MOLECULAR PHYLOGENETIC STUDY IN 16S RRNA GENE AMONG ACINETOBACTER BAUMANNII ISOLATES CHARACTERISTIC PRODUCING TO ESBLS GENES IN BURN INFECTION

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ABSTRACT *; This study was performed to describe relationship according to 16S rRNA gene sequence for the clinical Acinetobacter baumannii, ESBL-producing isolates were selected were identified in burn Infection in Iraq. Twenty-two months between January 2017 and September 2018, in a cross-sectional study, a total of Twenty-four suspected A. baumannii isolates were isolated from 177 patients hospitalized in our Burn Care Unit. Isolates were identified using conventional biochemical tests and on the basis of the bacterial 16S rRNA gene and chromosomal bla_{OXA-51} gene. Phylogeny and genotypic comparison of this methods, evidence given in this study, it is proposed that isolate is closely related to members of the genus Acinetobacter. Identification and annotation of genotype-varying in the 16S rRNA gene and characterization of their interaction can provide a basis for better understanding of the gene interaction. Computational identification of 16S rRNA gene of Acinetobacter sp. was performed using BPROM tool. The results showed 22 A. baumannii isolates, 11(50%) were ESBL producing isolates included all three genes together with the bla_{CTX-M}, bla_{TEM} and bla_{SHV} genes were detected in 17(77.2%), 12(54.5%) and 6(27.2%) isolates of test bacteria, respectively. A total of 11 out of 22 isolates carried all resistances genes, after sequencing 11 samples were noisy, hence they were excluded. According to phylogenetic analysis, we found that the isolates could be differentiated into groups by a single nucleotide difference within the 16S rRNA sequences also were closely related to strains from Indonesia. Despite the samples were from the same source (currency notes), we found that there is a broad sequence variation between them. The GenBank accession number for 16S rRNA gene of Acinetobacter sp.*

Keywords: Acinetobacter baumannii, ESBLs genes, 16S rRNA genes, Burn infection, Iraq.

1. INTRODUCTION

Phenotypic techniques include evaluation of biochemical characteristics and identification of antimicrobial resistance patterns [1]. These techniques are not powerful enough to identify and differentiate *Acinetobacter* strains and cannot determine the source and ways of distribution and effective measures for controlling the spread of infection and prevalent isolates [2]. Repetitive sequences between genes are often given the genetic relationships between genes or strains are carried out by molecular phylogeny by comparing of homologous DNA sequences such as mitochondrial DNA and ribosomal RNA, which is considered to be an absolute measure of relatedness [3] Within the Ribosomal RNA genes, 16S rRNA is the most universal and consists of highly conserved as well as it is variable regions, therefore it is the most valuable in phylogenetic and developmental [4]. Many studies on the genotypic identification and phylogenetic analysis of *A. baumannii* were performed via several highly specific and sensitive molecular markers including 16S rRNA gene [5]. Genus *Acinetobacter* is one cause of infections in hospitalized patients and especially those in burn infection [6]. Due to biofilm producing ability, they survive longer on dry surfaces or on instruments and disseminate inset hospital environments and cause nosocomial infections [7, 8]. The most common *Acinetobacter* spp. isolated from human samples is *Acinetobacter baumannii* [8]. This bacterium is resistant to many available antibiotics because it has been in contact with other gram-negative pathogens in hospital environments and also exposed to extensive bombardment with antibiotics, so most strains of *A. baumannii* are resistant to tetracycline, amoxicillin, clavulanic acid and penicillin [9]. Presence of resistance genes among in *Acinetobacter* spp. because of the ability to produce β- lactamases enzymes that are a major cause of bacterial resistance to the β -lactam family of antibiotics such as Penicillins Cephalosporins, Cephamicins and Carbapenems[10]. Since, developed third-generation Cephalosporins, e.g. Cefotaxime, Ceftazidime, Ceftriaxone, Cefoxitin, Cefepime, Cefixime and Cephalexin have been introduced as useful and effective drugs against most nosocomial infections and non-specific febrile diseases [11]. However, the excessive use of Cephalosporins in clinical practice has resulted in increased bacterial resistance to these antibiotics especially. These enzymes catalyze the hydrolysis of the amide bond of four-membered β -lactam ring and render the antibiotic inactive against its original cellular target, the cell wall Transpeptidase [11, 12]. Resistance to β-lactamase antibiotics among the *Acinetobacter* is a result of the expression of ESBL genes. Among the numbers of *A. baumannii* has the highest level of ESBLs [13]. Moreover; studies have shown that the most prevalent ESBL-producing bacterium is *A. baumannii* ESBLs arise mainly due to mutations in β-lactamase encoded by the bla_{CTX-M}, bla_{TEM} and bla_{SHV} [14]. The genes encoding β-lactamase enzymes are located on transferable plasmids [15]. Therefore, it can acquire resistance mechanisms from plasmids, integrin's, transposons, and other gram-negative, in addition to its inherent tendency to acquire resistance [15, 16]. Phenotypic and genotypic techniques are used to design effective strategies for controlling infections caused by this bacterium [17]. It was isolated and identified via conventional methods [18, 19], while present studies determining the frequency of resistance to β-lactamase antibiotics; the prevalence of different β-lactamases genes [20, 47], as well as 16S rRNA gene [21]. We planned this study to find out its impact and existing prevalence of EBSLs in *A.baumannii*. After the Isolation and Identification of bacteria, Moreover, we aim to study the relationships according to 16S rRNA gene sequences between our positive isolation disseminated in burn wounds and the closest strains from other countries over the world.

2. Materials and Methods

This prospective study was conducted of Institute of Genetic Engineering and Biotechnology for Postgraduate Studies,

University of Baghdad, Iraq, with the Burn Care Unit in Burn Specialist Hospital of Al Diwaniyah city, Iraq, from January 2017 to September 2018. A total of 290 burn samples (swab) of 177 hospitalized patients.

Microbiology Methods

Dissemination of *A. baumannii* in burn infections in Iraqi patients, isolates were identified after purification on nutrient media and MacConkey agar, routine tests such as producing acid in Oxidative, Fermentation glucose were conducted to identify suspected *A. baumannii* isolates. The identification of the isolates was accomplished by API 20NE.20NE.Then, Inherent genes of *Acinetobacter* strains including16S rRNA and tracking the bla_{OXA51}-like Carbapenemase gene[22], which is intrinsic to this species, using single PCR Table.1, after genotypic and phenotypic confirmation of samples, isolates were moved to an environment of 15% glycerol and 5% liquid Brain-Heart Infusion (BHI) medium for storage and preservation at -70°C.

Phenotypic detection of extended-spectrum β_lactamases (ESBLs)

Phenotypic identification of ESBL producing isolates have been carried out using the detected standard disk diffusion method of combined antimicrobial susceptibility pattern with seven antibiotic disks (Mast, UK) belonging to Cephems class. Antibiogram disks belonging to Cephems class. Briefly, Cefotaxime (30 µg), Cefepime (30 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg) Cefixime (5 µg) and Cephalexin (30 µg) was assessed using Mueller-Hinton agar disc diffusion test. The results were according to the CLSI criteria and manufacturer instruction, an increase in zone diameter of ≥5 mm in the presence of clavulanic acid indicated the presence of ESBL in the test organism. Klebsiella pneumoniae ATCC700603 was used as a positive control for ESBL production [23, 24].

Genotypic detection of extended-spectrum β_lactamases (ESBLs)

For the design of resistance-gene specific PCR primers, reference resistance gene nucleotide sequences were extracted from different databases: β-lactamase genes [26], NCBI [27]. A database ARG-DB (Antibiotic Resistance Gene Database) was for the extracted genes and all entries of ARG-DB were compared to each other by applying the BLAST algorithm [28]. Genes with more than 32 % sequence identity were clustered. Based on CLUSTAL W [29] alignments, a consensus sequence was calculated for each cluster and the gene showing the highest degree of identity to the consensus sequence was defined as representative for the respective cluster. Specific PCR primers were designed for all reference genes by means of the Primer3 program [30] and synthesized. The resulting PCR primer sequences are shown in Table 1 In silicon PCR amplification was performed on an online software http://insilico.ehu.eus/PCR/ [31] and resulting PCR product is computed automatically with the desired band size of a specific gene.

Bacterial genomic DNA extraction, briefly, one colony of a pure culture grown was re-suspended in 50 µl of LB broth and incubated at 37°C in an orbital shaker for 18 h. 50 µl of the bacterial suspension was centrifuged at 3000 rpm for 5 min and the sediment was used for DNA extraction. DNA extraction was performed using a Genomic DNA Extraction kit (Geneaied) according to manufacturers' instructions. The extracted bacterial DNA was checked by using the Nanodrop spectrophotometer (THERMO. USA) that measured DNA concentration (ng/µL) and checked the DNA purity by reading the absorbance at (260 /280 nm), and the supernatant containing DNA was stored at -20°C until tested. PCR reaction was carried out in a 25 µl volumes with 1 µl of extracted DNA, 12.5 µm of the PCR Master Mix containing 1.25 U of Taq Polymerase, 1 × PCR buffer containing 0.1 mM MgCl2 and 240 µM of each dNTP. Specifically designed primers used are including $bla_{\text{CTX-M,}}$ bla_{TEM} and bla_{SHV} were used for PCR amplification of the genes. PCR machine was installed for cycling according to Optimase Protocol Writer™ programme condition as 5 min of initial denaturation at 95°C, then 30 cycles at 95°C for 30 s, D°C for 1 min, 72°C for 40 sec , and finally 72°C for 5 min for amplification of incomplete segments(D: Differentiated) . About 10 µl of each PCR product was loaded into each well of the gel, and electrophoresis was carried out for 45 min at 100 V. The electrophoresis gel was placed on a UV transilluminator and picture was taken from the visible bands. The gel images were saved electronically for further analysis and typing of the isolates.

DNA Sequencing and Bioinformatics analysis

Amplification of the 16S rRNA gene ~1500 bp. was performed by using universal primers 27F (5'-AGAGTTTGATYMTGGCTCAG-3') and 1525R (5'-AGAAAGGAGGTGATCCAGCC-3') and the PCR products were sequenced using 27F, 1525R and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') with an ABI 3100 genetic analyses. PCR protocol as described elsewhere [25]. The PCR products were electrophoresed on a 2% agarose gel containing 0.5 µg/ml ethidium bromides to determine the size of the product. Both negative and reagent controls were included in each PCR run. In order to amplify the desired sequence, in a volume of 50 µl, 2.5 units of Taq polymerase, 50 pM of each primer, 200 µM of each four deoxynucleotides, 1 µl of template DNA, 1.5 µM of MgCl2, 10 mM of Tris-HCl (pH 8.3), and 50 mM of KCl, were used. PCR was performed using an Eppendorf gradient thermocycler involved initial denaturation cycle at 94°C for 5 minutes, followed by 35 amplification cycles (1 minute at 94°C, 1 minute at 58°C and 2 minute at 72°C) as well as final extension cycles at 72°C for 10 minutes[25]. After that, agarose gel electrophoresis was carried out against the obtained PCR amplicons via Amersham gel electrophoresis device according to the protocol described by Lee. P. Y *et al.* [32] and the PCR products were sent Macrogen Company-Netherlands for purification and nucleotide sequencing of both strands.

After obtaining the sequences, firstly they were checked and corrected them manually via Finch TV software [33]. From the received sequences, 11 sequences were identified as *A. baumannii* via nucleotide BLAST tool at NCBI [34]. After that, various bioinformatics tools were used including GenBank database to obtain the sequences of previously identified genomovars and strains with higher identity. In addition to that, BioEdit software [36] for the visualization

Table.1 Bacterial primers used in this study.

Primer	Primer sequence(Forward/Reverse)	Target(Sizes of amplification bp)	Annealing temperature	Ref
16S rRNA	F 5'-CAGCTCGTGTCGTGAGATGT-3' R 5'-CGTAAGGGCCATGATGACTT-3'	16SrRNA *Acinetobacter* spp.(~150)	55℃	[22]
OXA-51	F 5'-TCGTGCTTCGACCGAGTATG-3' R 5'-GAGGCTGAACAACCCATCCA-3'	$bla_{\text{OXA-51}}$ (~506)	53℃	This study
CTX	F 5'-ACCGCCGATAATTCGCAGAT-3' R 5'-CGGCCAGATAACCGCGATAT-3'	$bla_{\text{CTX-M}}$ (~600)	58.3℃	This study
TEM	F 5'-AACTGGATCTCAACAGCGGG-3' R 5'- TTCATTCAGCTCCGGTTCCC-3'	bla_{TEM} (~300)	59.3℃	This study
SHV	F 5'-TTCCCATGATGAGCACCTTT-3' R 5'-CGCTGTTATCGCTCATGGTA-3'	bla_{SHV} (~250)	57℃	This study

of multiple sequence alignment. Moreover, MEGA 7.07[37] was used for phylogenetic analysis and Unipro UGENE software [38] was used for the calculation of simple identity in per cent. The closest strains (sequences with higher identity in BLAST search) that we used for phylogenetic analysis with their accession numbers are listed Table 4 Nucleotide Sequence Accession Numbers the identified nucleotide sequences were deposited in the GenBank database [34] under the accession numbers that listed in Table 3.

3. RESULTS:

Bacterial Isolation and Characterization

During the study period, based on conventional phenotypic and biochemical features, 24(8.27%) suspected *A. baumannii* isolates [35]. These isolates of the *A.baumannii* bacteria were encoded. These were A_1, A_2, A_3, A_4, A_5, A_6, A_7, A_8, A_9, A_{10}, A_{11}, A_{12}, A_{13}, A_{14}, A_{15}, A_{16}, A_{17}, A_{18}, A_{19}, A_{20}, A_{21}, EA_{22}, EA_{23} and EA_{24}, to prepare for tests. Twenty-two isolates were positive for $bla_{\text{OXA-51}}$-like and identified as *A. baumannii* but two of them were negative consequently they were excluded Fig1 and Fig2.

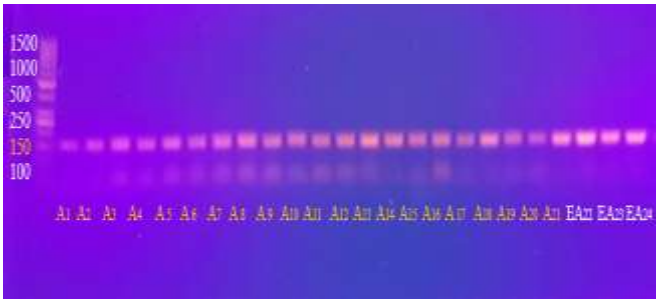

Figure 1: Result agarose gel electrophoresis of PCR amplified. Lane (A1-EA24) show a positive result with positive bands of 150 bp. products from amplified primers of 16S rRNA gene with extracted DNA .

Antimicrobial Susceptibility Test

On antibiogram tests, twenty-two *A. baumannii* strains were screened to be extended-spectrum β-lactamase-producing (ESBL) and most isolates were Cephems resistant (data not shown). In Cephems -resistant A. baumannii isolates, phenotyping showed resistance to Ceftriaxone (100%), Cefotaxime (91.6%), Cefoxitin (87.5%), Ceftazidime (83.3%), cefepime (95.8%), Cefoxitin (87.5%), Cefixime (79.16%) and Cephalexin (83.7%) were observed

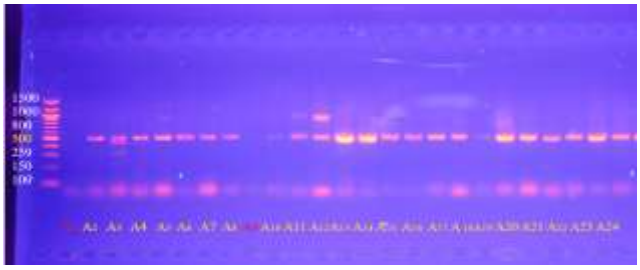

Figure 2: Agarose gel electrophoresis of PCR amplified $bla_{\text{OXA-51}}$ gene showing product size ~506 bp. That represents *Acinetobacter baumannii* isolates.

Table. 2. Ten out of 22 bacterial strains were resistant to all antibiotics tested in this study.

Table 2. Percentage of antimicrobial susceptibility rate of 24 *A.baumannii* isolates against seven antimicrobial agents.

Antibiotics code*	Resistance number isolates	Sensitivity number isolates
FOX	21 (87.5%)	3 (15.5%)
CTX	22 (91.6%)	2 (8.3%)
CRO	24 (100%)	0
CAZ	20 (83.33%)	4 (16.6%)
FEP	23 (95.8%)	1 (4.1%)
CFM	19 (79.16%)	5 (20.8%)
CL	20 (83.33%)	4 (16.6%)
Mean	**21 (88.7%)**	**3 (15.5%)**

* FOX- Cefoxitin; CTX – Cefotaxime; CRO – Ceftriaxone; CAZ –Ceftazidime; FEP- Cefepime; CFM- Cefixime; CL- Cephalexin (Grey shading indicates resistant strain)

Phenotypic and Genotypic ESBL Detection

Of the 22 *A. baumannii* isolates, 11 were ESBL producing and had been obtained from clinical samples, from surgical wounds. Three ESBL-negative isolates carried antibiotic-resistance genes. A total of 19 out of 22 isolated samples carried one, two or more ESBL genes. The results showed that the differences between phenotypic and genotypic identification methods were significant (P = 0.0001). Six bacteria had both the bla_{SHV} gene and the $bla_{\text{CTX-M}}$ gene. The ble_{SHV}, $ble_{\text{CTX-M}}$, and ble_{TEM} genes were detected in A_6, A_7, A_8, A_{10}, A_{11}, A_{12}, A_{13}, A_{16}, A_{18}, A_{20} and A_{21} bacterial isolates tested, respectively, Table 3. The frequency

Table. 3 Isolates were designated susceptible (S), intermediate (I), or resistant (R) according to antibiotic breakpoint guidelines of the CLSI for *Acinetobacter*spp. FOX- Cefoxitin; CTX – Cefotaxime; CRO – Ceftriaxone; CAZ –Ceftazidime; FEP- Cefepime; CFM- Cefixime; CL- Cephalexin (Grey shading indicates resistant strain).

A.baumannii Isolates	Phenotypic ESBL Detection							Genotypic ESBL Detection			Isolates Produced ESBLs
								ESBLs Genes			
	FOX	CTX	CRO	CAZ	FEP	CFM	CL	*bla*$_{CTX-M}$	*bla*$_{TEM}$	*bla*$_{SHV}$	
A_2	R	R	R	R	R	I	I		✓		
A_3	S	I	I	R	I	S	I				
A_4	S	R	R	R	R	R	S		✓		
A_5	S	I	R	R	R	S	S				
A_6	**R**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓		**AB_1**
A_7	**R**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓		**AB_2**
A_8	**R**	**R**	**R**	**I**	**R**	**R**	**R**	✓	✓	✓	**AB_3**
A_{10}	**R**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓	✓	**AB_4**
A_{11}	**R**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓		**AB_5**
A_{12}	**R**	**R**	**R**	**R**	**R**	**S**	**R**	✓	✓		**AB_6**
A_{13}	**R**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓		**AB_7**
A_{14}	R	R	R	R	R	R	R	✓			
A_{15}	R	R	R	R	R	R	R	✓		✓	
A_{16}	**R**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓	✓	**AB_8**
A_{17}	I	R	R	R	R	R	R	✓			
A_{18}	**I**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓		**AB_9**
A_{19}	S	R	I	S	S	I	S				
A_{20}	**R**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓	✓	**AB_{10}**
A_{21}	**R**	**R**	**R**	**R**	**R**	**R**	**R**	✓	✓	✓	**AB_{11}**
EA_{22}	R	R	R	S	R	R	R	✓			
EA_{24}	I	R	R	R	R	R	R	✓			
EA_{23}	S	R	R	R	R	R	R	✓			

of the *bla*$_{CTX\_M}$ gene was significantly higher than the other genes (P = 0.0001). Figure 3.

Nucleotide Sequences of 16S rRNA species.

Of these, all samples yielded growths of *Acinetobacter* subset of eleven bacterial isolates that were found resistant to any of the third generation Cephalosporins was selected for detailed screening for *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$. ~1500-bp 16S rRNA fragment was amplified from eleven isolates. Nucleotide sequence analysis of 16S rRNA of PCR products revealed that for all isolates, phylogenetic structure based on 16S rRNA. The phylogenetic tree based on the 16S rRNA nucleotide sequences is shown in Fig. 5

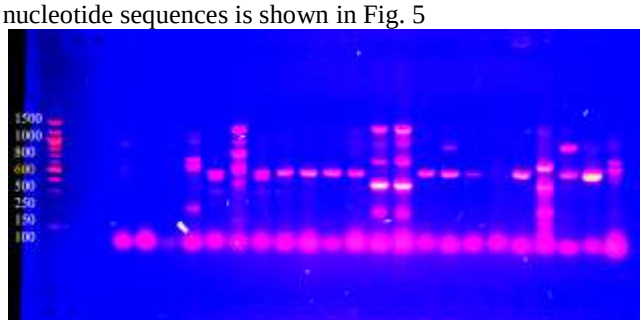

Figure 3: amplified *bla*$_{CTX-M}$ gene showing product size ~600bp. That represents *A. baumannii* isolates.

4. DISCUSSION

Genotypic-based identification eliminates the problem of variable phenotype and allows for more accurate identification of bacteria. The use of 16S rRNA in the classification of bacterial species has now been

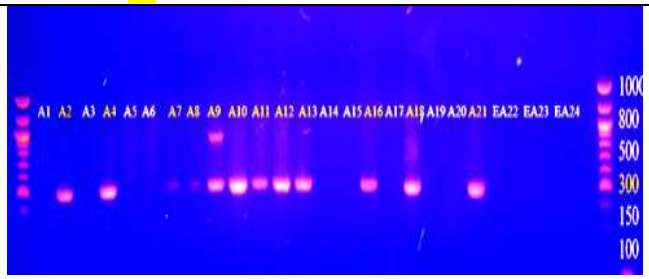

Figure 4: Amplified *bla*$_{TEM}$ gene showing product size ~300bp. that represents *A. baumannii* isolates.

well established and 16S rRNA gene sequencing is now the gold standard of bacterial identification. 16S rRNA genes are highly conserved among all organisms. However, all organisms possess various unique species-specific regions that allow for bacterial identification. The 16S rRNA genes from all of the isolates were amplified. Recently, more precise and accurate identification requires DNA-based methods, which are increasingly used; *bla*$_{OXA-51}$-like oxacillinases are chromosomally-encoded, have low-level carbapenemase activity and are widely distributed among *A. baumannii* strains [39]. Where our results revealed that the *ble*$_{OXA-51}$ gene was predominant in *A.baumannii* tests since this gene was detected in 91.6% of our isolates, although *Acinetobacter* spp. may harbour other genes *rpoB* as an accurate tool for identification [40]. The results of the present study confirmed results other studies, in which reported that in all thirty 56 MDR *A. baumannii* isolates from 1333 patients in five ICUs were amplified *bla*$_{OXA-51}$-like genes [47]. Also, Identification of *A. baumannii* strains was performed by biochemical and

bla$_{OXA-51}$ gene detection tests, which may not have appropriately discriminated all species from the *A. baumannii/calcoaceticus* complex [42]. In the current study, both phenotypic and molecular methods were identified *A. baumannii* isolates from burn and wounds. Results from them, 24 (8.27%) and 22 (7.5%), respectively, our finding represented extremely high rate. This was in line with a report by Joshi *et al*., with more than 75% MDR among their *Acinetobacter* isolates [43]. Similarly, a recent report from Mexico represented higher multidrug resistance as 96.6% among their tested *Acinetobacter* strains [44].

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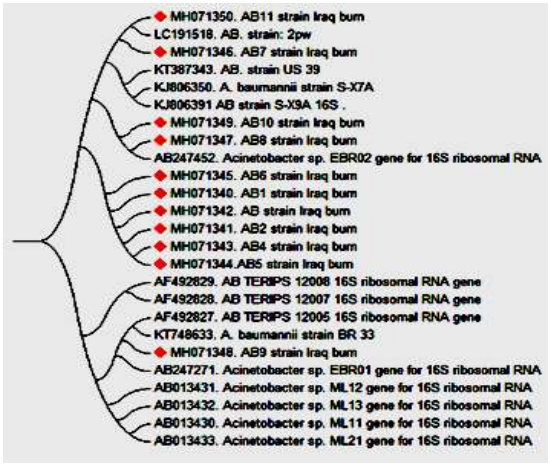

Figure 5. Molecular Phylogenetic Analysis on samples and strains with higher identity via 16S rRNA gene sequences.

Table .4 Eleven isolates with the source, region and accession number

No.& name	Source/Region	Accession number	GI: data base
AB_1	Iraq burn isolate	MH071350	GI: 1363752022
AB_2	Iraq burn isolate	MH071349	GI: 1363752023
AB_3	Iraq burn isolate	MH071348	GI: 1363752024
AB_4	Iraq burn isolate	MH071347	GI: 1363752025
AB_5	Iraq burn isolate	MH071346	GI: 1363752026
AB_6	Iraq burn isolate	MH071345	GI: 1363752027
AB_7	Iraq burn isolate	MH071344	GI: 1363752028
AB_8	Iraq burn isolate	MH071343	GI: 1363752029
AB_9	Iraq burn isolate	MH071342	GI: 1363752030
AB_{10}	Iraq burn isolate	MH071341	GI: 1363752031
AB_{11}	Iraq burn isolate	MH071340	GI: 1363752032

A likely explanation for the relatively low level of resistant *Acinetobacter* isolates in latter studies is probably the restricted use of antibiotics, which is in line with the practice regarding antimicrobial therapy in their countries, the strict isolation of patients with MDR isolates, and the immediate beginning of infection control measures when several patients are infected with the same strain. The uncontrolled use of antibiotics agents, especially antibiotics, for the treatment of infectious diseases has resulted in the emergence of highly antibiotic-resistant bacteria in the last few decades [10]. Third-generation Cephalosporins are some of the most effective antibiotics used against gram-negative bacterial infections. ESBLs play a very important role in the resistance to β-lactam antibiotics, via the destruction of the β-lactam cycle in the β-lactam antibiotic structure. The *bla*$_{CTX-M}$, *bla*$_{TEM}$ and *bla*$_{SHV}$ genes are the most common genes that encode ESBLs [11].

In the present study, ESBLs were detected based on phenotypic and molecular methods by combined antibiotic disks and PCR, respectively. Among 24 isolates, 21 (87.5%) were positive with the combined antibiotic disc method. On the other hand, 19 (79.1%) were positive for at least one of the resistance genes using PCR. The differences between our phenotypic and genotypic results and the frequency of resistance genes, and the results obtained in other studies [45, 20] may be due to different geographical locations and to the number and source of the isolated bacteria. On the other hand, our results', showing a high rate of isolation of antibiotic-resistant strains of *A. baumannii*, was consistent with other studies. This shows that the rates of resistant *A. baumannii* from different sources, especially in wounds, are high enough to interrupt effective antibiotic therapy. This also draws attention to the use of alternative ways to treat these bacterial infections. In the present study reported that 54.1% of ESBL positive isolates were carrying *bla*$_{TEM}$ genes, 25% *bla*$_{SHV}$, 70.8% *bla*$_{CTX-M}$, while 25% of isolates included all three genes together. The dissimilarity (87.5%) between the results of these two different methods might be related to the higher sensitivity of the molecular method, as well as to the effect of environmental factors or culture conditions, which can temporarily down-regulate some of the resistance genes [14]. In addition, in the current study, the frequency of the genes encoding ESBLs were 29 (60.4%), 10 (28.8%), and 9 (18.7%) for *bla*$_{SHV}$, *bla* $_{CTX-M}$, and *bla*$_{TEM}$, respectively. The negative amplification in the remaining isolates may be due to the presence of other ESBL genes, which we did not study further.

In this paper, 11 bacterial strains isolated from the burn care unit of Al Diwaniyah city, Iraq, were identified at the species level by sequencing the gene encoding 16S rRNA. It is sequence the whole 1,500-bp length to distinguish between particular taxa or strains [46], and usually required when describing a new species. From the MicroSeq databases of over 1,400 organisms for both the 500- and 1,500-bp lengths, we compared the 500- and 1,500-bp sequences for 100 organisms by using each length to generate dendrograms and found the relationships of species to be basically the same with either length. Fig.5 shows that dendrograms generated using the 1,500-bp 16S rRNA gene sequence of a group of clinical bacteria are similar but not identical. The existence of 11 different genetic profiles indicated genotypic variability and distribution. The diversity of genetic pattern among the isolates is due to a wide variety of hospitals, otherwise, several strains with identical genetic patterns and common origin were being definitely observed. Investigation of genetic similarity and diversity among different strains of a bacterial species by DNA fingerprinting is a useful method for detection of strains involved in outbreaks as well as the determination of epidemiological relationship among the isolates. Identification of an acceptable level of genetic diversity among the isolates by this technique indicated that this method is useful for studying and typing *A. baumannii* isolates, and isolates with different origins can be classified into different groups using this method. From the results of this study, it is concluded that repetitive sequences can be used for typing of *A. baumannii* strains.

Conclusion:

The availability of molecular techniques for fast and reliable genotypic characterization should increase our knowledge of

ecology, structure and dynamics of microbial communities. *A. baumannii* was identified as the most prevalent species with high ESBLs resistance. Other species showed lower frequencies ranged from 7 to 9 strains.

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REFERENCES:

[1] Wenzler E, Goff DA, Mangino JE. Impact of rapid identification of *Acinetobacter baumannii* via matrix-assisted laser desorption ionization time-of-flight mass spectrometry combined with antimicrobial stewardship in patients with pneumonia and/or bacteremia. Diagn Microbiol Infect Dis 2016;84:63-8.

[2] Patwardhan A RS, Roy A (2014) Molecular Markers in Phylogenetic Studies – A Review. J Phylogen Evolution Biol 2. doi: 10.4172/2329-9002-2-131.

[3] Bowman JS, Ducklow HW. Microbial communities can be described by the metabolic structure: a general framework and application to a seasonally variable, depth-stratified microbial community from the coastal west antarctic peninsula. PLoSONE. 2015; 10(8):1–18. https://doi.org/10.1371/journal.pone.0135868.

[4] Alemu A (2014) Microbial contamination of currency notes and coins in circulation: a potential public health hazard. Biomedicine and Biotechnology 2: 46-53.

[5] Patwardhan A RS, Roy A (2014) Molecular Markers in Phylogenetic Studies – A Review. J Phylogen Evolution Biol 2. doi: 10.4172/2329-9002-2-131

[6] WHO, "Multidrug-resistant *Acinetobacter baumannii* (MDRAB)," Fact Sheet 1, World Health Organisation, Metro Manila, Philippines, 2010.

[7] Richards A.M, Abu Kwaik Y, Lamont RJ. Code blue: *Acinetobacter baumannii*, a nosocomial pathogen with a role in the oral cavity. Mol Oral Microbiol 2015;30(1):2-15.

[8] K. J. Towner, "*Acinetobacter*: an old friend, but a new enemy," Journal of Hospital Infection, vol. 73, no. 4, pp. 355–363, 2009. View at Publisher.·

[9] Lin MF, Lan CY. Antimicrobial resistance in Acinetobacter baumannii: From bench to bedside. World J Clin Cases 2014;2(12):787-814.

[10] Szejbach A, Mikucka A, Bogiel T, Gospodarek E. Usefulness of phenotypic and genotypic methods for metallo-beta-lactamases detection in carbapenem-resistant *Acinetobacter baumannii* strains. Med Sci Monit Basic Res 2013;19:32-6.

[11] European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. In: Annual report of the European antimicrobial resistance surveillance network (EARS-net). Stockholm: ECDC. p. 2017.

[12] Bitterman R, Hussein K, Leibovici L, Carmeli Y, Paul M. Systematic review of antibiotic consumption in acute care hospitals. Clin Microbiol Infect. 2016;22(6):561 e7–561e19. doi: 10.1016/j.cmi.2016.01.026.

[13] Périchon B, Goussard S, Walewski V, Krizova L, Cerqueira G, Murphy C, Identification of 50 class D β-lactamases and 65 *Acinetobacter*- derived cephalosporinases in *Acinetobacter* spp. Antimicrob Agents Chemother 2014 58(2):936-49..

[14] Shahid M, Singhai M, Malik A, Shukla I, Khan HM, Shujatullah F, Tahira F. In vitro efficacy of ceftriaxone-sulbactam against *Escherichia coli* isolates producing $bla_{\text{CTX-M-15}}$ extended spectrum β-lactamase. J Antimicrob Chemother 2007; 60: 187-8.

[15] Bertini, A.; Poirel, L.; Mugnier, P.D.; Villa, L.; Nordmann, P.; Carattoli, A. Characterization and PCR-based replicon typing of resistance plasmids in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 2010, 54, 4168–4177.

[16]Woodford, N., Turton, J. F. & Livermore, D. M. Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev 35, 736–755 (2011).

[17]Hamidian M, Hall RM. 2016. *Acinetobacter baumannii* ATCC 19606 carries GIsul2 in a genomic island located in the chromosome. Antimicrob Agents Chemother. 61(1):e01991–16. pii: e01991-16

[18] Mindolli PB, Salmani MP, Vishwanath G, Hanumanthappa AR. Identification and speciation of *Acinetobacter* and their antimicrobial susceptibility testing. Al Ameen J Med Sci. 2010;3:345–9.

[19] Ahmed,S.S. E. Alp Genotyping methods for monitoring the epidemic evolution of *A. baumannii* strains J Infect Dev Ctries, 9 2015, pp. 347-354

[20] Kais K. G., Distribution of extended spectrum beta-lactamase (ESBL) genes among *Acinetobacter baumannii* isolated from burn infections. MOJ Cell SciRep.2018;5(3):42.46.DOI:10.15406/mojcsr.2018.05.00112

[21] Turton,J. Shah F. Ozongwu,J., Pike C. R., Incidence of Acinetobacter species other than A. baumannii among clinical isolates of Acinetobacter: evidence for emerging species J Clin Microbiol, 48 (2010), pp. 1445-1449

[22] Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research 22: 4673-4680.

[23] Krahn, T.; Wibberg, D.; Maus, I.; Winkler, A.; Bontron, S.; Sczyrba, A.; Nordmann, P.; Pühler, A.; Poirel, L.; Schlüter, A. Intraspecies transfer of the *chromosomally encoded* Acinetobacter baumannii $bla_{\text{NDM-1}}$ carbapenemase gene. Antimicrob. Agents Chemother. 2016, 60, 3032–3040.

[24] Clinical and Laboratory Standards Institute (CLSI) (2012) Performance standards for antimicrobial susceptibility testing. Twenty-second informational supplement. Tech Rep M100- S22; Fort Wayne, USA. 2012:1–230.

[25] Chang HC, Wei YF, Dijkshoorn L, Vaneechoutte M, Tang CT, Chang TC. Species-level identification of isolates of the *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex by sequence analysis of the 16S-23S rRNA gene spacer region. J Clin Microbiol 2005;43(4):1632-9.

[26] ß-Lactamase Classification and Amino Acid Sequences . 32) (http://www.lahey.org/Studies/)

[27] Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2013) GenBank. Nucleic acids research 41: D36-D42.

[28] Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive

multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research 22: 4673-4680.

[29] Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic acids research 22: 4673-4680.

[30] San Millán RM, Martínez-Ballesteros I, Rementeria A, Garaizar J, Bikandi J (2013) Online exercise for the design and simulation of PCR and PCR-RFLP experiments. BMC Res Notes 6: 513.

[31] Bikandi J, San Millán R, Rementeria A, Garaizar J (2004) In silico analysis of complete bacterial genomes: PCR, AFLP-PCR and endonuclease restriction. Bioinformatics. 20: 798-799.

[32] Lee PY, Costumbrado J, Hsu C-Y, Kim YH (2012) Agarose gel electrophoresis for the separation of DNA fragments. JoVE (Journal of Visualized Experiments): e3923-e3923.

[33] Finch TV software, (http://www.geospiza.com/ftvdlinfo.html).

[34] (http://www.ncbi.nlm.nih.gov/)

[35] Bruce et al., Microbiology: practical applications and infection prevention.2016

[36] Bio Edit software. (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

[37] Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA7: molecular evolutionary genetics analysis version 7.07. Molecular biology and evolution 30: 2725-2729

[38] Okonechnikov K, Golosova O, Fursov M (2012) Unipro UGENE: a unified bioinformatics toolkit. Bioinformatics 28: 1166-1167.

[39]Higgins P. G., Pérez-Llarena F. J., Zander E., Fernández A., Bou G., Seifert H. OXA-235, a novel class D *β*-lactamase involved in resistance to carbapenems in *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy*. 2013;57(5):2121–2126. doi: 10.1128/AAC.02413-12.

[40]Lee MJ, Jang SJ, Li XM, Park G, Kook JK, Kim MJ, Co mparison of *rpoB* gene sequencing, 16S rRNA gene sequencing, *gyrB* multiplex PCR, and the VITEK2 system for identification of *Acinetobacter* clinical isolates *Diagn Microbiol Infect Dis* 2014 78(1):29-34.

[41] Pagano M, Martins AF, Machado AB, Barin J, Barth AL. Carbapenem-susceptible Acinetobacter baumannii carrying the ISAba1 upstream blaOXA-51-like gene in Porto Alegre, southern Brazil. Epidemiol Infect. 2013;141:330–3. doi: 10.1017/S095026881200074X

[42] Chan JZ, Halachev MR, Loman NJ, Constantinidou C, Pallen MJ. Defining bacterial species in the genomic era: insights from the genus *Acinetobacter*. BMC Microbiol. 2012;12:302 doi: 10.1186/1471-2180-12-

[43] Jesús , M.D., Jose, M. C., Felipe Fernández- Cuenca, M.D and Anna Ribera, M.D. (2004)Clinical Features and Epidemiology of *Acinetobacter baumannii* Colonization and Infection in Spanish Hospitals. Infection Control and Hospital Epidemiology, Vol. 25, No. 10 .pp. 819-824.

[44] Ana M., Gonzalez-V., and VeronicaV. Antibiotic-Resistant Acinetobacter baumannii Increasing Success Remains a Challenge as a Nosocomial Pathogen. Hindawi Publishing Corporation Journal of Pathogens Volume 2016, Article ID 7318075, 10 pages http://dx.doi.org/10.1155/2016/7318075

[45] Mohammed S., Anuradha S., Farrukh S., Mohammad R., Abida M., Indu S., Haris M. K. $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} in Enterobacteriaceae from North-Indian tertiary hospital: high occurrence of combination genes. Asian Pacific Journal of Tropical Medicine (2011)101-105.

[46] Sacchi, C. T., A. M. Whitney, M. W. Reeves, L. W. Mayer, and T. Popovic. 2002. Sequence diversity of Neisseria meningitidis 16S rRNA genes and use of 16S rRNA gene sequencing as a molecular subtyping tool. J. Clin. Microbiol. 40:4520-4527

[47] Suellen R. A. C., Cássia S.M. G. , Adriana O. G.J., Lamaro C.,1. Maria C. P. André, 1 Ana Paula J. and André K. Acinetobacter baumannii strains isolated from patients in intensive care units in Goiânia, Brazil: Molecular and drug susceptibility profiles PLoS One. 2017;12(5):e0176790. doi:10.1371/journal.pone.0176790.