Genetic Variations in *rplB* Gene Associated with Multidrug Resistance *Acinetobacter baumannii* Isolated from Different Clinical Sources

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Abstract

In this study, two hundred specimens were collected from patients suffering from urinary tract infection, wound infection, otitis infection and respiratory tract infection (fifty specimens from each infection). After laboratory diagnosis by biochemical tests and confirmation by advance tests (VITEK- 2 Compact system), only twenty isolates were diagnosed as *Acinetobacter baumannii* (10%). Then sensitivity test was carried out of all these isolates by using twelve antibiotics, all isolates exhibited various levels of resistance to different antibiotics. Then DNA extraction of all twenty isolates followed by amplification of *rplB* gene aggainst using PCR with specific primers. DNA sequencing of all isolates was done, then alignmented sequences in NCBI and drew phylogenetic tree by using Geneious 9 software among locally isolates alone and then between them and high identity globally identified registred isolates in Gene Bank. Distribution of locally isolates in phylogenetic tree showed three different groups. The phylogenetic tree showed there were eight locally isolates differed from the standard isolates. From these locally isolates, one isolate (AE_12) was documented in NCBI under accession number (LOCUS KY818058) of nucleotides sequence and protein ID "ARV90996.1". [DOI: 10.22401/JNUS.21.3.14]

Keywords: *rplB* gene, 50S ribosomal protein, *Acinetobacter baumannii*, LOCUS KY818058, protein ID "ARV90996.1".

Introduction

Acinetobacter baumannii is a non-fermentative Gve- bacillus, and considered as a nosocomial pathogen. These bacteria contain genes encoded for high virulent factors and it has the ability to exhibit multidrug resistance by different mechanisms [1,2,3]. In classification of the genus *Acinetobacter* by Bouvet and Grimont, 1986, who recorded 12 genospecies (DNA groups) depend on techniques of DNA-DNA hybridization. Other molecular techniques were done to diagnose 33 named and unnamed species of *Acinetobacter* bacteria(4). This bacteria cause different infections included pneumonia, meningitis, urinary tract infections, bacterimia and wound infections [5,6].

Acinetobacter baumannii contain *rplB* housekeeping gene that encode to the 50S ribosomal protein L2. It is essential for ribosome activity and is a major component of the peptidyl transferase. This protein included in process activity (peptidyl transferase activity and binds to functionally important domains of 23S rRNA), one of the rRNA binding proteins, and contacts with the 16S rRNA in the 70S ribosome [7,8]. Therefore, *rplB* gene is very important housekeeping gene and could be use as phylogenetic marker in epidemiological study of *A. baumannii* strains multidrug resistance [9]. The objective of the present study is genetic variations in *rplB* gene associated with multidrug resistance *A. baumannii*.

Materials and Methods

Samples collection

In this study, two hundred specimens were collected from patients with UTI, wounds, otitis and respiratory tract infection. Fifty specimens were taken from each infection. From some hospitals (AL-Kindy teaching hospitals, Imam Ali hospitals, AL-Sader hospitals) in Baghdad during the period from September to December 2016.

Isolation and identification

Samples were cultured on MacConkey agar and incubated for 18-24 h at 37^{o}C. Non lactose fermenter isolates were cultured onto CHRO Magar Tm medium and incubated for 18-24 h at 37^{o}C. *Acinetobacter* appeared as a red colony after the incubation period. The isolates were tested by their morphological

characteristics and standard biochemical tests according to MacFaddin, (2000) [10]. Confirmation of *Acinetobacter* spp. isolates was carried out by VITEK- 2 Compact system to identification *Acinetobacter* isolates to species level according to manufactures' instructions (Biomerieux/ France).

Antibacterial sensitivity test

The isolates were tested against twelve types of antibiotics, they were Pipercilin100 µg, Ticarcillin 75 µg, Ceftazidime 30 µg, Cefepime 30 µg, Ceftriaxone 30 µg, Cefotaxime 30µg, Meropenem 10µg, Imipenem 10 µg, Amikacin 30 µg, Tetracycline 30 µg, Ciprofloxacin 5 µg, Levofloxacin 5 µg. The results were compared with National Committee for Clinical Laboratory Standard (CLSI, 2014)[11]

DNA extraction and PCR assay

Genomic DNA extraction of all twenty isolates was achieved by specific kit (wizard® genomic DNA purification kit, Promega company, USA) according to manufacturer's instructions. Amplification of the *rplB* gene was performed with specific primer pair Table (1). The cycling conditions were: Initial denaturation at 94ºC for 2 min, and 30 cycles of denaturation at 94ºC for 30 s, annealing at 50ºC for 30 s, extension at 72ºC for 30 S and a final extension at 72ºC for 5 min. A molecular marker (promega/ USA effective size range: 100 to 1500 bp) was used to assess PCR product size.

Table (1)
Sequence of oligonucleotides primer pair used for amplification of rplB gene.

Primer type	Sequence 5'→3'	Reference
Forward	GTAGAGCGTATTGAATACGATCCTAACC	Laure *et al*., 2010 [12]
Reverse	CACCACCACCATGCGGGTGATC	

Molecular analyses of *rplB* gene

A phylogenetic study was carried out using PCR products of all isolates from the amplification of *rplB* gene, they were sent for sequencing using ABI3730XL, automated DNA sequencer, by Macrogen Corporation-Korea. Results were analyzed using Geneious software. The sequenced DNA analyzed in BLASTn tool of NCBI GenBanK database. High identical strain compared with query sequences available in the GenBanK database and alignment match with very high identity to the homologues found in GeneBank. Gene sequences identities were also computed using the pairwise alignment by Geneious 9 software. Multiple sequence alignments were performed using Geneious alignment. phylogenetic analyses were concluded by the maximum likelihood method.

Results and Discussion

Isolation and identification

Identification of two hundred clinical samples was carried out by routine methods and confirmation by using VITEK- 2 Compact system, the results showed that only (20) isolates were *A. baumannii* from (200) clinical samples in percentage (10%). Eleven isolates were from urine, 4 isolates from wound, 3 isolates from sputum and 2 isolates from otitis.

In a local study carried out by Adnan *et al*. (2014)[13], the percentage of infection with these bacteria was (10.3%) in different clinical samples. Another local study by Mosafer, 2007[14] identified *A. baumannii* from different clinical sources and the percentage of isolates was (7%). The infection with *A. baumannii* increased significantly and found in different regions worldwide because this bacterium is an important nosocomial pathogens and has different virulence factors [15].

Antibacterial susceptibility test

Results showed that isolates exhibited various rates of resistance against 12 antibiotics, namely Pipercilin100 µg (85%), Ticarcillin 75µg (85%), Ceftazidime 30µg (90%), Cefepime 30 µg (80%), Ceftriaxone 30µg (85%), Cefotaxime 30µg (90%), Meropenem 10µg (60%), Imipenem 10µg (35%), Amikacin 30µg (30%), Tetracycline 30µg (85%), Ciprofloxacin 5 µg (90%),

Levofloxacin 5 µg (90%). The isolate AE_9 was more resistant than other isolates which were resistant to all antibiotics (100%) but the isolate AE_19 showed lower resistance than other isolates resisted 2 antibiotics (17%) as shown in Table (2).

The resistance bacteria against antibiotics increased through conjugation and bacterial mutation [16]. *Acinetobacter baumannii* contain an 86-kb resistance island, AbaR1, contains genes encoded to resistance as many as 25 antibiotics and 20 antiseptics and heavy metals[17]. This island variant in sequences of nucleotides lead to multidrug resistance [18]. There are endogenous functions in this bacteria increased resistance like overexpression process of β-lactamases (ADC and OXA-51-) affect on porins (CarO and Omp33–36) that increase bacterial resistant. The change in nucleotide sequence of gene (*GyrA* and *ParC*) targets and increase overexpression of resistance mechanisms (efflux systems)[19] lead to resistant levels. This resistance mechanism included different proteins (AdeB or AdeJ) that cause of destruction bacterial membrane stability and exit antibiotic to the outside of the cell [20, 21]. *A. baumannii* contain *adeABC* operon that increase resistance levels. Eflux pumb mechanism (AdeABC) play important role in distribution bacterial resistant in clinical sources samples [22].

Table (2

Antibiotic sensitivity test results of *A. baumannii* that distributed in clinical samples.

***A.baumannii* isolate**	**Antibiotics concentration (µg)**												**Percent of Resistance (%)**
	PI	**TI**	**CAZ**	**FEP**	**CRO**	**CTX**	**IPM**	**MEM**	**AK**	**TE**	**CIP**	**LEV**	
AE_1	-	+	+	+	-	-	-	-	-	-	-	-	35
AE_2	+	+	+	+	+	+	-	-	-	+	+	+	75
AE_3	+	-	+	-	+	-	-	-	-	+	-	+	42
AE_4	+	+	+	+	+	+	+	+	-	+	+	+	92
AE_5	+	+	-	+	+	+	-	+	-	-	+	-	59
AE_6	-	+	+	-	-	+	-	-	-	+	+	+	50
AE_7	+	+	+	+	-	+	-	-	-	+	+	+	67
AE_8	+	-	+	+	+	+	+	+	+	+	+	+	92
AE_9	+	+	-	-	+	+	-	-	-	+	+	+	59
AE_10	+	+	+	+	+	+	-	+	-	-	+	+	75
AE_11	+	+	+	+	+	+	+	+	+	+	+	+	100
AE_12	+	+	+	+	+	+	+	+	+	+	+	+	100
AE_13	+	+	+	+	+	+	-	+	-	+	+	+	84
AE_14	+	+	+	+	+	+	-	-	-	+	+	+	75
AE_15	-	+	+	+	+	+	-	-	-	+	+	+	67
AE_16	+	-	+	-	+	+	-	+	-	+	+	+	67
AE_17	+	+	+	+	+	+	-	+	-	+	+	+	84
AE_18	+	+	+	+	+	+	+	+	+	+	+	+	100
AE_19	+	+	+	+	+	+	+	+	+	+	+	+	100
AE_20	+	+	+	+	+	+	+	+	+	+	+	+	100

Footnote

(+): Resistance, (S): Sensitive

(PI)=Pipercilin 100µg, (**TI**)=Ticarcillin 75µg, (**CAZ**)=Ceftazidime 30µg (**FEP**)=Cefepime 30µg, (**CRO**)=Ceftriaxone 30µg, (**CTX**)=Cefotaxime 30µg, (**IPM**)=Imipenem 10µg, (**MEM**)=Meropenem 10µg, (**AK**)=Amikacin 30µg, (**TE**)=Tetracycline 30µg, (**CIP**)=Ciprofloxacin 5µg, (**LEV**)=Levofloxacin 5µg.

Detection of *rplB* gene

Detection of *rplB* gene in all isolates carried out to investigate the presence of this gene. The results showed that all isolates were positive to the presence of this gene as shown in Fig.(1).

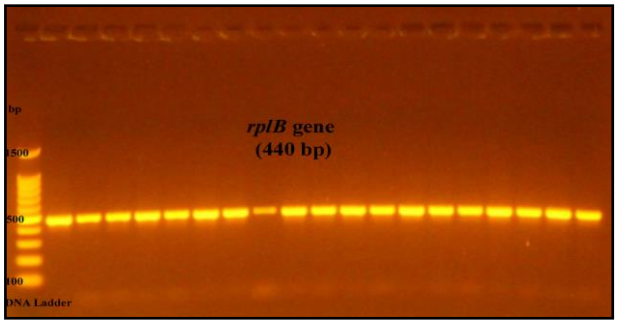

Fig.(1): Gel electrophoresis for amplified rplB gene from Acinetobacter baumannii on agarose gel (1%), 50V for 1 h.

Sequencing of *rplB* gene

Sequencing of rplB gene of all isolates was done by sending PCR products of amplification of rplB gene to Macrogen Corporation-Korea using ABI3730XL, automated DNA sequencer. Then sequenced DNA in NCBI GenBanK database and the results were analyzed by using Geneious 9 software to draw phylogenetic tree.

Genetic variation of *rplB* gene within *A. baumannii* isolates

Phylogenetic tree of the rplB gene carried out by using Geneious 9 software for all (20) locally isolates of multidrug resistance A. baumannii from all sources of isolation included urine, wound, sputum and otitis infections. The results in phylogenetic tree showed 3 groups of isolates as shown in Fig.(2) and Table (3). The isolates in group one combined between wound, sputum and urine sources. Group two included in wound and urine sources. Third group of isolates included in urine, otitis infection and sputum.

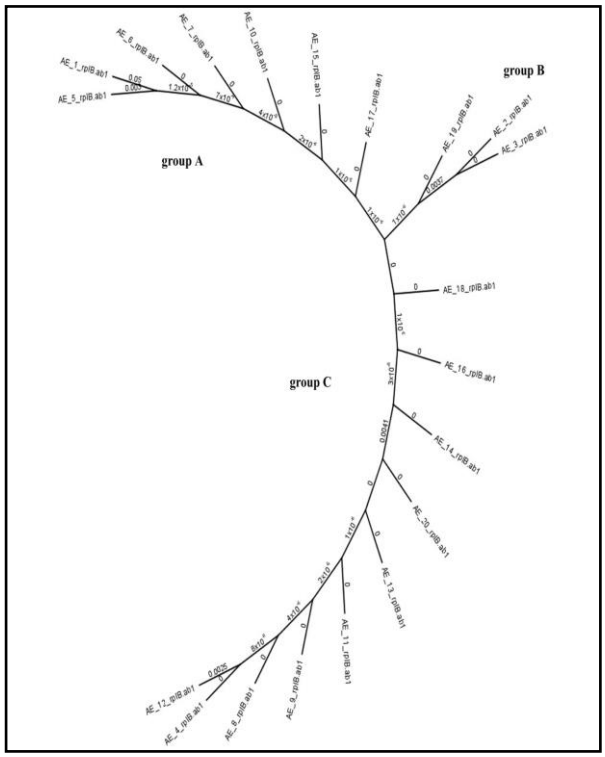

Fig.(2): Unrooted phylogenetic tree of rplB gene within Acinetobacter baumannii isolates structured with the maximum probability by Geneious 9 software program.

Table (3)
Distribution of A. baumannii isolates from clinical sources in phylogenetic tree of rplB gene.

Location in phylogenetic tree	Isolate symbol	Source of isolation
Group A	AE_5	Wound
	AE_1	Urine
	AE_6	wound
	AE_7	Sputum
	AE_10	Urine
	AE_15	Urine
	AE_17	Urine
Group B	AE_19	Urine
	AE_2	Wound
	AE_3	Urine
Group C	AE_18	Urine
	AE_16	Urine
	AE_14	Otitis
	AE_20	sputum
	AE_13	Otitis
	AE_11	Urine
	AE_9	wound
	AE_8	sputum
	AE_4	Urine
	AE_12	Urine

Outbreak of multidrug resistance *A. baumannii* among Gram-negative bacteria is a growing clinical problem worldwide. These bacteria now have become an important nosocomial pathogen that easily adapted to the hospital environment and is able to acquire resistance to almost all currently used antibiotics, including carbapenems which rate of imipenem resistance increased over two fold. This will lead to spreading of strains of this bacteria[23,24]. The increasing clinical importance of *A. baumannii* has stimulated using molecular identification methods for these strains. Like housekeeping *rplB* gene provide a higher degree of resolution as the latter genes evolved and considered as the gold standard. Sequencing of housekeeping genes in general and protein-encoding genes used in the identification of species or strains and detect of phylogenetic relationships among different strains of *A. baumannii* [25, 26].

Genetic variation of *rplB* gene among locally and global *A. baumannii* isolates

Comparison of all locally *A. baumannii* isolates with ten high identity global isolates in gene bank under accession numbers (CP010781, CU459141, LN997846, LN865143, CP012952, CP009256, LT605059, CU468230, CP012035, CP008706) was carried out to detect similarity of genetic relationship between locally and global isolates. The results in phylogenetic tree Fig.(3) showed six groups of isolates. The first group A contain only locally isolate (AE_1). The second group B included only locally isolates (AE_11, AE_4, AE_20). The third group C included only locally isolates, they were (AE_12, AE_8, AE_9, AE_13). The results in group D showed identity between locally isolates (AE_6, AE_7, AE_10, AE_14, AE_16, AE_19) and standard isolates in GeneBank (CP010781, CU459141, LN997846). The local isolates in group E (AE_18, AE_2, AE_3) have similarity with standard isolates (LN865143, CP012952, CP009256). The standard isolates in group F (LT605059, CU468230, CP012035, CP008706) were identical with locally isolates (AE_15, AE_5, AE_17). These results showed that the first three groups (A, B, C) included only eight locally isolates not identical with standard isolates in GenBank. That's might due to found genetic variation between these isolates and standard isolates. Therefore, the locally isolates in first three groups mentioned above may be new isolates in Baghdad city.

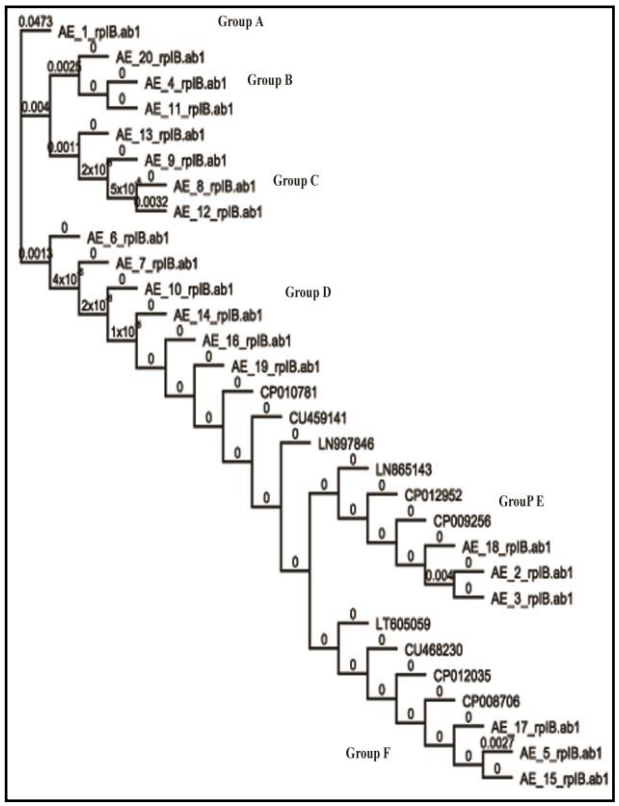

Fig.(3): Unrooted phylogenetic tree of rplB gene between Acinetobacter baumannii and standard isolates (AE are locally isolates and CP010781, CU459141, LN997846, LN865143, CP012952, CP009256, LT605059, CU468230, CP012035, CP008706 are global isolates in gene bank). The tree was structured with the maximum probability by Geneious 9 program.

Documentation sequence of a locally isolated strain of *rplB* gene in NCBI

The documentation of sequence of *rplB* gene and sequence of amino acids encoded by it from locally *A. bumanii* in GenBank was achieved. AE_12 isolate was selected due to dissimilarity with global isolates as shown in Fig.(3). The nucleotides sequence of *rplB* gene documented under accession number (LOCUS KY818058) and protein ID "ARV90996.1".

Conclusion

Distribution of isolates in phylogenetic tree depend on sequence of *rplB* gene showed 3 groups of isolates. The isolates in group one

were combined between wound, sputum and urine sources. Second group included in wound and urine sources. Third group included in urine, otitis infection and sputum. In comparison of locally isolates with global isolates documented in Gene Bank, there are eight isolates different from the standard isolates. Only one isolate (AE_12) from these locally isolates was selected and documented in NCBI under accession number (LOCUS KY818058) of nucleotides sequence and protein ID "ARV90996.1".

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