MOLECULAR DETECTION AND ANALYSIS OF MUT L GENE FROM *Pseudomonas aeruginosa* IN URINARY TRACT INFECTION PATIENTS

A. S. Hamzah
Lecturer
Middle Technical University, Technical Institute/Kut, Iraq.
aalaatad@yahoo.com

ABSTRACT

This study was aimed to detect and analysis of *mutL* gene from *Pseudomonas aeruginosa* in UTI patients .Two hundred midstream urine specimens were collected from UTI patients. Laboratory diagnosis carried out depend on morphological and biochemical tests and confirmed by using VITEK- 2 Compact system. The results showed twenty isolates of *Pseudomonas aeruginosa* from two hundred urine specimens in percentage (10%). Genome extracted from all twenty isolates by specific kit , then amplification of *mutL* gene in PCR by specific primers. Sequencing process of product PCR of all twenty isolates was done. *MutL* gene sequencing of all twenty isolates was alignment in NCBI and the phylogenetic tree was drew by using Geneious 10 software among sequence of locally isolates. The results of analysis sequence of *mutL* gene from locally isolates in phylogenetic tree showed seven different groups. Analysis comparison sequencing of *mutL* gene between locally and global isolates showed eight different groups in phylogenetic tree included three groups (A,G,H) identical with global isolates and five groups (B,C,D,E,F) were not identical with global isolates , therefore selected one isolate (SD-9) from these five locally groups and documented as anew Iraqi isolate in national GenBank and accepted under accession number (LOCUS MF898429) of nucleotides sequence and protein ID(AUI39222.1).

Key words: Iraqi isolate , amplification , phylogenetic tree , sequencing of nucleotides , gel electrophoresis

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الكشف والتحليل الجزيئي لجين MUT L من بكتريا *Pseudomonas aeruginosa* في مرضى التهاب المجاري البولية

علاء سالم حمزة

مدرس

الجامعة التقنية الوسطى ، المعهد التقني / كوت ، العراق.

المستخلص

هدفت هذه الدراسة للكشف و التحليل الجزيئي لجين *mutL* من بكتريا *Pseudomonas aeruginosa* في مرضى التهاب المجاري البولية . جمعت مئتي عينة من المجرى الوسطي للإدرار. أجري التشخيص المختبري بالإعتماد على الصفات المظهرية والفحوصات الكيموحيوية وتم تأكيد التشخيص بإستخدام VITEK– 2 Compact system . بينت النتائج وجود عشرين عزلة بكترية من اصل مئتي عينة ادار وبنسبة 10% . تم استخلاص المادة الوراثية ومن ثم تضخيم الجين *mutL* بإستخدام برايمرات خاصة . اجريت عملية تحديد تسلسل التعاقبات النيوكليوتيدية لناتج ال PCR لجميع العزلات البكتيرية العشرين . تم مطابقة تسلسل التعاقبات النيوكليوتيدية لجين *mutL* لجميع العزلات البكتيرية في NCBI ورسمت الشجرة الوراثية بأستخدام برنامج Geneious 10 بين تسلسل القواعد النيتروجينية للعزلات المحلية . بينت نتائج تحليل التعاقبات النيوكليوتيدية لجين *mutL* بين العزلات المحلية في الشجرة الوراثية وجود سبعة مجاميع مختلفة. بينت مقارنة تحليل التعاقبات النيوكليوتيدية لجين *mutL* مع العزلات العالمية وجود ثمانية مجاميع مختلفة في الشجرة الوراثية تضمنت ثلاثة مجاميع (A,G,H) متطابقة مع العزلات العالمية وخمسة مجاميع (B,C,D,E,F) لم تتطابق مع العزلات العالمية، لذلك اختيرت عزلة واحدة (SD–9) من تلك المجاميع الخمسة المحلية وسجلت كعزلة عراقية جديدة في بنك الجينات العالمي وتم قبولها تحت الرقم التسلسلي (LOCUS MF898429) وتسلسل الأحماض الامينية للبروتين تحت الرقم التسلسلي (AUI39222.1).

الكلمات المفتاحية: العزلة العراقية، تضخيم، الشجرة الوراثية، تسلسل القواعد النيتروجنية، الترحيل الكهربائي على الهلام
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INTRODUCTION

Urinary tract infecion is very important disease caused by different types of bacteria like *P. aeruginosa* especially patients with catheterization of the urinary tract is the major cause of nosocomial acquired-UTI (16). *P. aeruginosa* is opportunistic pathogen can utilize catheters and attach to the surface of catheter to form biofilm (12,14) . This non fermentative gram negative bacteria consider causative agent of about (10%–20%) of nosocomial infections (septicemia in intensive-care units, cystic fibrosis, burn and wound infections) (19) and about 9% of all healthcare-associated infections because increasing resistance level against antibiotics . *Pseudomonas* bacteria included more than 120 species that are all-over in moist environments such as water and soil ecosystems and infective to plants, animals and humans (6, 10) . Mismatch repair protein is encoded by *mutL* gene in *P.aeruginosa* . This protein is one types of the MMRS (mismatch repair system) . The function of this protein is an important in correction of replication errors that escape from polymerase proofreading activity and for preventing homeologous recombination events (4). The correction pathway started by linking mutL protein with mutS protein and this complex activate process of correction (activates the strand discriminating endonuclease MutH, which cleaves the newly synthesized, unmethylated daughter strand at the nearest hemimethylated d(GATC) site, and thereby marks it for a removal and a repair–synthesis process that involves a variety of other proteins) (13,17). So, this study aimed to achieve molecular detection and analysis of *mutL* gene from *Pseudomonas aeruginosa* in UTI patients through detect sequencing and comparison between locally isolates and between locally and globally isolates.

MATERIALS AND METHODS

Collection of Specimens: In this study, two hundred urine specimens from patients with UTI in some hospitals in Baghdad were teaching lab of Baghdad hospitals, AL-Kindy teaching hospitals and Imam Ali hospitals during the period from September to December 2017.

Laboratory diagnosis: The specimens of midstream urine were cultured on MacConkey agar and blood agar media and incubated for 18-24 hrs at 37^{o}C. selected non fermentatative bacrerial isolates of lactose on MacConkey agar and used biochemical tests according to Harly and Prescott. (2002) (8) . VITEK- 2 Compact system was carried out to confirm diagnosis *Pseudomonas* isolates to species level according to manufactures'instructions (Biomerieux/ France).

Extraction of genetic material

Genetic material was extracted by wizard® genomic DNA purification kit (Promega , USA) was carried out according to manufactures'instructions.

Amplification and gel electrophoresis of *mutL* gene

Amplification process of *mutL* gene was carried out by specific primers and program conditions according to Barry *et al*.,(2004) (3) as shown in table (1) and gel electrophoresis against 100bp DNA ladder (Promega,USA).

Table 1. Primers and program conditions for amplification of *mutL* gene

Primer type (Sequence 5'→3')	Expected amplicon size	Program conditions
Forward : CCAGATCGCCGCCGGTGAGGTG Reverse : CAGGGTGCCATAGAGGAAGTC	940 bp	Initial denaturation at 96°C for 1 min , 30 cycles of denaturation at 96°C for 1 min, primer annealing at 55°C for 1 min , extension at 72°C for 1 min , final extension step of 72°C for 10 min.

Sequencing of *mutL* gene

Analysis of phylogenetic tree of *mutL* gene

PCR products of *mutL* gene of all twenty isolates were sent for sequencing using ABI3730XL, automated DNA sequencer, by macrogen corporation-Korea. Analysis of phylogenetic tree was performed , which the results of sequencing of all twenty isolates were analyzed by using Geneious 10 software. The sequenced DNA were analyzed by BLASTn tool of NCBI GenBanK database . The highly strain identity was established by comparing the query sequences with those available in the GenBanK database. The

confirmation of species was done when the closest alignment match has very high identity to the homologues found in Gene-Bank. DNA sequences identities were also computed using the pairwise alignment by Geneious 10 software. Multiple sequence alignments were performed using Geneious alignment. phylogenetic analyses were inferred by the maximum likelihood method.

RESULTS AND DISCUSSION

Isolation and identification:The specimens of midstream urine were cultured on MacConkey agar and blood agar media incubated for 18-24 hrs at 37^{o}C. Then chose bacterial isolate that non fermentatative of lactose on MacConkey agar and used morphological and biochemical tests. Results showed the isolates were Gram stain negative, oxidase positive, citrate positive , urea hydrolysis positive, catalase positive, bluish green pigmentation positive, indole production negative, voges proskaeur negative and methyl red negative . Then confirmed laboratory diagnosis of *Pseudomonas aeruginosa* by using VITEK- 2 Compact system according to manufactures' instructions (Biomerieux/ France). Results showed twenty isolates of *Pseudomonas aeruginosa*. Locally studies on isolation and identification of this bacterium from urine and other clinical sources included study by (23) who isolated and identified this bacterium from different clinical sources (four isolate from midstream urne, bacteremia, eye infection and otitis media). Other locally study carried out by Klrissa and Mohammad , 2016 (12) which isolated this bacteria from otitis media (28 isolates). From the global studies (11) who conducted that percentage of this bacteria in midstream urine was 27% . Distribution of infection with *Pseudomonas aeruginosa* belong to ability of this bacterium to produce different virulence factors. These virulence factors encoding by virulence genes located in the chromosome of *Pseudomonas aeruginosa* that enable it to play a great role in causes of the urinary tract infection (2, 7, 22).

Molecular detection of *mutL* gene

Gel electrophoresis was done of all PCR product against 100bp DNA ladder and the results showed that all twenty *Psudomonas aeruginosa* isolates contain *mutL* gene as shown in figure 1.

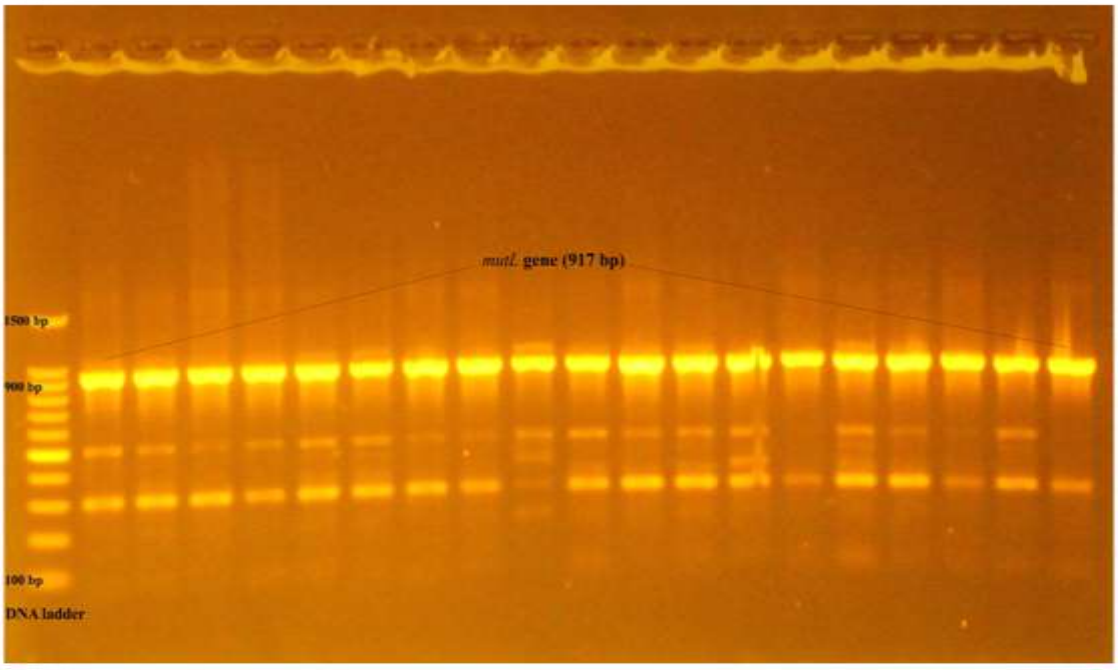

Figure 1. Gel electrophoresis for amplified *mutL* gene from *Pseudomonas aeruginosa* on agarose gel (1%) , 50V for 1 hour.

Sequencing of *mutL* gene from *Pseudomonas aeruginosa*

Results of sequencing *mutL* gene of all twenty isolates showed 917 bp as shown in figure 2. All these sequence analyzed by Geneious 10 software to analysis of phylogenetic tree in next steps.

Figure 2. Alignments sequence of *mutL* gene from Iraqi bacterial isolate *Pseudomonas aeruginosa* (query) against standard JQ316664.1 in GenBank.

Analysis sequence of *mutL* gene in Iraqi bacterial isolate *Pseudomonas aeruginosa*

The results in genetic tree showed there are genetic relationship between isolates as shown in figure 3. The results conducted different seven groups were isolates (SD-8_mutL, SD-14_mutL, SD-15_mutL)in group A. Second group B included isolate (SD-10_mutL), Third group C included isolates (SD-13_mutL, SD-17_mutL), fourth group D included isolate (SD-9_mutL), fifth group E included isolates (SD-1_mutL, SD-18_mutL, SD-19_mutL), sixth group F included isolates (SD-6_mutL, SD-12_mutL, SD-11_mutL, SD-16_mutL) and seventh group G included isolates (SD-7_mutL, SD-4_mutL, SD-2_mutL, SD-20_mutL, SD-5_mutL, SD-3_mutL).

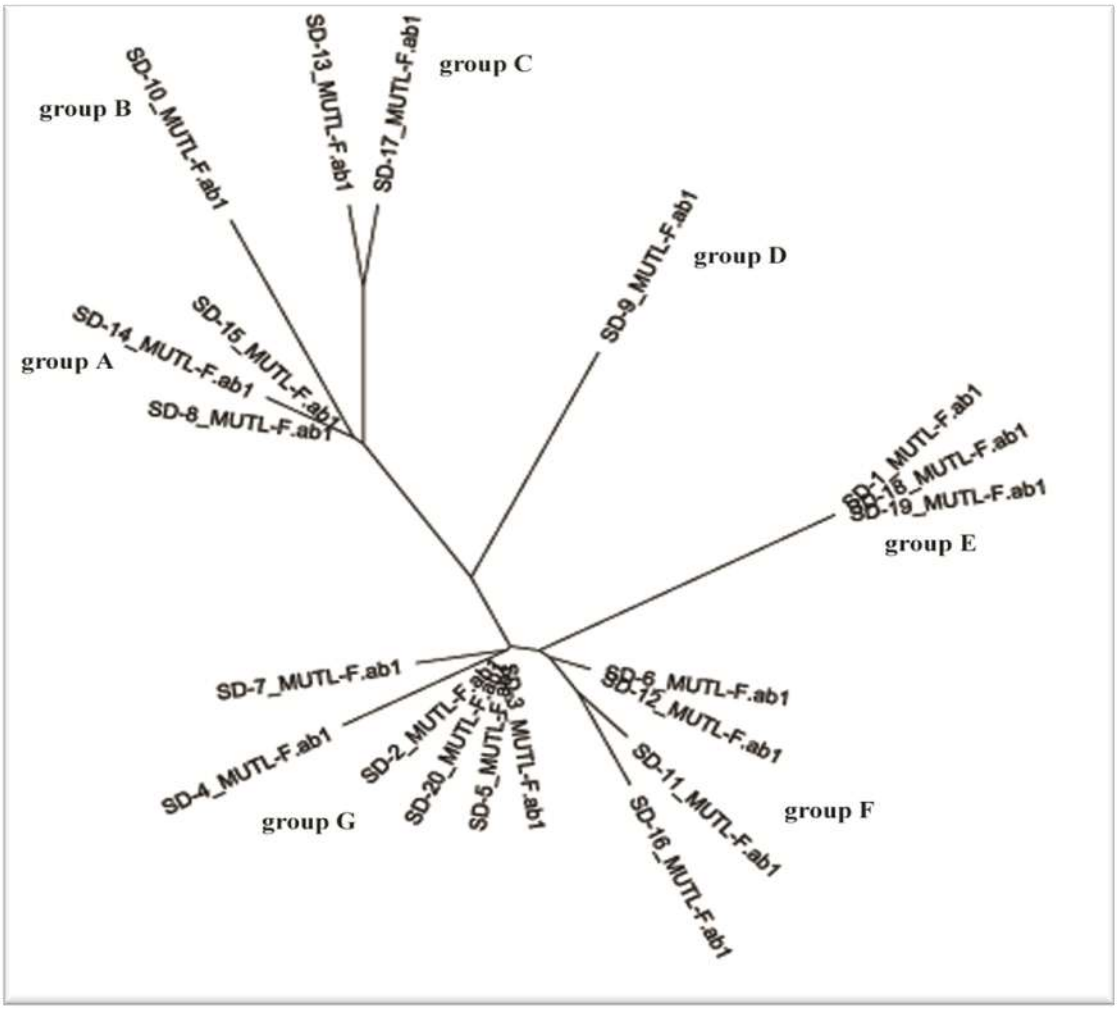

Figure 3. Unrooted phylogenetic tree of *mutL* gene among locally *Pseudomonas aeruginosa* isolates. The tree was organized with the maximum eventuality method using Geneious 10 program

Analysis comparison sequence of *mutL* gene in Iraqi and global *Pseudomonas aeruginosa* isolates: Analysis of sequences *mutL* gene in locally and global *Pseudomonas aeruginosa* performed through drawing phylogenetic tree by using Geneious 10 program against ten high identity isolates that documented in gene bank under accession numbers (LN870292, KX784363, KX784362, KX784360, CP022002, CP021380, JQ316665, CP007147, CP013993, CP012066). The results in phylogenetic tree were showed eight groups. First group included locally isolates (SD-1, SD-18, SD-19) that identity with global isolates in gene bank under accession number (LN870292, KX784363, KX784362, KX784360, CP022002, CP021380, JQ316665). There are five groups not identity with global registered isolates, which contained only locally isolates were group B (SD-8, SD-14,SD-15), group C (SD-13, SD-17), group D (SD-10), group E (SD-9) and group F (SD-6, SD-12, SD-11, SD-16). Seventh group G included (SD-3, SD-5) that identity with global isolate under accession number (CP007147), eighth group H included locally isolates (SD-2, SD-20, SD-7, SD-4) that identity with global isolates under accession number (CP013993, CP012066) as shown in figure 4 and table 2. Locally *Pseudomonas aeruginosa* isolates in five groups (B, C, D, E, F) that not identity with global isolates because the genetic variation may be new isolates in UTI patients , So selected one group of them was group E (SD-9) to register as a new isolate in global gene bank and it is accepted under accession numbers (LOCUS MF898429) of nucleotides sequence and protein ID(AUI39222.1). Product of *mutL* gene (mismatch repair protein) play important role in correction process of replication errors that escape from

polymerase proofreading activity by DNA polymerase and protect *Pseudomonas aeruginosa* from mutation that may lead to death of bacterial cell (1,4,20). All that's established to distribute these pathogenic bacteria that considered a major healthcare problem and appearance resistant to many antibiotic and most challenging pathogen which It is one of the most important causative agents of nosocomial infections and difficult to eradicate due to acquired resistance to many antibiotics (5,9). *Pseudomonas aerugiosa* in UTI patients associated with high mortality in hospitalized patients, which increases significantly in those with severe from chronic renal failure, advanced liver disease and diabetes mellitus patients as well as infected other patiants because possess different virulent gene in pathogenecity island that's encoded to the different high virulence factors (15,18).

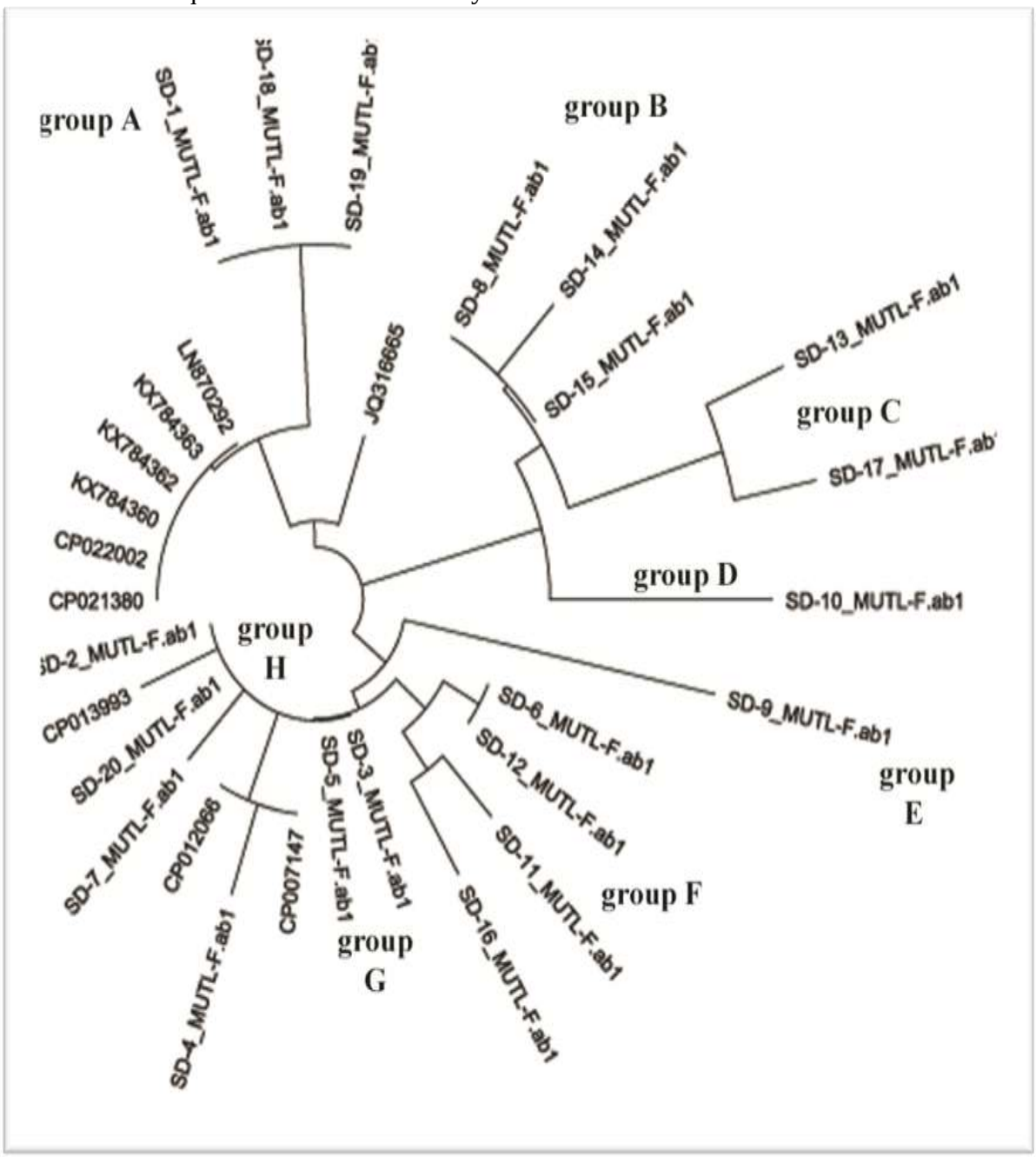

Figure 4. Unrooted phylogenetic tree of *mutL* gene among *Pseudomonas aeruginosa* and Global isolates were LN870292, KX784363, KX784362, KX784360, CP022002, CP021380, JQ316665 that documented in GeneBank. The tree was structured with the maximum endurance method using Geneious 10 program

Table 2. Classification of locally and identical global *Pseudomonas aeruginosa isolates* in phylogenetic tree according to nucleotides sequence of *mutL* gene.

Symbol of group	Symbol of locally isolates	Accession number of global isolates in GeneBank
Group A	SD-1 SD-18 SD-19	LN870292 KX784363 KX784362 KX784360 CP022002 CP021380 JQ316665
Group B	SD-8 SD-14 SD-15	Not identical
Group C	SD-13 SD-17	Not identical
Group D	SD-10	Not identical
Group E	SD-9	Not identical
Group F	SD-6 SD-12 SD-11 SD-16	Not identical
Group G	SD-3 SD-5	CP007147
Group H	SD-2 SD-20 SD-7 SD-4	CP013993 CP012066

Documentation in NCBI

The comparison of sequence *mutL* gene between Iraqi and global isolates shown some of locally isolates (eleven isolates) in phylogenetic tree were un similarity with global isolates, therefore one of these locally isolates (SD-9) was selected and documented in NCBI as anew Iraqi isolates and accepted under accession number (LOCUS MF898429) of nucleotides sequence and protein ID(AUI39222.1).

Conclusion In analysis sequence of *mutL* gene from Iraqi bacterial isolate *Pseudomonas aeruginosa* in UTI patients concluded found genetic variation in sequence of this gene which the isolates distributed in seven different groups in phylogenetic tree. After Analysis comparison sequence of *mutL* gene in locally and global *Pseudomonas aeruginosa* isolates that documented in NCBI , the phylogenetic tree showed nine locally isolates idendity with global isolates and eleven locally isolates not identical with global isolates, So selected one isolate (SD-9) from these eleven locally isolates and registered in global gene bank under accession number (LOCUS MF898429) of nucleotides sequence and protein ID(AUI39222.1) as anew isolate.

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