

Genotyping of *fusA* Gene from Clinical Isolates *Acinetobacter baumannii* in Baghdad

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Abstract:

This study aims at detecting the differences in genotyping of coding region *fusA* gene in clinical isolates of *Acinetobacter baumannii* from Baghdad, Iraq. Collected two hundred clinical samples (50 samples from urine, 50 samples from wound, 50 samples from sputum and 50 samples from otitis infections). Laboratory diagnosis for bacterial isolates carried out by some biochemical tests and confirmed by using VITEK- 2 compact system. The results appeared that twenty isolates of *Acinetobacter baumannii* in all these samples. Genotyping study was performed of coding region *fusA* gene of the extracted genome of all bacterial isolates and used specific primers in achieved amplification process of this target gene. DNA sequencing of this gene and alignment of sequencing in NCBI was achieved and drew phylogenetic tree by using Geneious 9 software among locally isolates alone and then among locally isolates and high identity global isolates in GenBank. The results in phylogenetic tree of *fusA* gene in locally isolates showed 4 groups of isolates included more than one source of isolation. The results in phylogenetic tree of the locally and global isolates showed that are four different groups and each group included some locally isolates and global isolates except group A (AE_22, AE_26) and group E (AE_35, AE_32, AE_33) that not identity with global isolates. The nucleotides sequence of *fusA* gene from localized isolate (AE_35) was registered in national GenBank under accession number (LOCUS KY818057) and protein ID "ARV90995.1.

Keywords: *fusA* gene, Elongation factor EF-G, Genotyping, (LOCUS KY818057), Protein ID "ARV90995.1.

Introduction:

The bacterium *A. baumannii* is an opportunistic pathogen (Gram-negative bacilli bacteria) which causing world wide nosocomial infections included different of hospital acquired infection (UTI, endocarditis, surgical-site infections, meningitis, septicemia, and ventilator-associated pneumonia among patients in ICU) (1, 2, 3). These bacteria develop resistance against different antibiotics like carbapenems group that's most effective antimicrobial agents for the treatment of infections caused by multidrug resistant bacteria (MDR) (4, 5, 6).

fusA gene is a housekeeping gene in *A. baumannii*, encodes for the elongation factor EF-G that catalyzes the GTP-dependent ribosomal translocation step during translation elongation by catalyzing the translocation of peptidyl-RNA from the A to P site of *A. baumannii* ribosome. During this step, the ribosome changes from the pre-translocational to the post-translocational state as

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the newly formed A-site-bound peptidyl-tRNA and P-site-bound deacylated tRNA move to the P and E sites, respectively Catalyzing process of the coordinated movement of the two tRNA molecules, the mRNA and conformational changes in the ribosome required of product of this gene based on the importance of *fusA* gene in translation regulation (7, 8, 9). So, this study aim at detecting genetic variation and genotype of *fusA* gene in clinical isolates of these bacteria.

Materials and Methods:

Samples collection

In this study two hundred clinical samples were collected (were 50 samples from urine, 50 samples from wound, 50 samples from sputum, 50 samples from otitis) in some hospitals (AL-Kindy Teaching Hospitals, Imam Ali Hospitals, AL-Sader Hospitals) in Baghdad during the period from September into December 2016.

Isolation and identification

The samples were cultured onto MacConkey agar and incubated for 18-24 hrs at 37°C. The non lactose fermenting isolates were cultured onto CHRO Magar TM medium and incubated for 18-24 at 37 °C, *Acinetobacter* appears as a red colonies after the incubation period. The isolates were tested by morphologic characteristics and standard biochemical tests according to MacFaddin, (2000) (10). Then confirmation of *Acinetobacter* spp. isolates was carried out by VITEK- 2 Compact

system to identification *Acinetobacter* isolates to species level according to manufactures' instructions (Biomérieux/ France).

DNA extraction and PCR assay

DNA of all twenty *A. baumannii* isolates from two hundred clinical samples was extracted by wizard® genomic DNA purification kit (Promega, USA) according to manufactures' instructions. Amplification of the *fusA* gene was performed with specific primers, (Table 1).

Table 1. Sequence of oligonucleotides primers used for amplification coding region of *fusA* gene

Primer type	Sequence 5'→3'	Reference
Forward	ATCGGTATTTCTGCGCACATCGAT	Laure <i>et al.</i> , 2010 (11)
Reverse	CCAACATACGCTGAACACCTTTGTT	

The reaction program of PCR (initial denaturation at 94 °C for 2 minutes, and 30 cycles of denaturation at 94°C for 30 second, annealing at 50°C for 30 second, extension at 72°C for 30 second and a final extension at 72°C for 5 minutes) was done. A molecular marker (promega/ USA effective size range: 100 to 1500 bp) was used to assess PCR product size.

Sequencing of *fusA* gene

Sequencing of *fusA* gene of all twenty isolates was carried out by sending PCR products of amplification *fusA* gene to macrogen corporation-Korea using ABI3730XL, automated DNA sequencer.

Genetic Analyses of *fusA* gene

Phylogenetic study was carried out which the results were analyzed by using Geneious 9 software. The sequenced DNA were analyzed by BLASTn tool of NCBI GenBank database. Then comparing was done between the query sequences and those documented in the GenBank database. The confirmation of bacterial isolates was carried out which the closest alignment match has a very high identity to the homologues in Gene- Bank. The sequencing of target gene that identities were also computed using the pairwise alignment by Geneious 9 software. Multiple sequence alignments were performed using Geneious alignment and the phylogenetic analyses were done by the maximum likelihood method.

Results and Discussion:

Isolation and identification

In this study collection of two hundred clinical samples (fifty samples from urine, fifty samples from wound, fifty samples from sputum and fifty samples from otitis) was done. Then laboratory diagnosis of these samples carried out by routine methods and confirmation by using VITEK- 2 Compact system. There were twenty confirmed isolates of *A. baumannii* in all these samples in percentage (10%) included 11 isolates from urine , 4 isolates from wound , 3 from sputum and 2 from otitis. In locally study carried out by Adnan *et al.* (2014) (12) the percentage of infection with this bacteria was (10.3%) in different clinical samples. Another locally study by Mosafar, 2007 (13) isolated *Acinetobacter baumannii* from different clinical sources with different percentage of infection. Distribution of infection with this bacteria in different region in worldwide because this bacterium an important nosocomial pathogens and has different virulence factors confirmed the local rate of infections (14).

Detection of *fusA* gene

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

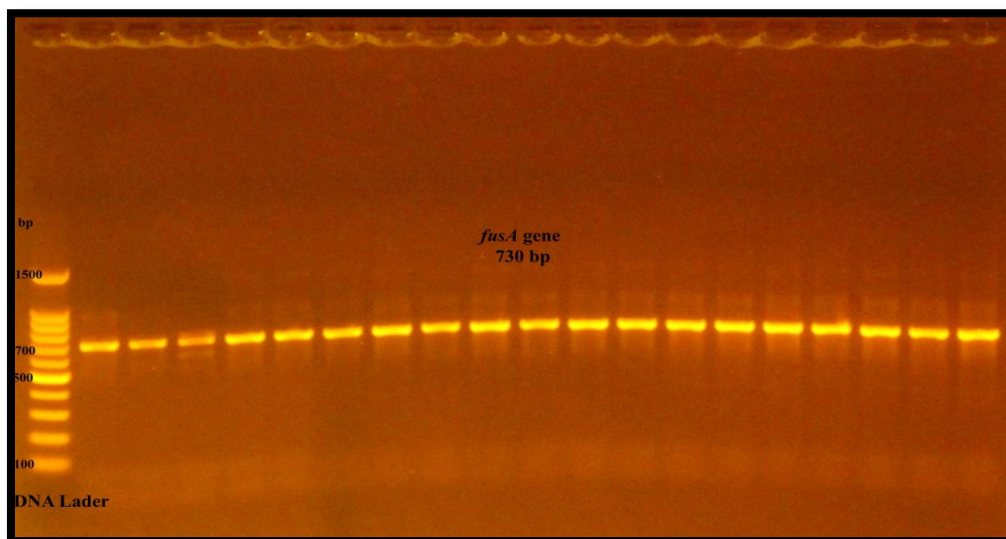


Figure 1. Gel electrophoresis for amplified *fusA* gene of *A. baumannii* on agarose gel (1%), 50V for 1 hour.

Sequencing of *fusA* gene

Analyses of sequencing *fusA* gene of all isolates were carried out by BLASTn tool of NCBI GenBank database and results of sequencing of all isolates were analyzed by using Geneious 9 software to draw phylogenetic tree among locally isolates alone and among local and global isolates that documented in gene bank.

Genotyping of *fusA* gene in locally isolates

Phylogenetic study of the *fusA* gene was done for all (20) clinical isolates *A. baumannii* from all sources of isolation included urine, wound, sputum and otitis infection. The results in phylogenetic tree found 4 groups of isolates as shown in Figure (2) and Table (2). The isolates in group a included urine and wound sources. Group B included wound and sputum sources. Group C contain otitis and urine sources, the group D included urine, otitis infection and wound sources.

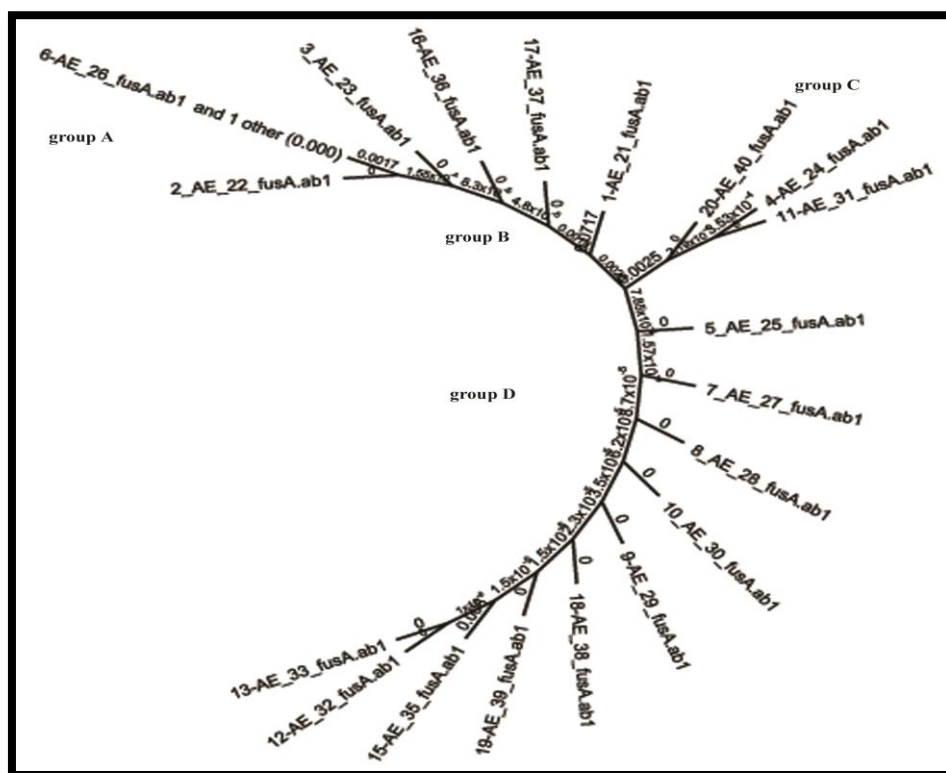


Figure 2. Unrooted phylogenetic tree of *fusA* gene within *Acinetobacter baumannii* isolates structured with the maximum probability method by Geneious 9 software.

Table 2. Location of clinical *A. baumannii* isolates in phylogenetic tree of *fusA* gene

No. of isolate	Source of isolation	Location in phylogenetic tree
AE_21	Sputum	Group B
AE_22	Wound	Group A
AE_23	Wound	Group B
AE_24	Urine	Group C
AE_25	Wound	Group D
AE_26	Urine	Group A
AE_27	Wound	Group D
AE_28	Urine	Group D
AE_29	Urine	Group D
AE_30	Urine	Group D
AE_31	Urine	Group C
AE_32	Urine	Group D
AE_33	Urine	Group D
AE_34	Otitis	Group D
AE_35	Urine	Group D
AE_36	Sputum	Group B
AE_37	Sputum	Group B
AE_38	Urine	Group D
AE_39	Urine	Group D
AE_40	Otitis	Group C

These bacteria consider a high virulence pathogen that led to spreading of epidemiology of infections produced by this bacteria and increasing multidrug resistance. Drug resistance in *Acinetobacter baumannii* is mainly caused by the emergence and produced of extended spectrum betalactamase, fluoroquinolone resistance and the prevalence of multidrug resistance and carbapenem-resistant strains. This problem shows that data in the world organization (National Healthcare Safety Network) in united states centers for disease control and prevention (CDC), in which there are high rates of carbapenem resistance *Acinetobacter baumannii* throughout the USA and increased nosocomial infections (15,16,17). All that leads to the

pathogenesis of these bacteria. The study of housekeeping gene like *fusA* gene in clinical isolates is very important tool in identification of distribution *A. baumannii* (18). So phylogenetic tree of this gene was done in this study to understand the epidemiology of *Acinetobacter baumannii* in Baghdad.

Genotyping of *fusA* gene in locally isolates comparison with global isolates in GenBank

For studying genotyping of *fusA* gene in locally isolated *Acinetobacter baumannii* and comparison with standard isolates. All locally isolates compared with eight standard global isolates documented in gene bank under accession numbers were (CP014528, CP007712, CP006768, CP012006, LT605059, CP003847, CP009256, CP018677). The results in phylogenetic tree (Figure 3) show that there are four groups of isolates. The first group A included locally isolates (AE_22, AE_26) and group E (AE_35, AE_32, AE_33) were not identity with global isolates. The second group B included locally isolates (AE_23, AE_36, AE_37, AE_21, AE_25, AE_27, AE_28, AE_30, AE_39) similarity with global isolates (CP014528, CP007712). The locally isolates in third group C (AE_40, AE_24, AE_31) were identified with global isolates (CP006768, CP012006, LT605059). Group D included locally isolates that were (AE_29, AE_38) and global isolates were (CP003847, CP009256, CP018677). These results in phylogenetic tree Figure (3) showed each group including local isolates and global isolates except group A (AE_22, AE_26), in another meaning each group from the locally isolates identity with specific global isolates except group A. That's may be due to found genetic variation between these isolates and global isolates lead to distribution new isolates (AE_22, AE_26) in Baghdad governorate.

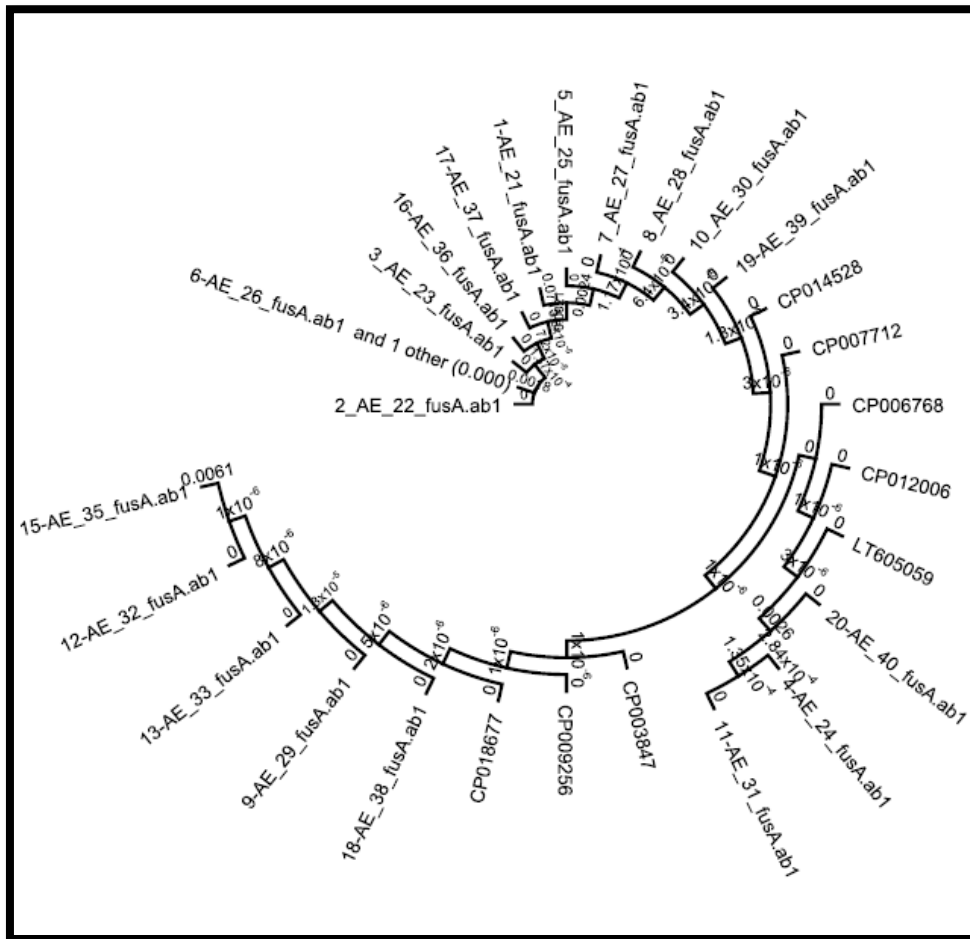


Figure 3. Unrooted phylogenetic tree of *fusA* gene between *Acinetobacter baumannii* locally and global isolates (AE refer to locally isolates but CP014528, CP007712, CP006768, CP012006, LT605059, CP003847, CP009256, CP018677 are global isolates) . This phylogenetic tree was structured with the maximum probability method by Geneious 9.

Registration locally sequencing of *fusA* gene in GenBank

The registration sequence of *fusA* gene from local *A. bumannii* in national GenBank was done. The local isolate (AE_35) that not identical with global isolates as shown in Figure 3, was selected to the registration. The nucleotides sequence of *fusA* gene was registered under accession number (LOCUS KY818057) and protein ID "ARV90995.1.

Conclusion:

In genotyping of *fusA* gene from clinical *Acinetobacter baumannii* isolates, the results in phylogenetic tree among local isolates found 4 groups of isolates. From the comparison between local and global isolates , it can be concluded that there are four different groups of local isolates and each group identified with certain global isolates except group A (AE_22, AE_26) and group E (AE_35, AE_32, AE_33) which may consider new isolates. The nucleotides sequence of *fusA* gene from isolate (AE_35) was registered in national GenBank under accession number (LOCUS KY818057) and protein ID "ARV90995.1.

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النمط الوراثي لجين *fusA* من عزلات سريرية لبكتريا *Acinetobacter baumannii* في بغداد

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الخلاصة:

هدفت هذه الدراسة لتحديد الاختلافات في التنميط الوراثي للمنطقة المشفرة من جين *fusA* لبكتريا *Acinetobacter baumannii* المعزولة من عينات سريرية مختلفة العراق، بغداد. جمعت 200 عينة سريرية من مصادر سريرية مختلفة (50 عينة من الأدرار، 50 عينة من الجروح، 50 عينة من الفسح و 50 عينة من التهاب الأذن). تم إجراء التشخيص المختبري للعزلات البكتيرية بواسطة بعض الفحوصات الكيوكيوية وتأكيدها باستخدام VITEK-2 compact system. وظهرت النتائج الحصول على 20 عزلة لبكتريا *A. baumannii* في عدد العينات الكلية. اجريت دراسة النمط الوراثي للمنطقة المشفرة من جين *fusA* للجينوم الذي تم استخلاصه من جميع العزلات البكتيرية واستخدام برايمرات خاصة لعملية تضخيم هذا الجين المطلوب. و اجريت عملية تحديد تسلسل التعاقبات النيوكليوتيدية لجميع العزلات ومن ثم مطابقتها على موقع ال NCBI وتم رسم الخارطة الوراثية باستخدام برنامج Geneious 9 بين العزلات المحلية لوحدها ومن ثم بين العزلات المحلية والعالمية الأكثر تطابق معها في بنك الجينات. اظهرت النتائج في الشجرة الوراثية للجين *fusA* للعزلات المحلية تكون اربعة مجاميع مختلفة من العزلات البكتيرية وكل مجموعة تتضمن اكثر من مصدر للعزل. اظهرت نتائج مقارنة العزلات المحلية مع العزلات العالمية وجود اربعة مجاميع مختلفة وكل مجموعة تحتوي عدد من العزلات المحلية والعزلات العالمية ماعدا المجموعة A (AE_22 , AE_26) والمجموعة E (AE_35, AE_32, AE_33) لا تتطابق مع العزلات العالمية المسجلة في بنك الجينات. لذلك سجلت العزلة المحلية (AE_35) في بنك الجينات العالمي تحت الرقم التسلسلي LOCUS KY818057 لتسلسل القواعد النيتروجينية والرقم التسلسلي protein ARV90995.1 ID لتسلسل الأحماض الأمينية للبروتين.

الكلمات المفتاحية: جين *fusA* ، عامل الأستطالة EF-G ، الموقع KY818057 ، رقم البروتين ARV90995.1 .