Molecular detection of *Escherichia coli* local isolate from pregnant Iraqi women

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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 pregnant Iraqi women and to compare their genetic relatedness utilizing phylogenetic analysis. Urinary tract infection (UTI) in pregnant women is especially important because it may produce complications that may be serious to the mother and fetus. Seventy-five samples were collected aseptically in sterile disposable containers. The microscopic examination of the urine plays an important role in the diagnosis of UTI based on the presence or none of pus cell, crystals, Epithelium cell and RBCs, as well as the appearance of the bacteria in the urine sediment under the microscope in large numbers confirming its presence in the urine. However, the culture remains the basic rule in diagnosing urinary tract infections. The specimens were labeled and transported to the microbiology laboratory. A calibrated sterile platinum wire loop was used to transfer 1µl of the uncentrifuged urine specimen and streaked on MacConkey agar and EMB agar during the period from November 2017 to February 2018.

The DNA was extracted from E. coli and the PCR technique 16srRNA gene was detected by the specific primers. Using electrophoresis after adding red safe stain, the bands size appeared 1250 base pairs, then the samples are sent to conduct the sequencing. The results showed three variations: two Transition and once Transversion. The results of phylogenetic analysis showed proximity and the genetic dimension among themselves and the world with more than 99% compatibility values.

Keywords: *E. Coli*, UTI, Pregnancy, 16S rRNA gene.

Introduction

Urinary tract infections (UTI) is considered as the second most common clinical problem[30]. It is concurred from the previous forty decades that UTI is more common in pregnant ladies than in non-pregnant by around 4– 10 times[14]. The physio-anatomical and functional perturbation that happens to the urinary system amid pregnancy, specifically a decrease in the urine concentration by virtue from the physiological increase in plasma volume, equal to 70% of pregnant ladies evolves glucosuria, which facilitates bacterial growth in the urine[23]. Bacteriuria may show as asymptomatic or symptomatic[9]. Asymptomatic infections are usually attributed to pregnant women and the elderly[4]. Various factors including low socioeconomic status, multiparty, age, sexual behavior, urinary tract anomalies, and previous treatment for UTI, in addition to diabetes, sickle cell anemia, and immuno-compromised patients had been linked to asymptomatic bacteriuria[23].

Untreated UTI can be associated with serious obstetric complications. These comprise of caesarean and preterm deliveries with low birth weight infants, intrauterine growth retardation, preeclampsia, maternal sepsis and respiratory insufficiency[21,22]. Among diverse pathogens that cause UTIs during pregnancy, *E. coli* and *Klebsiella pneumoniae* with its multidrug resistant strains are considered as predominant especially the resistance to carbapenem antibiotics[16,27,33]. Many studies have shown a significant relationship between the recovery rate of *E. coli* from (cervical) vaginal cultures and pre-term labor[17,25]. Maternal genitourinary infection is a leading cause of pregnant complications worldwide[2]. In this study, we describe a PCR method based on the DNA sequence from the 16S rRNA subunit for the detection of *Escherichia coli* local isolate from pregnant Iraqi women.

Material and Methods

Samples Collection: Seventy- five samples of *Escherichia coli* were obtained from pregnant Iraqi women. We obtained the samples from External Laboratories in Baghdad, Iraq, during the period of November 2017 to February 2018.

Sample Collection and Bacterial Identification: Total of 75 samples were collected aseptically in sterile disposable containers. Specimens were labeled and transported to the microbiology laboratory. A calibrated sterile platinum wire loop was used to transfer 1µl of the uncentrifuged urine specimen and streaked on MacConkey and EMB agar. All inoculated plates were incubated at 37°C for 18-24 hours. Whole isolates were identified utilizing classical biochemical ways, while an investigation of *E. coli* strains was carried out biochemically and by molecular techniques (PCR) using primers for 16S rRNA.

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.

PCR Amplification: The 16S rRNA gene was amplified using primer. Forward (5'-AGAGTTTGATCCTGGCTCAG -3') and primer Reverse (5'- GGTTACCTTGTTACGACTT -3') [26]. The PCR amplification is performed in a total volume of 25µl containing 1.5µl DNA, 5µl Master Mix PCR (intron, Korea), 1µl of each primer 10 pmol and then nuclease-free water is added into a tube to a total volume from 25µl. Thermo cycling conditions were as follows: initial denaturation at 5 min at 95°C followed by 40 cycles of denaturation 95°C for 45 sec, annealing at 52°C for1 min , extension at 72°C for 1 min and a final extension of 72°C for 7 min. The PCR products were separated on 1% agarose gel.

The gel is left to run for 90min with a 70volt/65 Am current. Following electrophoresis, visualization was conducted with a UV transilluminator after red safe stain staining.

Sequencing: The sequencing of 16S rRNA gene was performed at Macrogen Inc. using their ABI 3730xl genetic analyzer (Applied Biosystems, US). Homology search was conducted using Basic Local Alignment Search Tool (BLAST) program which is available at the National Center Biotechnology Information (NCBI) online at (http://www.ncbi.nlm.nih.gov) and Bio Edit program.

Results and Discussion

Precise identification from bacteria is serious for clinical care and general health surveillance to understand the pathobiology of clinical syndromes and better use of specific antibiotic and infection control strategies for patients and populations. Several have questioned whether standard culture-based approaches can correctly recognize between isolates given that many diverse species can share the same biochemical phenotypes. Indeed, some species not considered "difficult" to identify based on routine culture-based testing were recognized to be misidentified upon sequent sequence-based molecular identification[8]. In this study, we used high quality sequences generated from 16S rRNA gene to detect *Escherichia coli* local isolate from pregnant Iraqi women.

Biochemical tests: The results of the biochemical tests showed positive results for Kovac's Reagent (Indole ring formed), Catalase, Methyl Red, Lactose fermentation and motion test and were negative for both Voges-Proskauer and Oxidase as well as the citrate test[6]. These tests were confirmed by the results of the diagnosis of API 20E for Enterobacteriaceae family produced by Biometrix company.

DAN Extraction: Figure 1 shows the electrophoresis after the extraction process and visualization was conducted using UV transilluminator.

PCR Amplification: The electrophoresis results showed that the size band was 1250 bp using the ladder 10000bp in seen as fig. 2.

Sequence Alignment of 16S rRNA Gene: The results obtained showed that 3 variations: first Transversion T>A, second Transition T>C, and lastly G>A Transition have shown 99% compatibility as showed in table 1 and fig. 3. *Escherichia coli* strain E22 16S ribosomal RNA gene showed partial sequence under sequence ID: KY780357.1 .

Submission of local Iraq isolate in NCBI: Where possible, nearly full lengths of *16S rRNA* gene sequences were selected to be 813bp preferentially. INSD registration that passed the validations steps was then utilized to create reference sequence entries. Ongoing work will add to this set as more type strains are published and it is available for download at NCBI: https://www.ncbi.nlm.nih.gov/nuccore/1316235700.

Phylogenetic Tree Structuring: The phylogenetic tree diagrammatic by (MEGA and NCBI) software version 6.0 is shown in fig. 4. The isolated sequences have shown 99% compatibility. Neighbor-joining tree was constructed for phylogenetic analysis. These alignments appeared for the genetic distance and other global strains by partial sequence similarity in 16S rRNA gene for translating specific region. The genetic dimension between Iraq and the isolates of the world is detailed according to the Phylogenetic tree. Hierarchical cluster analysis determines the following clusters: large cluster divided into several necks. The last neck is divided into two branches the first branch *Escherichia coli* from India: Sambalpur"ID: KF991482.1 "the genetic dimension was by 0.009, and the second branch including Iraq ID: MG722676 " the genetic dimension was by 0.002 it is close to Saudi Arabia, India, and Egypt "ID: KY780357.1, ID: KJ540213.1, ID: FR719725.1" respectively; the genetic dimension was by 0.001.

Figure 4 revealed only sequences that showed the highest identity (>95%) and maximum coverage (>99%).

Submission of Local Iraqi Isolates in NCBI: The16SrRNA gene was registered after the correspondence of NCBI and obtain accession number.

Table 1
Represent Type of Polymorphism of 16srRNA Gene from *Escherichia coli* Isolate

No. Of sample	Type of substitution	Location	Nucleotide	Sequence ID
Isolate1	Transition	145	T>C	ID: KY780357.1
	Transversion	419	T>A	
	Transition	421	G>A	

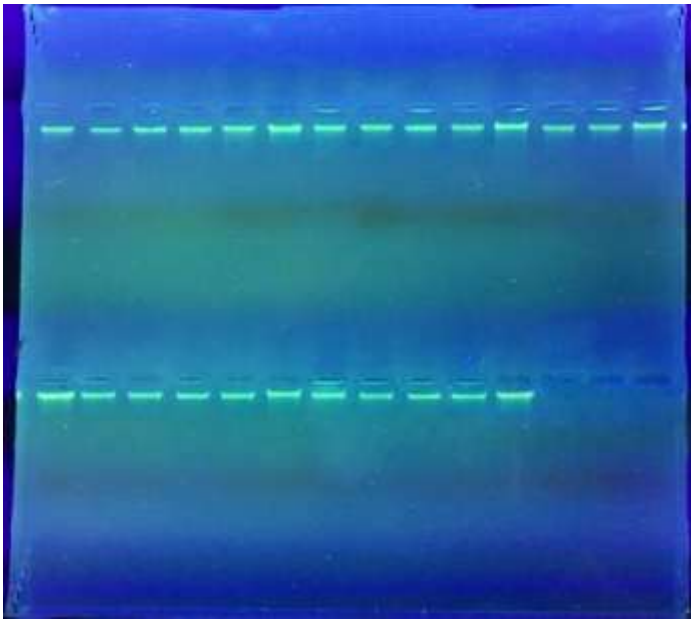

Fig. 1: Gel electrophoresis of genomic DNA extraction 1% agarose gel at 5 vol /cm for 1:15 hour

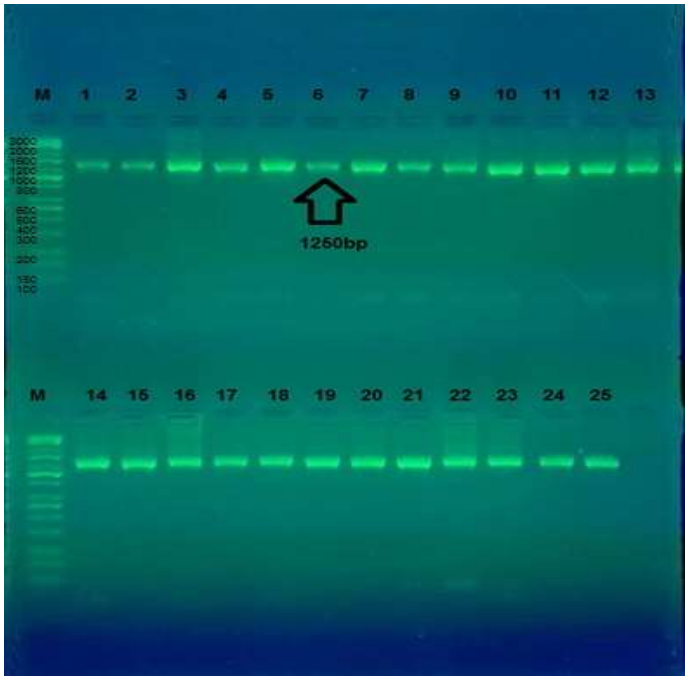

Fig. 2: Gel electrophoresis of PCR product *(*1250bp), for *Escherichia coli* 2% agarose gel at 5volt /cm for 2 hours. Lane 1-25: PCR product positive for 16S rRNA genes

Score	Expect	Identities	Gaps	Strand
1546 bits (804)	0.0	810/813(99%)	0/813(0%)	Plus/Plus

```
Query  121  GCATAACGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  139  GCATAATGTCGCAAGACCAAAGTGGGGGACCTTCGGGCCTCATGCCATCAGATGTGCCCA  198

Query  361  GCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGATAAGGTTAATAACCTTGTC  420
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  379  GCCTTCGGGTTGTAAAGCACTTTCAGCGGGGAGGAAGGCGTTGAGGTTAATAACCTTGTC  438
```

Fig. 3: Sequencing of *Escherichia coli an* obtained from Gene Bank

Table 2
Sequencing ID in Gene Bank, And Compatibility of DNA Sequences Obtained from National Center Biotechnology Information (NCBI)

Accession	Gene	Country	Source	Compatibility
ID: KY780357.1	16SrRNA	Saudi Arabia	*E. coli*	99%
ID: KJ540213.1	16SrRNA	India	*E. coli*	99%
ID: FR719725.1	16SrRNA	Egypt	*E. coli*	99%
ID: KF991482.1	16SrRNA	India: Sambalpur	*E. coli*	98%
ID: MH040909.1	16SrRNA	Ecuador: Manabi	*E. coli*	98%
ID: KT287074.1	16SrRNA	China	*E. coli*	97%
ID: FJ789678.1	16SrRNA	South Korea: Seoul area, Han River	*E. coli*	97%
ID: KU052648.1	16SrRNA	Nigeria: Ogun State, southwestern	*E. coli*	96%
ID: MH346261.1	16SrRNA	South Africa	*E. coli*	96%
ID: KX575718.1	16SrRNA	Saudi Arabia: Al-Ahsa	*E. coli*	97%
ID: MG554686.1	16SrRNA	Iran	*E. coli*	96%
ID: MG198700.1	16SrRNA	Sudan	*E. coli*	96%
ID: KY367395.1	16SrRNA	Poland	*E. coli*	96%
ID: MH111682.1	16SrRNA	Turkey	*E. coli*	96%
ID: JQ781569.1	16SrRNA	Portugal	*E. coli*	96%
ID: GU811877.1	16SrRNA	Germany	*E. coli*	96%
ID: KP789329.1	16SrRNA	USA	*E. coli*	95%

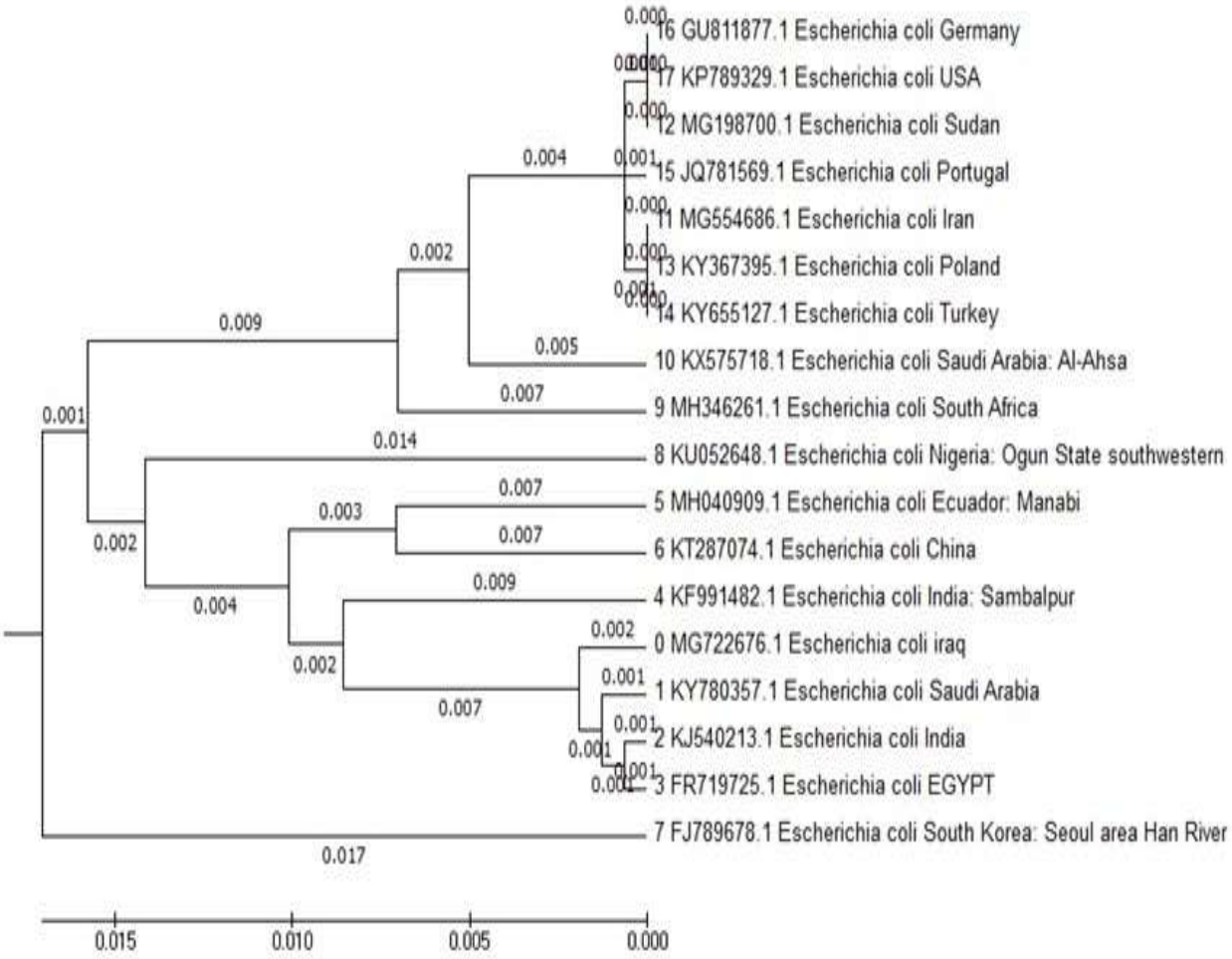

Fig. 4: Neighbor-joining tree *Escherichia coli* of 16S rRNA gene

Conclusion

We conclude from this research that the early diagnosis of pregnant women with urinary tract infection offers the possibility of reducing the risk to the mother and fetus. Three variations appeared: first Transversion, second Transition, and lastly Transition, as well as showing the compatibility 99% percentage between the local and global isolates.

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