from the patient's bed among them.

The fifth group (E) consists of two clusters, E1 and E2, and the E1 included the No. 8 isolated wound sample, which was associated with the rest of the isolates belonging to the cluster E2, each of the isolates (1, 2, which taken form respiratory tract plus isolates No. 4,5,7 isolated from wounds, plus No. 6 which was hospital tool sample) was 80%.

The last group (F) included one isolate (17) sampled from the hospital environment (ICU); gave the lowest similarity with the rest of the isolates.

As mentioned above, the two *A. baumannii* ICU isolates on both sides of the Figure (7) which had cluster A and cluster F, this may be explained that both isolates are gnomically different from each other which makes them distinguishable from one another. Also, we have group C which contains clinical isolates only, indicating that all of them have similar genetic material in 70% similarity, the same thing was identical in cluster D and cluster E which contained clinical isolates except isolate No. 11 and No. 6 which were clustered to them respectively. The suggestion is both isolates may be transferred from clinical sites to the patient's bed and hospital tool respectively in the short time before sampling, here the hospital-acquired infections would emerge among medical staff, output patients, input patients, and their companions.

Due to the variation, the differences in similarity ratios between subspecies of the same species may be Due to different sources of sampling as well as possible mutations that result in genetic variations over time. [25].

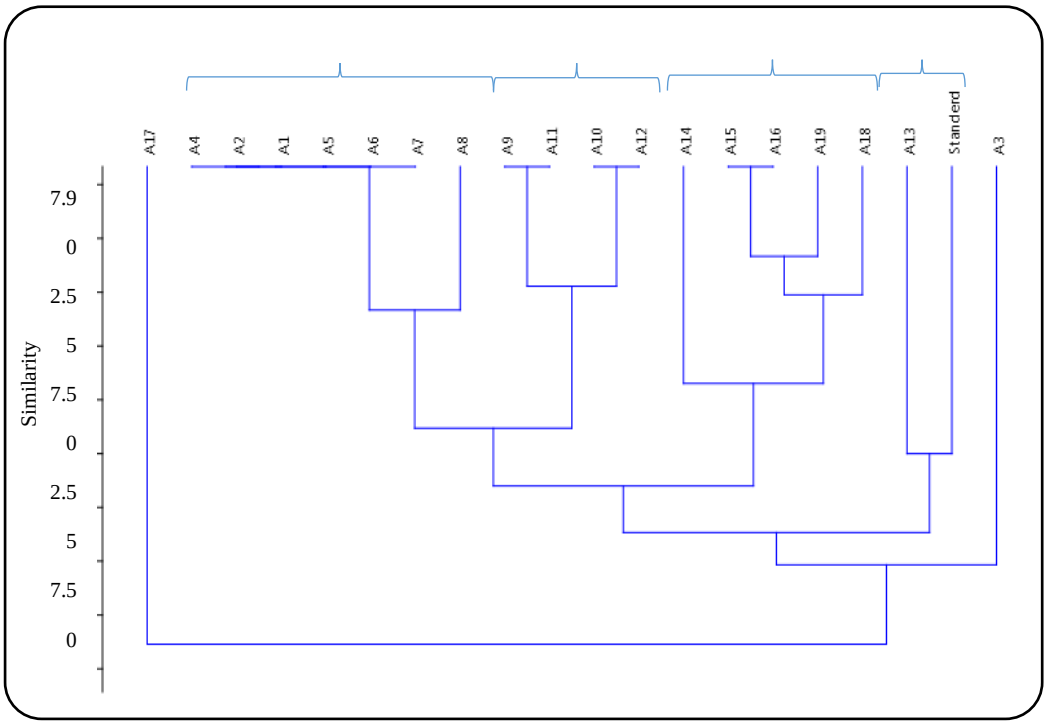

Figure 7. The evolutionary tree diagram of *A.baumannii* isolates

The results of our study coincided with the results of Akalin and coworkers (2006). The isolates showed 12 different genotypes from 120 *A.baumannii* isolates over a period of three years.

Our results are lower than the value obtained by Ferreira and his group (2011)[24], who found seventeen genotypes obtained from 124 isolates distributed in 30 clusters and 25 isolates containing a single band and the molecular sizes ranged between (1600-211) base pairs, also Ahmed in Baghdad 2017 [26] where the number of genotypes of *A.baumannii* isolates reached eighteen variants. Li and coworkers (2016) obtained 17 genotypes from 127 isolates of *A.baumannii* bacteria [27].

Others also found four genotypes of this bacteria when they analyzed them using the ERIC- 2 at molecular weights (300,600,900,1500) base pairs, this agrees with the present study on standard isolate ATCC 19606 *A.baumannii* and the size of the bands of their study ranged from (300,2500) base pair and the isolates possessed 4-8 individual packages [23, 28].

The number of genotypes obtained in the present study was higher than that reported by Aljindan and coworkers (2018) and Ece and coworkers 2015 [29, 25], the number of genotypes was 7, distributed in 5 clusters and 2 clusters respectively. Variation in the genotypes of our local isolates indicates variations in *A.baumannii* strains, The genetic variations observed in the present study may be due to the diversity of the sampling in terms of the source of isolation, the different hospital, isolation period, and location, moreover, this variation refers to the wide geographical distribution of these bacteria in the hospital environment due to cross-transmission within patients of the hospital. This is consistent with the study of Maleki and coworkers (2016), which showed the presence of genetic variation between *A.baumannii* strains and their different distribution among hospitals in Iran [30].

The differences in patterns refer to the clonal diffusion, which may be due to the prolonged survival of *A.baumannii* in the hospital environment and the movement of patients between hospitals [31].

4. Conclusion

A.baumannii isolates differ according to the virulence factors they own, and that depends on many factors like their habitat (clinical or environmental) as was clearly observed in the current study. The study also revealed a vast variety of genotypes of our local *A.baumannii* depending on ERIC-2 primer, which may be due either be to the bacteria having an adaptive and flexible genome or this bacterium may be continuously receiving new genes via horizontal transfer gene methods.

Genotyping is of vital importance in epidemiological studies to aid in finding the source of infection and controlling it. Healthcare institutions and workers must be well trained and enlighten in order to limit the spread of hospital-acquired infections especially *A. baumannii* which has become a global pathogenic bacteria due to the emergence of multi-drug resistance (MDR) and extensive-drug resistance (XDR) isolates.

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Conflict of interest:

No conflict of interest or common interest has been declared by the author.

The Declaration of Ethics Committee Approval

The author declares that this document does not require an ethics committee approval or any special permission. Our study does not cause any harm to the environment.

The Compliance to Research and Publication Ethics

This work was carried out by obeying research and ethics rules.

Authors' Contributions

N.H.A: Conceptualization, Methodology, Formal analysis, Writing - Original draft preparation (%50)
Gh. A. M.: Conceptualization, Methodology, Resources, Investigation (%50)

References

[1] Tacconelli, E.; Carrara, E.; Savoldi, A.; Harbarth, S.; Mendelson, M.; Monnet, D.L.; Pulcini, C.; Kahlmeter, G.; Kluytmans, J.; Carmeli, Y., "Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis", *Lancet Infect. Dis*. 18, 318–327, 2018.

[2] Antunes LCS, Visca P, Towner KJ., "*Acinetobacter baumannii*: evolution of a global pathogen", *Pathog Dis*, 71:292–301, 2014.

[3] Darvishi, M., "Virulence factor Profile and Antimicrobial Resistance of *Acinetobacter baumannii* Strain isolated from various References 114 infection Recovered from immunosuppressive patients", *Biomedical Pharmacology Journal*, 9, 1057-1062, 2016.

[4] Kim, M.H.; Jeong, H.; Sim, Y.M.; Lee, S.; Yong, D.; Ryu, C.M.; Choi, J.Y., "Using Comparative Genomics to Understand Molecular Features of Carbapenem-Resistant *Acinetobacter baumannii* from South Korea Causing Invasive Infections and Their Clinical Implications", *PLoS ONE,* 15, 2020.

https://doi.org/10.1371/journal.pone.0229416

[5] Barbe, V., Vallenet, D., Fonknechten, N., Kreimeyer, A., Oztas, S., Labarre, L., & Ornston, L. N., "Unique features revealed by the genome sequence of Acinetobacter sp. ADP1, a versatile and naturally transformation competent bacterium", *Nucleic acids research*, 32, 5766-5779, 2004.

[6] Hamidian M, Nigro SJ., "Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii", Microb Genom* 5(10):e000306, 2019.

[7] Ahmad, N. H., & Mohammad, G. A., "Identification of *Acinetobacter baumannii* and Determination of MDR and XDR Strains", *Baghdad Science Journal*, 17(3), 726-732, 2020.

[8] Li, P., Li, H., Lei, H., Liu, W., Zhao, X., Guo, L., & Bai, C. "Rapid detection of *Acinetobacter baumannii* and molecular epidemiology of carbapenem-resistant A. *baumannii* in two comprehensive hospitals of Beijing, China", *Frontiers in microbiology*, 6, 950 -997, 2015.

[9] Ali, H. M., Salem, M. Z., El-Shikh, M. S., Megeed, A. A., Alogaibi, Y. A., & Talea, I. A. "Investigation of the virulence factors and molecular characterization of the clonal relations of multidrug-resistant *Acinetobacter baumannii* isolates", *Journal of AOAC International*, 100, 152-158, 2017.

[10] Kareem, S. M., Al-Kadmy, I. M., Al-Kaabi, M. H., Aziz, S. N., & Ahmad, M., "*Acinetobacter baumannii* virulence is enhanced by the combined presence of virulence factors genes phospholipase C (plcN) and elastase (lasB)", *Microbial pathogenesis*, 110, 568-572, 2017.

[11] Mohajeri, P., Sharbati, S., Farahani, A., & Rezaei, Z., "Evaluate the frequency distribution of non-adhesive virulence factors in carbapenemase-producing *Acinetobacter baumannii* isolated from clinical samples in Kermanshah", *Journal of natural science, biology, and medicine*, 7, 58-64, 2016.

[12] Braun, G., & Vidotto, M. C., "Evaluation of adherence, hemagglutination, and presence of genes codifying for virulence factors of *Acinetobacter baumannii* causing urinary tract infection", *Memórias do Instituto Oswaldo Cruz*, 99, 839-844, 2004.

[13] Johnson JR, Stell AL, "Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogenic and host compromise", *Journal of Infected Disease*. 181, 261-272, 2000.

[14] Zong, Z., Lü, X., Valenzuela, J. K., Partridge, S. R., & Iredell, J., "An outbreak of carbapenem-resistant *Acinetobacter baumannii* producing OXA-23 carbapenemase in western China", *International Journal of antimicrobial agents*, 31, 50-54, 2008.

[15] McConnell, M. J., Pérez-Ordóñez, A., Pérez-Romero, P., Valencia, R., Lepe, J. A., Vázquez-Barba, I., & Pachón, J., "Quantitative real-time PCR for the detection of *Acinetobacter baumannii* colonization in the hospital environment", *Journal of clinical microbiology*, 50, 1412-1414, 2012.

[16] Bandana, K., Jashandeep, K. and Jagdeep, K., 'Phospholipases in Bacterial Virulence and Pathogenesis", *Advances in Biotechnology & Microbiology*, 10, 106-113, 2018.

[17] Antunes, L., Visca, P., & Towner, K. J., "*Acinetobacter baumannii*: evolution of a global pathogen", *Pathogens and Disease*, 71, 292-301, 2014.

[18] Stahl, J., Bergmann, H., Göttig, S., Ebersberger, I., & Averhoff, B., "*Acinetobacter baumannii* virulence is mediated by the concerted action of three phospholipases", *D. PloS one*, 10(9): 0138360, 2015.

[19] Yu, H., He, X., Xie, W., Xiong, J., Sheng, H., Guo, S., Zhang, K., "Elastase LasB of *Pseudomonas aeruginosa* promotes biofilm formation partly through rhamnolipid-mediated regulation", *Canadian Journal of Microbiology*, 60, 227-235, 2014.

[20] Cathcart, G. R., Quinn, D., Greer, B., Harriott, P., Lynas, J. F., Gilmore, B. F., Walker, B., "Novel Inhibitors of the *Pseudomonas aeruginosa* Virulence Factor Pseudolysin/LasB: A Potential Therapeutic Approach for the Attenuation of Virulence Mechanisms in Pseudomonal Infection", *Antimicrobial agents and chemotherapy*, 55, 2670–2678, 2011.

[21] AL-Kadmy, I. M., Ali, A. N. M., Salman, I. M. A., Khazaal, S. S., "Molecular characterization of *Acinetobacter baumannii* isolated from Iraqi hospital environment", *New microbes and new infections*, 2, 51-57, 2018.

[22] Momtaz, H., Seifati, S. M., Tavakol, M., "Determining the prevalence and detection of the most prevalent virulence genes in *Acinetobacter baumannii* isolated from hospital infections", *International Journal of Medical Laboratory*, 2, 87-97, 2015.

[23] He, X., Lu, F., Yuan, F., Jiang, D., Zhao, P., Zhu, J., Lu, G., "Biofilm formation caused by clinical *Acinetobacter baumannii* isolates is associated with over-expression of the AdeFGH efflux pump", *Antimicrobial agents and chemotherapy* 59, 4817-4825, 2015.

[24] Ferreira, A., Fuentefria, D., Barth, A., Corção, G., "Molecular characterization of clinical multiresistant isolates of Acinetobacter sp. from hospitals in Porto Alegre, Brazil", *Revista da Sociedade Brasileira de Medicina Tropical*, 44, 725-730, 2011.

[25] Ece, G., Erac, B., Cetin, H. Y., Ece, C., Baysak, A., "Antimicrobial susceptibility and clonal relation between *Acinetobacter baumannii* strains at a Tertiary Care Center in Turkey", *Jundishapur journal of microbiology*, 8, 3-15, 2015.

[26] Ahmed, R. Z., Study the phenotype and genotype of some virulence factors of *Acinetobacter baumannii* isolated from different clinical samples, M.Sc. thesis. Baghdad University. Baghdad, Iraq, 2017.

[27] Li, P., Li, H., Lei, H., Liu, W., Zhao, X., Guo, L., Bai, C. "Rapid detection of *Acinetobacter baumannii* and molecular epidemiology of carbapenem-resistant A. *baumannii* in two comprehensive hospitals of Beijing, China", *Frontiers in microbiology*, 6, 950 -997, 2016.

[28] Medina-Presentado, J. C., Seija, V., Vignoli, R., Pontet, J., Robino, L., Cordeiro, N. F., Savio, E.,"Polyclonal endemicity of *Acinetobacter baumannii* in ventilated patients in an intensive care unit in Uruguay", *International Journal of Infectious Diseases*, 17, 422-427, 2013.

[29] Aljindan, R., Alsamman, K., & Elhadi, N., "ERIC-PCR genotyping of *Acinetobacter baumannii* isolated from different clinical specimens", *Saudi Journal of Medicine and Medical Sciences*, 6, 13, 2018.

[30] Maleki, A.; Vandyousefi, J.; Mirzaie, Z.; Ghafourian, S.; Kazemian H. Sadeghifard, N., "Molecular Analysis of the isolates of Acinetobacter baumannii isolated from Tehran Hospitals Using ERIC-PCR Method", *Modern Medical of Laboratory*, 1, 16-1 2016.

[31] Hammoudi, D., Moubareck, C. A., Hakime, N., Houmani, M., Barakat, A., Najjar, Z., & Sarkis, D. K., "Spread of imipenem-resistant *Acinetobacter baumannii* co-expressing OXA-23 and GES-11 carbapenemases in Lebanon", *International Journal of Infectious Diseases*, 36, 56-61, 2015.