DOI: 10.24996/ijs.2018.59.1A.2

ISSN: 0067-2904

Phylogenetic analysis of *cpn60* gene from locally isolated *Acinetobacter baumannii*

Alaa Salim Hamzah
Middle Technical University, Technical Institute/Kut, Iraq.

Abstract

This study was aimed to analysis phylogenetic tree of the *gene cpn60* in *Acinetobacter baumannii* that was identified in Baghdad. Study included collection two hundred specimens (fifty from UTI, fifty from wound infection , fifty from respiratory tract infection and fifty from otitis infections) . In primary laboratory diagnosis and confirmed by using VITEK- 2 Compact system, twenty isolates of this bacterium were indentified (10%) from total specimens. Extraction of geneteic material to detect target gene by amplification this target gene. DNA sequencing of all isolates was done. Then alignment of sequencing in NCBI and draw phylogenetic tree by use Geneious 9 software among sequence of locally isolates . The results in phylogenetic tree of *cpn60* gene in locally isolates showed 4 groups of isolates different with difference source of isolation. Then phylogenetic tree for locally isolates and high identity global isolates in gene bank was drew and its results showed 12 locally isolates not identity with standard isolates. So, chosen isolate (AE_29) isolate from these 12 isolates and documented in national GenBank as anew isolate under accession number (LOCUS KY818056) of nucleotides sequence and protein ID "ARV90994.1" .

Keywords: *cpn60* gene, *Acinetobacter baumannii* , chaperonin , phylogenetic analysis , GenBank , LOCUS KY818056 , protein ID "ARV90994.1"

التحليل الوراثي لجين *cpn60* من بكتريا *Acinetobacter baumannii* المعزولة محلياً

علاء سالم حمزة

الجامعة التقنية الوسطى، المعهد التقني، الكوت، العراق

الخلاصة

هدفت هذه الدراسة لتحليل الشجرة الوراثية للجين *cpn60* من بكتريا *Acinetobacterbaumannii* المعزولة من اربع مصادر سريرية مختلفة في بغداد . جمعت 200 عينة تضمنت 50 عينة من الأدرار و50 عينة من الجروح و50 عينة من القشع و50 عينة من التهاب الأذن . وبعد اكمال التشخيص الاولي وتأكيد التشخيص بإستخدام VITEK– 2 Compact system تم الحصول على 20 عزلة بكترية من اصل 200 عينة وبنسبة 10% . تمت عملية استخلاص الدنا ومن ثم تضخيم الجين بإستخدام برايمرات خاصة . اجريت عملية تسلسل التعاقبات النيوكليوتيدية ومن ثم مطابقتها مع الNCBI و بأستخدام برنامج Geneious 9 رسمت الشجرة الوراثية من تسلسلات العزلات المحلية . اظهرت نتائج الشجرة الوراثية للجين *cpn60* أن العزلات المحلية شكلت اربع مجاميع مختلفه من العزلات البكتيرية تختلف بأختلاف موقع العزل . وقد رسمت الشجرة الوراثية مرة اخرى بين العزلات المحلية والعزلات العالمية الأكثر تطابقاً معها والمثبتة في بنك الجينات

Email: aalaatad@yahoo.com

العالمي واظهرت نتائج مقارنة العزلات المحلية مع العزلات القياسيةوجود 12 عزلة محلية لا تتطابق مع العزلات القياسية .لذلك تم اختيار واحدة من هذه العزلات وهي (AE_29) لتسجل في بنك الجينات العالمي كعزلة جديدة حيث تم تسجيل تسلسل القواعد النتروجينية تحت الرقم التسلسلي (LOCUS KY818056) وتسلسل الاحماض الأمينية المشفرة لها تحت الرقم التسلسلي ("protein ID "ARV90994.1) .

Introduction

Acinetobacter baumannii is very important opportunistic pathogen has some characteristic nonmotile and Gve- bacterium. This bacteria caused different disease: pneumonia, meningitis , UTI and bacteremia and found in intensive care units (ICUs) because its ability of multidrug resistance (MDR) for antibacterial agents [1, 2].

Acinetobacter baumannii infections are very resist therapy and often cause level of mortality because it is resist antibacterial group like β-lactams , tetracyclines , aminoglycosides as well as fluoroquinolones. Genetic determinant that responsible on the resistant are often located in resistant islands (AbaRs) that target the (comM) genes , this region of resistant island (AbaRs) variation in structure [3,4] .

cpn60 gene encodes of 60 kDa chaperonin protein (also known as *GroEL*) . The structures of protein was determined through amino acid sequences , in vivo, a chaperones required to make mature protien to their natural case in stable optimization, increase protein quantity may be cause aggregation unless transiently exposed hydrophobic regions are protected [5,6].

Chaperones are play a very important role (protect of protein) during stresses such as heat shock that lead to the partial unfolding of proteins. The chaperonins structure (tetradecamers made up of 60 kDa subunits arranged in two heptameric rings with a central cavity where protein folding can occur). [7]. The aim of this study is phylogenetic analysis of *cpn60* gene from locally isolated *Acienetobacter baumannii* in Baghdad governorate.

Materials and methods

Samples collection

This study included collecting two hundred clinical specimens (fifty specimens were collected UTI, fifty were collected wound, fifty were collected from respiratory tract infection and fifty were collected otitis) in some hospitals in Baghdad during the period from September into December 2016.

Isolation and identification

The samples were cultured onto MacConkey agar and CHROM agar media incubated for 18-24 hrs at 37^{o}C. Bacterial isolates were tested by their morphological characteristics and standard biochemical tests . Then confirmation of *Acinetobacter* spp. isolates was carried out by VITEK- 2 Compact system for identification *Acinetobacter* isolates to species level according to manufactures' instructions (Biomerieux/ France).

DNA extraction and PCR assay

DNA of all isolates was extracted by wizard® genomic DNA purification kit (Promega , USA) according to manufactures' instructions. Amplification of the *cpn60* gene was performed with specific primer Table-1.

Table 1- Sequence of oligonucleotides primers used for amplification of *cpn60* gene

Primer type	Sequence 5'→3'	Expected amplicon size	Reference
Forward	ACTGTACTTGCTCAAGC	405 bp	Laure *et al*., 2010 [8]
Reverse	TTCAGCGATGATAAGAAGTGG		

The cycling conditions were: Initial denaturation at 94 ºC for 2 minutes, and 30 cycles of denaturation at 94ºC for 30 second, annealing at 50ºC for 30 second, extension at 72ºC for 30 second and a final extension at72ºC for 5 minutes. A molecular marker (promega/ USA effective size range: 100 to 1500 bp) was used to assess PCR product size.

Phylogenetic analysis of *cpn60* gene

Phylogenetic study carried out , PCR products of all isolates from the amplification of *cpn60* gene were sent for sequencing using ABI3730XL, automated DNA sequencer , by macrogen corporation-Korea . Then the results analyzed using Geneious software. The sequenced DNA were analyzed in NCBI GenBanK database and compared with high identity strain available in the GenBanK database . The species was confirmed when the closest alignment match has very high identity to the homologues found in Gene- Bank . Gene *cpn60* sequences identities were also computed through Geneious 9 program. Multiple gene sequence alignments were acheived by Geneious alignment and phylogenetic analyses concluded by the maximum probability method.

Results and discussion

Isolation and identification

From two hundred collected clinical samples non lactose fermenting isolates were cultured on to CHROM agar medium and incubated for 18-24 at 37 ^{o}C, *Acinetobacter* appears as ared colonies after the incubation period. Then the isolates were tested by morphological characteristics, standard biochemical tests according to MacFaddin, (2000) [9] and confirmed by VITEK**.** Twenty *A. baumannii* were obtained in different specimens with 10%, as shown in Table-2.

Table 2- Distribution of *Acinetobacter baumannii* isolates in clinical samples

Clinical samples	No. of samples	No. of isolates	Percentage (%)
Urine	50	11	22
Wound	50	4	8
Sputum	50	3	6
otitis	50	2	4

In locally study carried out by Adnan *et al*. (2014) [10] the percentage of infection with this bacteria was (10.3%) in different clinical samples . Another locally study by Mosafer, 2007 [11] isolated *Acinetobacter baumannii* from different clinical sources and the percentage of infection was (7%) . The infection with *Acinetobacter* increased significantly and continuous in different region in worldwide because this bacterium an important nosocomial pathogens and has different virulence factors [12].

Detection of *cpn60* gene

Detection of *cpn60*gene in all isolates carried out by investigate the presence of *cpn60* gene and the results showed that all isolates were positive to presence of this gene, as shown in Figure-1.

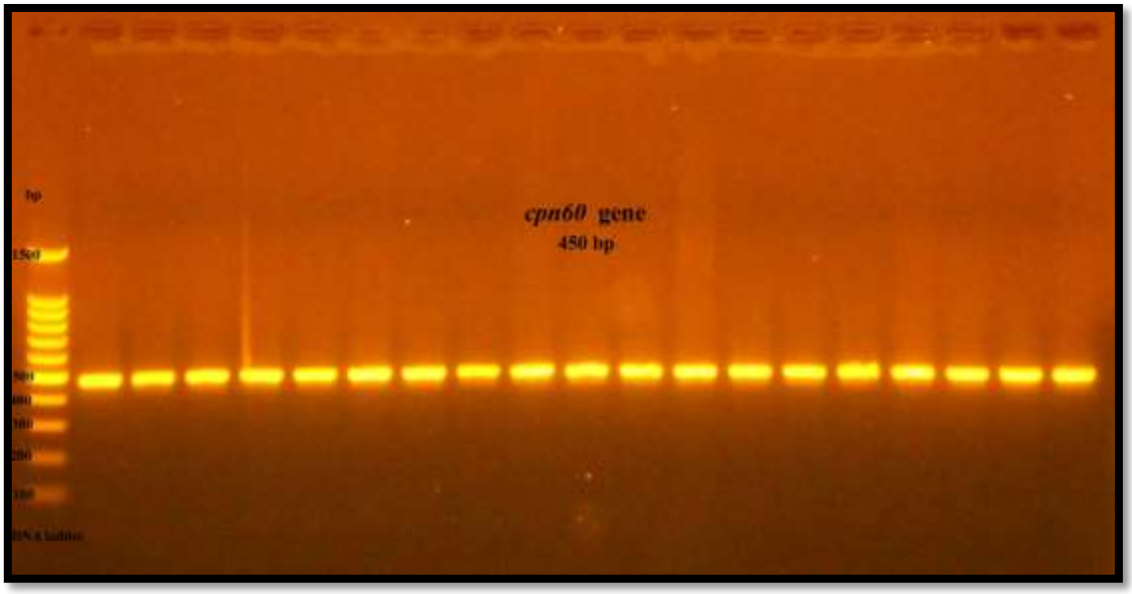

Figure 1- Gel electrophoresis for amplified *cpn60* gene from *Acinetobacter baumannii* on agarose gel (1%), 50V for 1 hour.

Sequencing of *cpn60* gene

The sequencing of PCR products amplified of *cpn60* gene were sent for sequencing using ABI3730XL, automated DNA sequencer , by macrogen corporation-Korea .The sequenced nucleotides of this gene analyzed in NCBI GenBanK database as shown in Figure-2. Then the results analyzed using Geneious 9 software to draw phylogenetic analysis in further experiment.

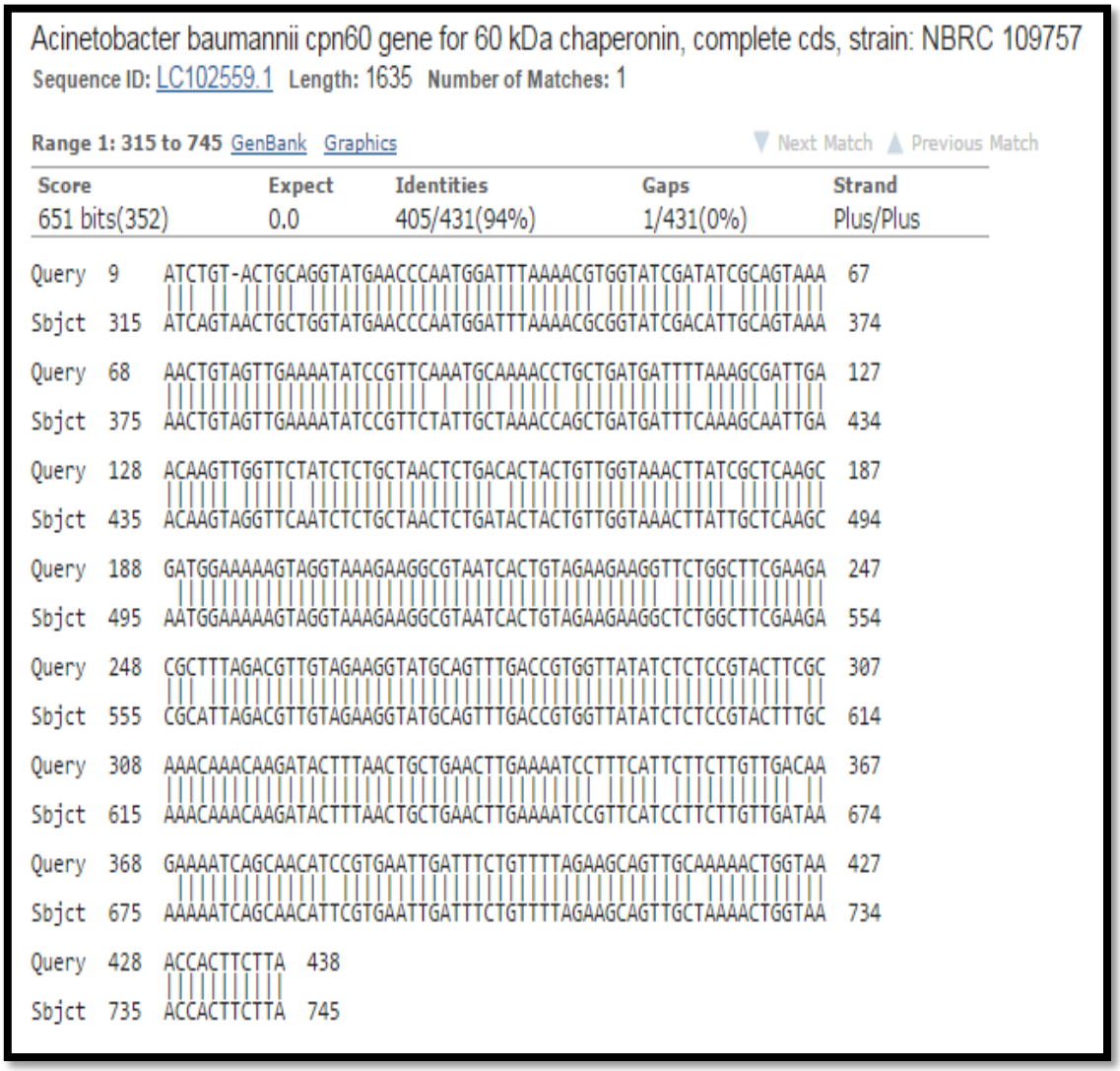

Figure 2-alignments sequence of *cpn60* gene from locally *Acinetobacter baumannii* (query) with global LC102559.

Phylogenetic analysis within locally isolates

Phylogenetic analysis of the cpn60 gene was done among all twenty *Acinetobacter baumannii* isolates by analyzed multiple sequence alignments using Geneious 9 software . The results in genetic tree showed there are genetic relationship between isolates different with difference source of isolation; the 11 isolates (55%) from urine were (AE_39, AE_38, AE_35, AE_33, AE_32, AE_31, AE_30, AE_28, AE_29, AE_26, AE_24) located in specific group in phylogenetic tree, the 4 isolates (20%) from wound infection were (AE_27,AE_25,AE_22,AE_23) located in one group, while 3 isolates (15%) from sputum were (AE_21,AE_37,AE_35) located in different group and 2 isolates (10%) from otitis were (AE_34, AE_40) located in specific group in phylogenetic tree, as shown in Figure-3.

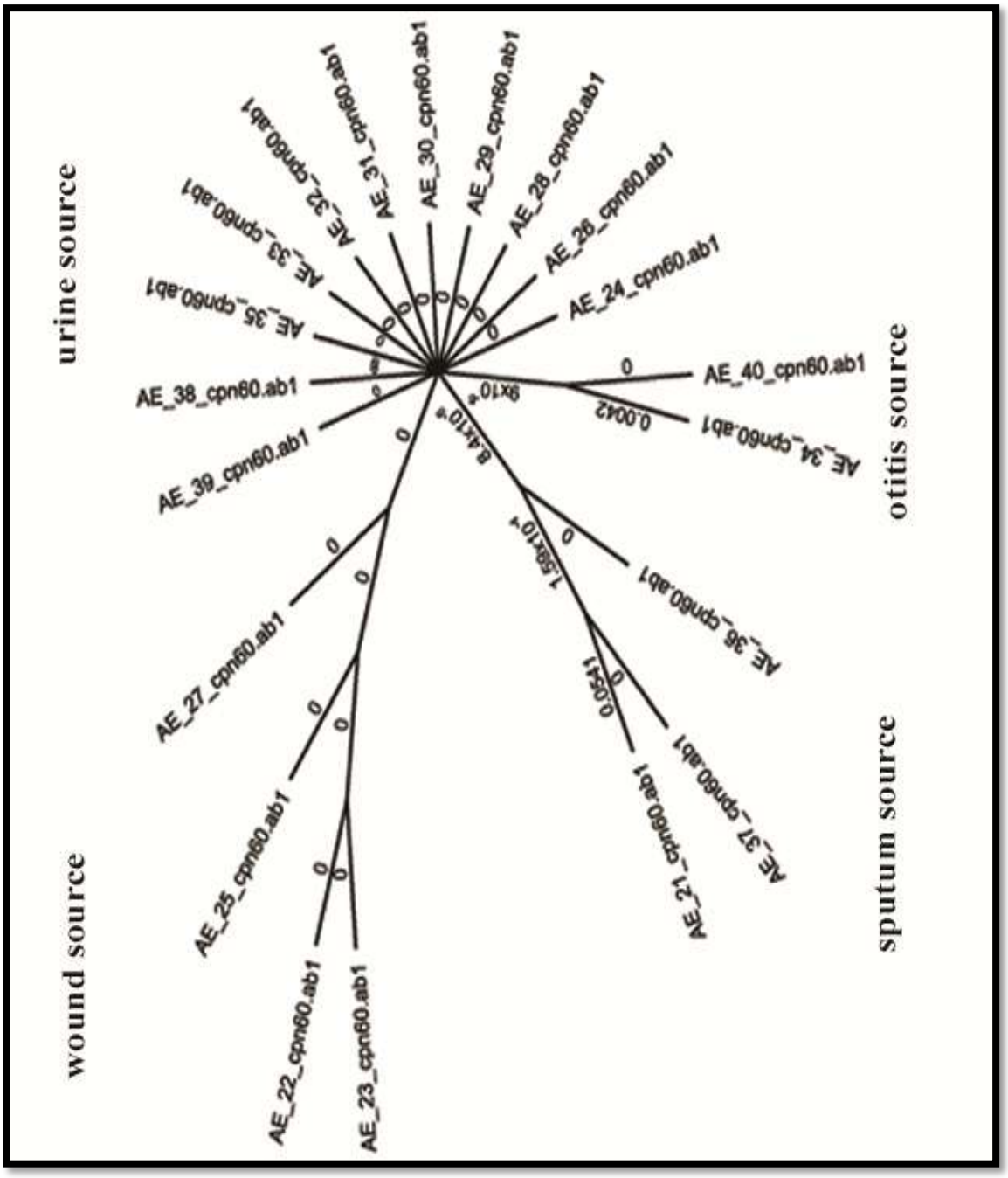

Figure 3- Unrooted phylogenetic tree of *cpn60* gene within *Acinetobacter baumannii* isolates and the tree structured with the maximum probability by Geneious 9 program.

Phylogenetic analysis between locally and global isolates

Phylogenetic tree of all locally*Acinetobacterbaumannii*and global isolates was done by comparison with ten high similar standard global isolates in gene bank , the accession numbers were (LT605059 , LN997846 , LN865143 , LC102559 , KU215659 , KU165787 , KC553433 , KC553376 , KC553302 , KC207588) to detect identity of genetic relationship between them . The results in Figure-4 showed formation four different groups were A, B, C and D . The three groups were A, B and C included only locally isolates in phylogenetic tree but the isolates in group D were eight locally isolates and ten standard isolates. This results may be refer to the locally isolates in group D identity with standard isolates but the locally isolates in group A, B and C not identity with standard isolates were documented in gene bank. The locally isolates in first three groups perhaps are new isolates in Baghdad city.

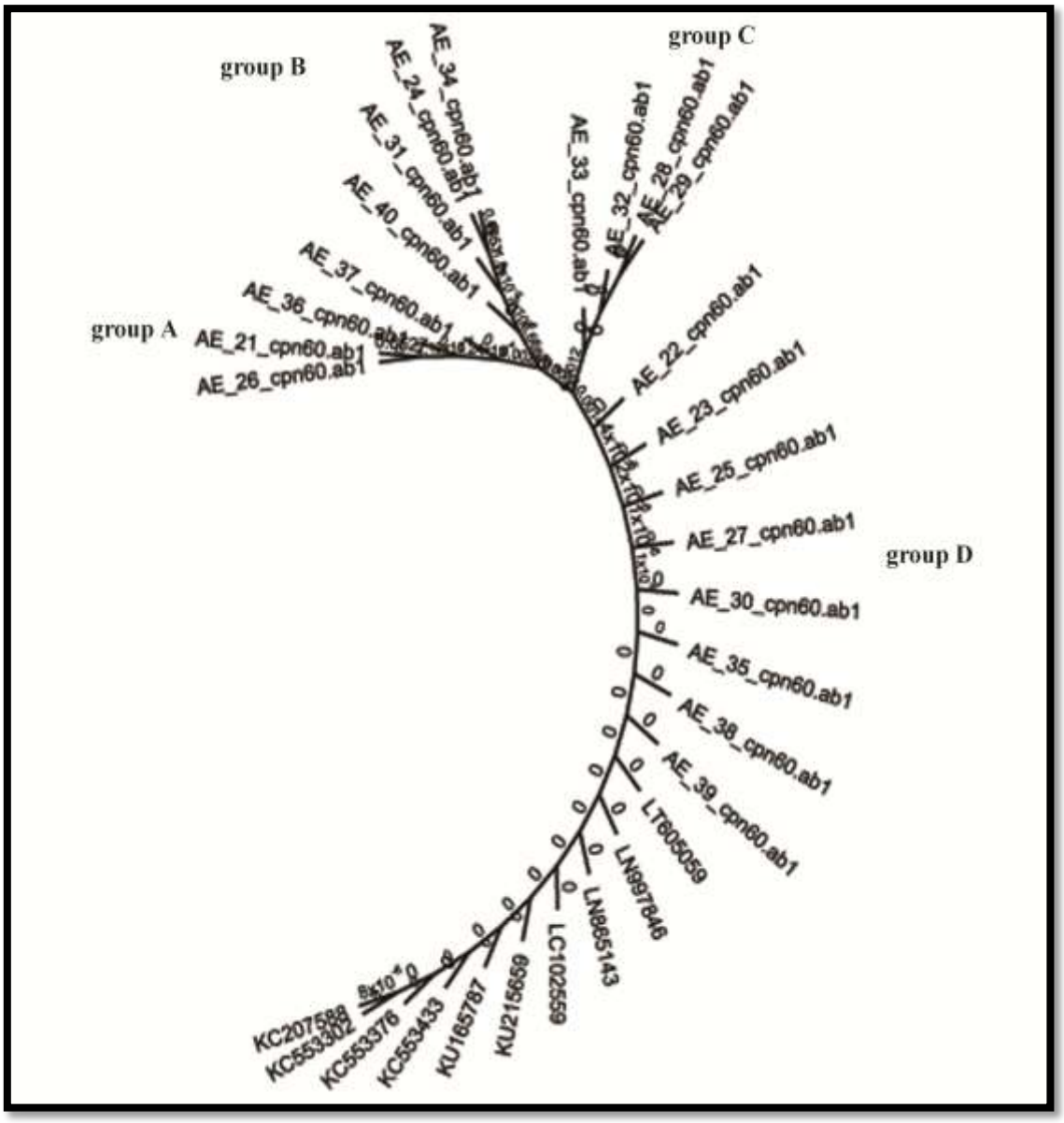

Figure 4- Unrooted phylogenetic tree of cpn60 gene between *Acinetobacter baumannii* and standard isolates. The tree designed with the maximum probability by Geneious 9. (AE symbol of locally isolates but LT605059, LN997846, LN865143, LC102559, KU215659, KU165787, KC553433, KC553376, KC553302, KC207588 are standard isolates).

*Acinetobacterbaumannii*is an important nosocomial pathogen in various countries in Europe, Asia, the UnitedStates, and Latin America. There are several researchers emphasized the genetic heterogeneity among epidemiology strains of this bacteria [13,14] .Iinfections are mostly associated with epidemic spread andprevalence strains arefrequently multidrug resistant (MDR). A most concerningdevelopment is the increasing resistant strains to antimicrobial agents and theMDR is often associated with isolates that belong to the international clones . So the phylogeneic analysis of genetic variation between strains is needed for global epidemiological understandingand as a foundation for studying the relationshipsbetween genotype and phenotype of epidemic potential*Acinetobacterbaumannii*isolates [15, 16].

Recording new locally isolates in national GenBank

The recording sequence of *cpn60* gene from locally *A. bumannii* in NCBI carried out to the isolates that not identity with global isolates to certain that these locally isolates are new isolates . Then one isolate (AE_29) was chosen from group C in Figure-4 that not identity with global isolates

to record in NCBI .The results showedaccepting sequence of nucleotides and sequence of amino acids in GenBank as a new isolate under accession number (LOCUS KY818056) of nucleotides sequence and protein ID "ARV90994.1" .

Conclusion

In phylogenetic tree among locally isolates shown occur genetic variation between isolates lead to formation 4 groups depend on source of isolation. In the phylogenetic tree between locally and standard isolates concluded 12 locally isolates not identity with standard isolates , that's may be suggest this 12 locally isolates are new isolates especially AE_29 isolate accepted in NCBI under accession number (LOCUS KY818056) of nucleotides sequence and protein ID "ARV90994.1" .

References

1. Perez, F., Hujer, A.M. and Hujer, K.M. **2007**. Global challenge of multidrugresistant *Acinetobacter baumannii*. *Antimicrob Agents Chemother*, **51**: 3471–84.
2. Dijkshoorn, L., Nemec, A. and Seifert, H. **2007**. An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol*, **5**: 939–51.
3. Post, V., White, P.A. and Hall, R.M. **2010**. Evolution of AbaR-type genomic resistance islands in multiply antibiotic-resistant *Acinetobacter baumannii*. *J Antimicrob Chemother* , **65**: 1162–70.
4. Krizova, L., Dijkshoorn, L. and Nemec, A. **2011**. Diversity and evolution of AbaR genomic resistance islands in *Acinetobacter baumannii* strains of European clone I. *Antimicrob Agents Chemother*, **55**: 3201–6.
5. Lin, Z. and Rye, H.S. **2006**. GroEL-mediated protein folding: making the impossible, possible. *Crit Rev Biochem Mol*, **41**: 211–239.
6. Ellis, R.J. **2007**. Protein misassembly: macromolecular crowding and molecular chaperones. *Adv Exp Med Biol*, **594**: 1–13.
7. Horwich, A.L., Fenton, W.A., Chapman, E. and Farr, G.W. **2007**. Two families of chaperonin: physiology and mechanism. *Annu Rev Cell Dev Bi*, **23**: 115–145.
8. Laure, D., Virginie, P., Alexandr, N., Lenie, D. and Sylvain, B. **2010**. The Population Structure of *Acinetobacter baumannii*: Expanding Multiresistant Clones from an Ancestral Susceptible, *Genetic Pool*, **5**: 4 -11.
9. MacFaddin؛ J.F. **2000**. *Biochemical tests for identification of medical bacteria*؛ 3rd ed.؛ Lippincott Williams and Wilkins, USA.
10. Adnan, H. ., Ali, M. M. and Ali, S. K. **2014**. Emergence of plasmid mediated *aac(6´)-Ib-cr* Gene in Flouroquinolon- resistant *Acinetobacter* spp. *QMJ*, **10**: 17– 23.
11. Mosafer, H. K. **2007**. Effect of Crude Fimbriae Extract of *Acinetobacter baumannii* on Biotic and Abiotic Surfaces,M. SC.thesis, Dpartment of Biology, College of Science, Al-Mustansiriya University.
12. Chua, M. M. and Alejandria, M. M. **2008**. The Epidemiology of *Acinetobacter* Infections Among Critically Ill Adult Patients Admitted at the University of the Philippines - Philippine General Hospital. *Philippine Journal of Microbiology and Infectious Diseases*, **37**: 38-53.
13. Landman, D., Quale, J. M., Mayorga, D., Adedeji, A., Vangala, K., Ravishankar, J., Flores, C. and Brooks, S. **2002**. Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, N.Y.: the pre antibiotic era has returned. *Arch. Intern. Med*., **162**: 1515–1520.
14. Van Looveren, M. and Goossens, H. **2004**. Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clin. Microbiol. Infect*. **10**: 684–704.
15. Hujer, K.M, Hujer, A.M, Hulten, E.A, Bajaksouzian, S. and Adams, J.M. **2006**. Analysis of antibiotic resistance genes in multidrug-resistant *Acinetobacter* spp. isolates from military and civilian patients treated at the Walter Reed Army Medical Center. *Antimicrob Agents Chemother*, **50**: 4114–4123.
16. Hoffmann, M.S, Eber, M.R. and Laxminarayan, R. **2010**. Increasing resistance of Acinetobacter species to imipenem in United States hospitals, 1999-2006. *Infect Control Hosp Epidemiol*, **31**: 196–197.