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RESEARCH ARTICLE

Molecular Identification of Isolate from *Escherichia coli* Isolates from Dialysis Patients

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Abstract

The aim of this present study to was to detect the 16S rRNA gene sequences of *Escherichia coli* local isolate from dialysis patients and to compare their genetic relatedness utilizing phylogenetic analysis. A total of (75) clinical samples (Urine) were collected from in dialysis patients but found thirty specimens of *E. coli* admitted at Baghdad Medical City, Baghdad Teaching Hospital during the period from October, 2017 to January, 2018 platinum calibrated sterile wire loop to transfer 1µl of the uncentrifuged urine specimen and streaked on MacConkey agar incubated at 37°C for 18-24 hours. The results of phylogenetic analysis showed that Proximity and the genetic dimension among themselves and the world with more than 98% to 99%compatibility values.

Keywords: *E. coli*, UTI, 16S rRNA gene.

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INTRODUCTION

Clinically, urinary tract infections (UTIs) are classified as uncomplicated or complicated; Complicated UTIs are known as UTIs associated with agents that compromise the urinary tract or steward defence, involving urinary obstruction, urinary retention raised by neurological disease, immunosuppression, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, indwelling catheters or another drainage devices (Flores-Mireles *et al*., 2015). Urinary tract contagion caused by bacteria associated with an increased antimicrobial resistant types are common in all age groups. Most urinary tract infections are initiated by organisms that gain entrance of the natural environment to the bladder through the urethra and are more prevalent in women than men, Urinary pathogens vary depending upon age, sex, catheterization, hospitalization and previous exposure for antimicrobials (Biadglegne and Abera, 2016). *Escherichia coli* is facultative anaerobe, Gram-negative bacilli, belong to Enterobacteriaceae. The lower intestine of human is the natural habitat of this bacterium, which resident in commensalism manner. However pathogenic *Escherichia coli* strains play a critical role in severe infections to human being (Farrokh *et al*., 2012). *E. coli* gives rise to maximal recurrent infections occur in human such as urinary tract infections, intestinal diseases, neonatal meningitis and septicemia (Motayo BO1 *et al*., 2012). In this study aim to the determine the role of 16S rRNA genes in detection of *E. coli* isolates in dialysis patients.

MATERIALS AND METHODS

Samples Collection

A total of (75) clinical samples (Urine) were collected from in dialysis patients but found thirty specimens of *E. coli* admitted at Baghdad Medical City/ Baghdad Teaching Hospital during the period from October, 2017 to January, 2018.

Isolation and Identification of *Escherichia coli*

According to the diagnostic procedures recommended by MacFaddin and Benson (McFadden, 2000, Benson., 2001)Urine specimens were collected from the patients at admission to the hospital in a sterile container. A loopful of uncentrifuged urine samples was cultured onto blood agar, MacConkey agar and EMB media incubated for 24 hrs at 37°C, Fig. 1.

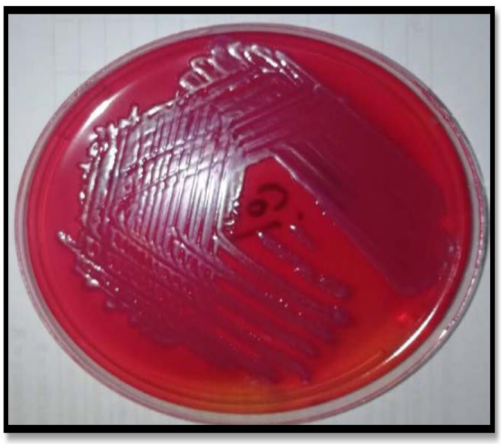

Fig. 1. Identification of *E.coli* in MacConkey agar

DNA extraction

Genomic DNA was extracted from clinical isolates of *E. coli* using (DNA mini kit that was supplied by G- spin DNA extraction kit, Korea) according to manufacturer's instructions, primer were used in this study were obtained from microgen company (16S rRNA) Forward strand primers 5'- AGA GTTT GAT CCT GGC TCAG -3' and Reverse strand primers 5'- CTT GTG CGGG CCCCC GTC AATTC -3', Fig. 2.

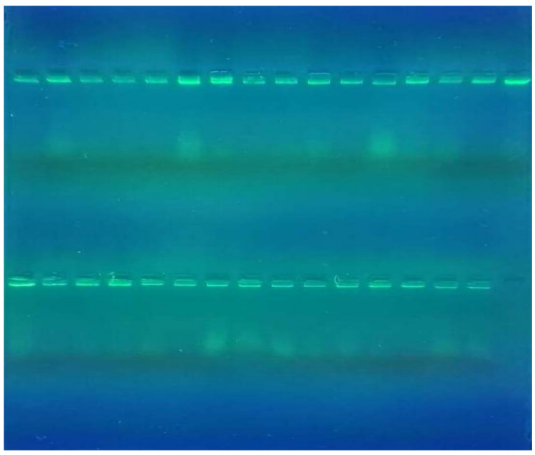

Fig. 2. Gel Electrophoresis of genomic DNA on 1%agarose gel, (70 Volt/30 minute)

PCR Amplification

The 16S rRNA gene was amplified using primer F (5'- AGA GTTT GAT CCT GGC TCAG -3') and primer R (5'- CTT GTG CGGG CCCCC GTC AATTC -3'). The PCR amplification is performed in a total volume of 25ml containing 1.5ml DNA, 5ml Master Mix PCR (intron, korea), 1ml of each primer 10 pmol then nuclease-free water is added into a tube to a total volume from 25ml. Thermo cycling conditions were as follows: initial denaturation at 3 min at 95°C, followed by 35 cycles of denaturation 95°C for 45 sec, annealing at 62°C for 45sec, extension at 72°C for 45 sec and a final extension of 72°C for 7 min. The PCR products were separated on 2% agarose gel. The gel is left to run for 90 min with a 70 volt/65Am current. Following electrophoresis, visualization was conducted with a UV transilluminator after red stain staining**.**

Sequencing

The sequencing of 16S rRNA gene was performed at Macrogen Inc., by using NCBI BLAST.

RESULTS AND DISCUSSION

16S rRNA Genetic Polymorphism

One and half µl of genomic DNA were used for each PCR reaction. A conventional PCR protocol was used to analyze simultaneously the presence of 16S rRNA gene. The presence of the 16S rRNA gene was identified by 850bp, Fig. 3.

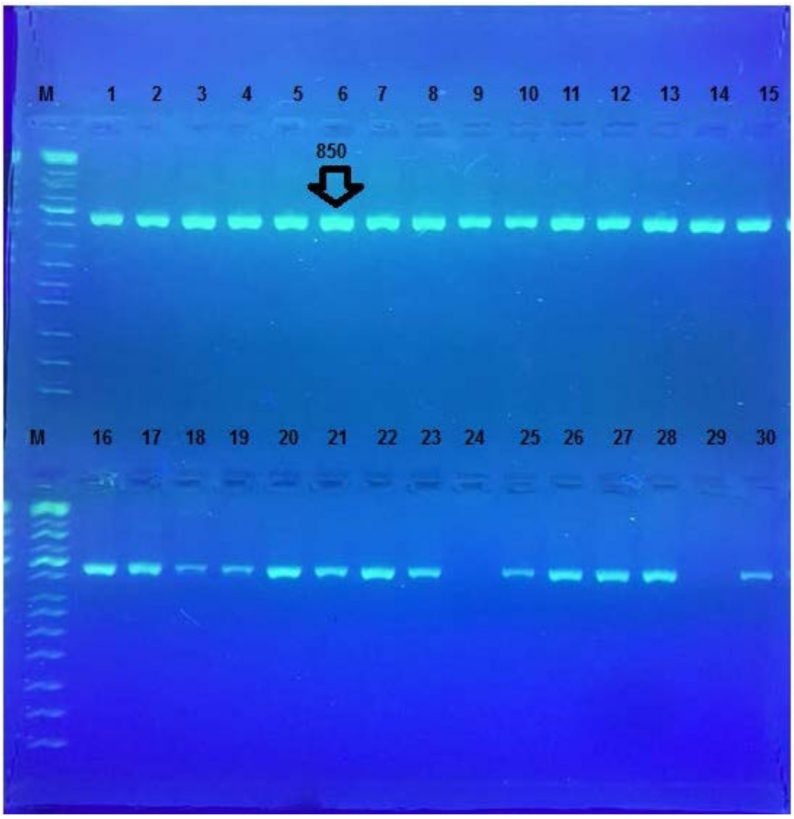

Fig. 3. Agarose gel electrophoresis for16S rRNA gene (850bp). Bands were fractionated by electrophoresis on a 2% agarose gel (2 h., 5V/cm, 1X TBE) and visualized under U.V. light after staining with red stain. Lane: 1 (M: 100bp ladder)

The sequencing of amplified product of 16S rRNA gene for Study Groups

The sequencing of amplified product of 16S rRNA gene by direct sequencing. Our sequences were compared with the reference sequence of in national center biotechnology information (NCBI) Gene Bank.

A- G>C , T>A , A>T , G>A , T>G ,and G>T genotyping.

Table 1. Represent type of polymorphism of 16S rRNAgene from *E. coli* isolate

No. of Sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate 1	Transversion	63	G>C	ID: HQ163793.1
	Transversion	66	T>A	
	Transversion	68	A>T	
	Transversion	489	G>C	
	Transition	492	G>A	
	Transversion	503	T>G	
	Transition	509	G>A	
	Transversion	536	G>T	
	Transversion	549	G>C	
	Transversion	580	G>C	

After alignment of product amplification of 16S rRNA gene for isolate 1 having two Transition G>A, eight Transversion G>C , T>A , A>T , T>G, and G>T from the Gene Bank, found that part of 16S rRNA gene having 98% compatibility with standard in Gene Bank 16S rRNA gene as shown in Table (1) (Singh SK.,2009).

B- G>A , C>T, T>C, G>T, and G>C genotyping.

After alignment of product amplification of 16S rRNA gene for isolate 2 having four Transition G>A, C>T, T>C , three Transversion G>T, and G>C from the Gene Bank, found that part of 16S rRNA gene having 99% compatibility with standard in Gene Bank 16S rRNA gene, Table 2.

C- G>C , G>T, A>C, and G>A genotyping.

Table 2. Represent type of polymorphism of 16S rRNAgene from *E. coli* isolate

No. of Sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate 2	Transition	10	G>A	ID: : KP244274.1
	Transition	43	C>T	
	Transition	91	T>C	
	Transversion	537	G>T	
	Transversion	540	G>C	
	Transversion	550	G>C	
	Transition	568	G>A	

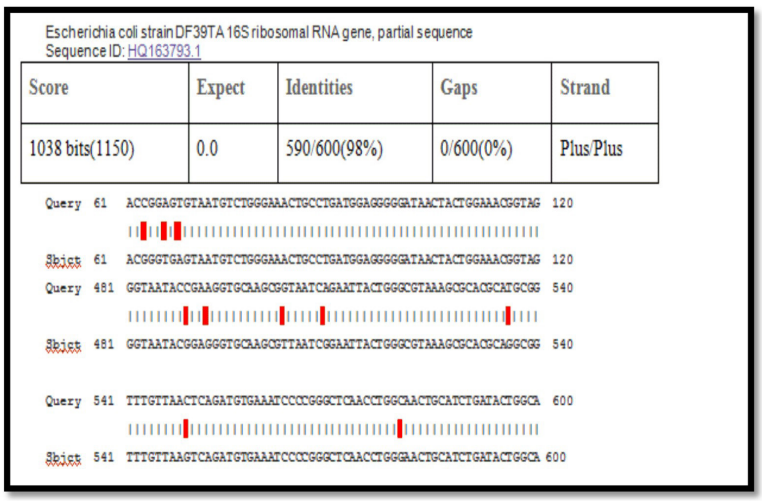

Fig. 4. Sequence analysis of16S rRNA gene for *E.coli* isolate

After alignment of product amplification of 16S rRNA gene for isolate 3 having three Transition G>A , nine Transversion G>C , G>T, A>C from the Gene Bank, found that part of 16S rRNA gene having 98% compatibility with standard in Gene Bank 16S rRNA gene, Table 3 (Ramesh *et al.,* 2015).

D- M(A,C)>A ,and G>A genotyping.

After alignment of product amplification of 16S rRNA gene for isolate 4 having two Transition G>A, one Transversion M(A,C)>A from the Gene Bank, found that part of 16S rRNA gene having 99% compatibility with standard in Gene Bank 16S rRNA gene, Table 4 (Kohl KD *et al.,*2018).

Phylogenetic tree structuring

The phylogenetic tree diagrammatic by Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0. Sequences that showed the highest identity (>98%) and maximum coverage (>99%), by comparison between isolated from dialysis patients Neighbor-joining tree was constructed for phylogenetic analysis. The genetic dimension between Iraq and the isolates of the world is detailed according to the Phylogenetic tree and the comparison table. Hierarchical cluster analysis determine the following clusters: large Cluster divided into several neck: first root the USA: Iraq and India the genetic dimension

Table 3. Represent type of polymorphism of 16S rRNAgene from *E. Coli* isolate.

No. Of sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate 3	Transversion	447	G>C	ID: : KP244274.1
	Transversion	537	G>T	
	Transversion	91	G>C	
	Transversion	550	A>C	
	Transversion	553	G>C	
	Transition	554	G>A	
	Transition	609	G>A	
	Transition	619	G>A	
	Transition	627	G>A	
	Transversion	635	G>C	
	Transversion	641	G>C	
	Transversion	643	G>C	
	Transversion	646	G>C	

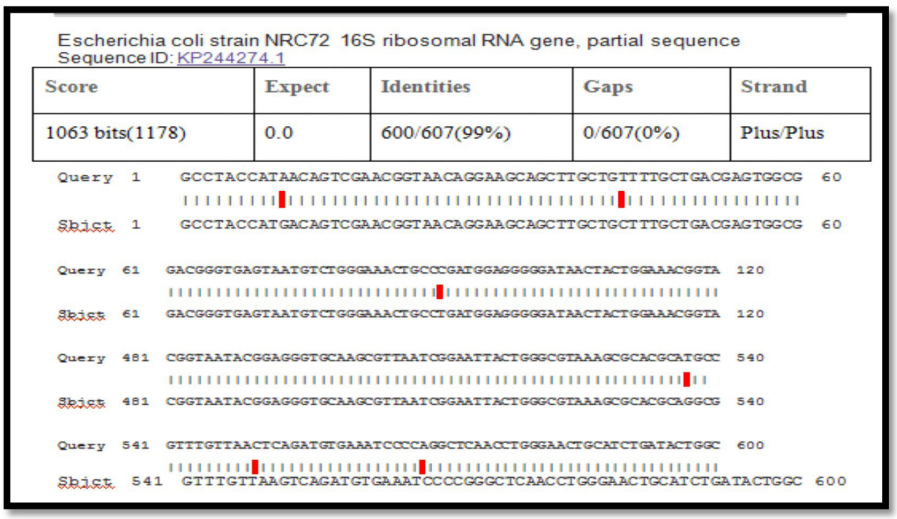

Fig. 5. Sequence analysis of 16S rRNA gene for *E.coli* isolate

Table 4. Represent type of polymorphism of 16S rRNAgene from *E. Coli* isolate.

No. Of sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate 4	Transversion	228	M(A,C)>A	ID: : KP789327.1
	Transition	638	G>A	
	Transition	667	G>A	

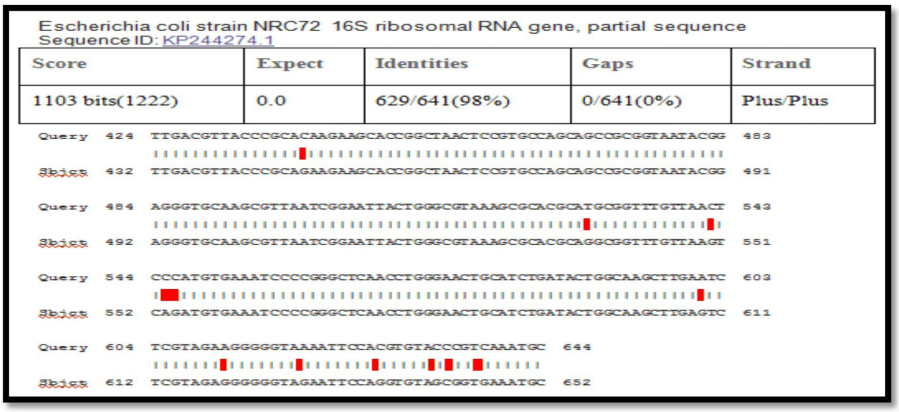

Fig. 6. Sequence analysis of 16S rRNA gene for *E.coli* isolate

was by 2.3 it is close to UNITED KINGDOM ''ID: FN356960.1'', third root including Iraq isolate *E.coli* the genetic dimension was by 2.7 it is close to Bangladesh ''ID: MG857757.1''. The last cluster is divided into two branches the first branch Iraq the genetic dimension was by 3.5 it is close to Sweden "ID: CP029579.1"

When comparison between *Escherichia coli* isolated from dialysis patients recorded in the National Center Biotechnology Information (NCBI) and isolated from different source have under sequence (ID: HQ163793.1**,** ID: EU420950.1**,** ID: MF179674.1**,** ID: KY655125.1**,** ID: FN356960.1**,** ID: KP789332.1**,** ID: MG857757.1**,** ID: LM997164.1, ID: CP029973.1**,** ID: CP029579.1, ID: CP026473.1**)** (Shahi *et al.,* 2016, Wang, C *et al.,*2008, Farag *et al.,*2017, Bozcal *et al.,*2018, Ogue *et al.,*2009, Momtaz *et al.,*2017, Johansen *et al.,*2014, Zurfluh

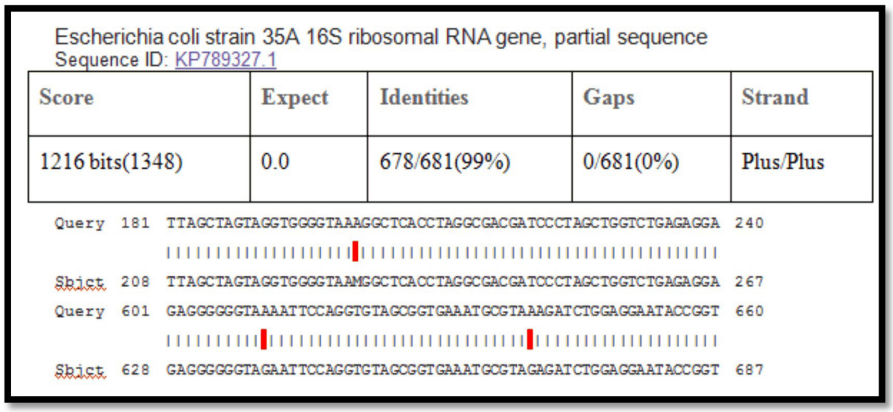

Fig. 7. Sequence analysis of 16S rRNA gene *E.coli* isolate

*et al.,*2017, Kim *et al.,*2017) respectively with source of isolation and showed compatibility the highest identity (>98%) and maximum coverage (>99%) and score (1038-1216), and expect 0.0 with gene bank.

Submission of local Iraq isolate in NCBI

The 16S rRNA gene were registered after the correspondence of the National Center for Biotechnology Information and obtained accession number and became a reference to Iraq and the Middle East and the world. Ongoing work will add to this set as more type strains are publishedand it is available for download at NCBI: https://www.ncbi.nlm.nih.gov/nuccore/MH628651.1, MH628665.1, MH628664.1, MH 628646.1.

CONCLUSION

The efficiency of genetic methods in the detection of accurate and rapid bacterial isolates proved near the Iraqi isolates *E. coli* of isolates Sweden

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