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Molecular detection of 16srrna gene in *escherichia coli* isolated from urinary tract infection patients

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Abstract
The present study aimed to isolate and to diagnose of Uropathogenic Escherichia coli which caused (UPEC). From 75 isolation were collected from children whom caused UPEC during 2015. Fifty of total isolates diagnosis as E. coli and entirely were used to detect 16SrRNA gene by polymerase chain reaction (PCR) technique and DNA sequencing. Disc diffusion method was used to detect antibiotic sensitivity against of 8 antibiotics. The results of present study showed different in resistant percentage against Penicillin, Tetracycline, Amoxicillin, Nalidixic acid, Trimethoprim-Sulphamethoxazo, Nitrofuranation, Gentamycin, Nitrofuranation and Chloramphenicol. The resistant percentage of the isolates which: 50 (100%), 48 (96%), 47 (94.8%), 38 (76%), 30 (60%), 22 (44%), 11 (22%), 9 (18%) respectively. The results of PCR showed all *E. coli* samples had the targeted gene and 28 samples recorded as new strains Wid which documented in NCBI.

Keywords: E. coli, 16SrRNA gene, PCR, DNA sequencing

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INTRODUCTION

Escherichia coli was unique of the best widely studied Gram-negative microbes in bacteriology science. The goal bacteria was linked with intestinal and further-intestinal infections in humans and numerous animals (Kaper et al. 2004).

In addition, the extraintestinal pathogenic E. coli (ExPEC), containing 'uropathogenic E.coli (UPEC), were related with urinary tract infections (UTI)'. UPEC causes approximately 90% of public-acquired UTI and up to 50% of nosocomialUTIs (Kucheria et al. 2005). It had been valued that catheter-linked UTIs characterize one of the greatest mutual causes the nosocomial infections (Dwyer et al. 2013); so highest UTI cases were described in women, children, aging people, and immune-compromised persons (Foxman et al. 2010). A mixture of numerous danger factors described the most reasonable cause of like the phenomenon, together with physiological and anatomical changes, a lively sex life, age, and the close proximity of the urethra, vagina, and rectum in women (Nowicki 2002). That was mostly accepted that the host normal-flora in fecal is the chief source of UPEC isolates (Moreno et al. 2008).

Another method for overcoming the limitations of using culture for bacterial identification, the metagenomic sequencing analysis was introduced in the diagnosis of microbial infections (Didelot et al. 2012). Also, the use of 16S ribosomal RNA genes was one of sequence based microbiome studies which used as molecular marker for classification the bacteria (Srinivasan et al. 2015). The long of bacterial 16SrRNA gene was about 1500 bp, and which contains variable and conserved regions that progress at diverse rates.

The existence of any cell was related with the sequences of the ribosomal RNA, which highly conserved gene through the evolution, because of the requiring of all cells to complex inter, also to intra-molecular interactions to preserve the mechanism of protein-syntheses (Sacchi et al. 2002).

The old-style culture techniques in clinical laboratories of microbiology remain the crucial approach which used for identification of most isolates of bacteria; these procedures were time-consuming, and caught involve various biochemical tests, also might be expensive, predominantly for the fastidious bacteria.

The introduction of automated instruments in identification had resulted in superior reliability (Jin et al. 2011). Besides, a lot of research laboratories absence the funds essential for invest in these tools, and often rely on work-intensive biochemical test or 16SrRNA sequencing to confirm the bacterial species type. The aim of this study to diagnosis the Escherichia coli that caused UPEC and to determine the antibiotic sensitivity against 8 antibiotics and to detect the 16rRNA gene in E. coli by PCR and DNA sequencing.

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Table 1. Primer sequences used for amplification of 16SrRNA gene

Gene	Primer Sequences (5´- 3´)	Product size	Reference
16SrRNA	F: AGAGTTTGATCMTGGCTCAG R: CCGTCAATTCATTTGAGTTT	919bp	Momtaz et al. (2013)

MATERIAL AND METHODS

Urine Culture

1- The bacterial number in urine/ml was important differential diagnosis of bacterial species in urinary tract, by using Calibrated Inoculating Loops to transfer 0.01 and 0.1 ml of urine. The positive results was detect of 105 cell/ml of one species of bacteria.

2- The urine samples was cultured on Blood-agar and MacConkey-agar and incubated at 37O for 24h.

Laboratory Methods

All *E.coli* recovered from 75urine samples which collected from children in AL-Habbuby Teaching-Hospital in Thi-Qar province through the time from February-November, 2018 and identified depending on cultural properties (LAB/ United Kingdom), microscopical examination followed by biochemical tests (Catalase, Methyl-Red, Oxidase, Indole, Voges-Proskauer Test, CitrateUtilization, Sugar Fermentation and CO2, H2S Production Test (Harley and Prescott 2002, Brooks et al. 2007). The confirmed examination was performed via using API system (BioMerieux/France).

Antibiotic Sensitivity Test

To detect the E. coli sensitivity against Penicillin (10µ/disc), Tetracycline (30µ/disc), Amoxicillin (30µ/disc), Nalidixic acid (30µ/disc) , Trimethoprim-Sulphamethoxazo (25µ/disc), Nitrofuranation (300µ/disc), Gentamycin (10µ/disc), Nitrofuranation and Chloramphenicol (30µ/disc). Antibiotic (Bioanalyse, Turkey) by using the disc diffusion method designated by Kirby (1966). 'The diameters of inhibition zone were measuredand interpreted depending on Clinical Laboratory Standard Institute (2011).

Preparation of Bacterial DNA

The *E. coli* chromosomal DNA extraction was carried out on entirely E. coli isolates using Genomic DNA Extraction kit (Geneaid/Korea).

PCR Diagnosis of 16SrRNA Gene

The specific primer pairs of 16SrRNA gene described in **Table 1**. The PCR cycling conditions of 16SrRNA gene: initial denaturation at94°C for three minute, trailed by thirty five cycles of denaturation at 94°C for thirty second, annealing at 55°Cfor 30sec, extension at72°C for 1 min and final extension for 10 min (Kirby 1966). The agarose-gel-electrophoresis of product of PCR performed thru using 1.4% and the presence of a 919 bp band indicate a positive result for 16SrRNA gene. The ending volume of PCR reaction tubing was twenty µl, consist of five µl of Pre-Mix (Bioneer/Korea), one µl of each forward and reverse of the primers specific for any gene, five µl of DNA and the volume was accomplished thru adding distilled water.

DNA Sequencing

Thirty product of PCR of *E.coli* to 16srRNA gene, were chosen for sequencing of both forward and reverse primer for current gene, then sent to the research laboratory for sequencing in(Macrogen, Korea)'. NCBI (BLAST) was chief to blast log. The sample sequences labeled as (Wid1 to Wid 28) for goal gene were edited, aligned, and matched with the reference sequences using finch-TV program (Hall 1999).

RESULTS AND DISCUSSION

Antibiotic Sensitivity Test

The results that documented in **Fig. 1** showed all *E. coli* isolates had high resistant rate to most antibiotic in this study, these isolates recorded complete resistant to penicillin (100%), while slightly resistant against chloramphenicol (18%). The results showed E. coli isolates resistant against AMX, P, STX, NA, F in low percentage (16%). Also Moore et al. (2017) showed the microbial resistance to antibiotics was increased problematic in Asia countries.

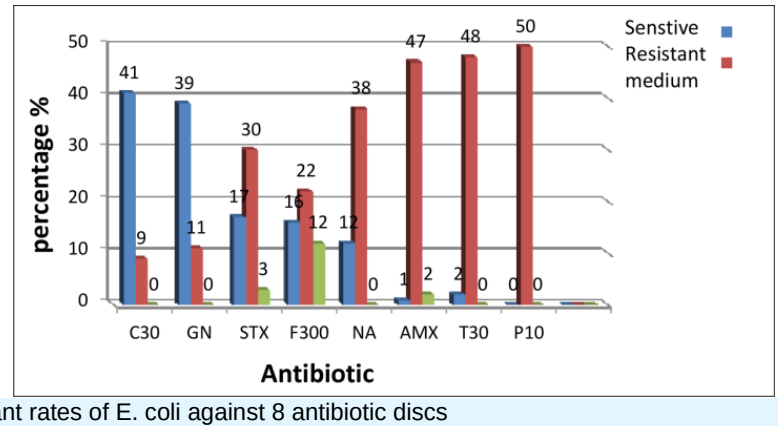

Fig. 1. Antibiotic resistant rates of E. coli against 8 antibiotic discs

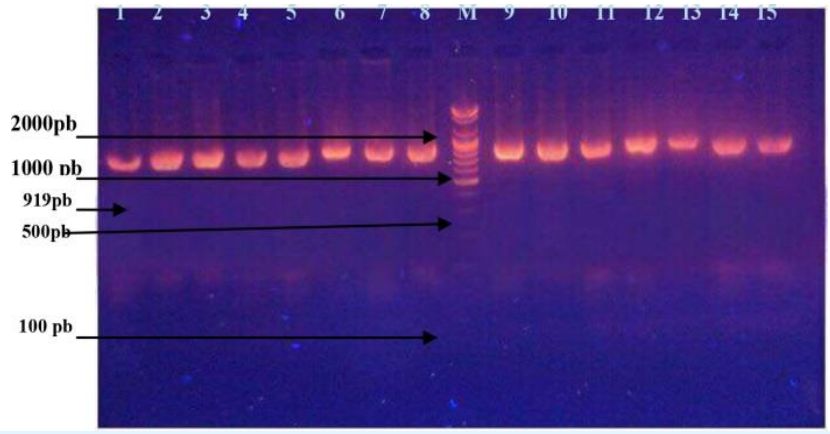

Fig. 2. Agarose gel electrophoresis of 16SrRNA gene amplification, M: ladder, 1- 15: positive results

Fig. 3. The evolutionary relations of E. coli, phylogeny of the 16SrRNA gene through distance-based-analysis via Tamura-Nei distance estimates of aligned nucleotide sequences resultant from PCR results.

The study performed by Sultana et al. (2015) that recoded high resistant rat against Penicillin group like Penicillin, Ampicillin and Amoxcillin, and showed resistant percentage 100%.

The *E.coli* isolates were resistance toward Tetracyclin (96%), these percentage were agreed with Tawfiq (2005) that recorded the percentage of resistance was 92.5 against Tetracyclin, while the research performed by Momtaz et al. (2013) showed the *E.coli* isolates were showed the resistancy to Tetracyclin was (73.98%).

The antimicrobial sensitivity results exhibited that eight of isolates (53.33%) were vulnerable to azithromycin, also ten isolates (66.67%) were sensitive to streptomycin antibiotic, while 13 isolates of *E. coli* (86.67%) were susceptible to gentamicin, 12 bacterial isolates (80%) were sensitive toward norfloxacin, eleven isolates of goal bacteria (73.33%) were susceptible to tetracycline antibiotic and ten E. coli (66.67%) were sensitive towards streptomycin. Furthermore, 12 isolates of *E.coli* isolates (86.67%) were resistant against amoxycillin and eleven isolates (73.33) were resistant toward erythromycin (Islam et al. 2016).

Molecular Results

The Polymerase Chain Reaction results of 16srRNAgene in E. coli showed completely samples harbored the current gene; also Himi et al. (2015) recorded 81/150 of E. coli (54%) had this gene. While 15 samples from 20 were recorded the E. coli had 16srRNA gene. The PCR technique was the accurate method to identify E. coli bacteria through the detection the16srRNA gene, this method used after traditional techniques like cultural, morphological characterization, staining method, and biochemical tests (Islam et al. 2016, Ramezanpour et al. 2019).

The phylogeny of *E. coli* according to the neighbor-relating of sites of 16SrRNA gene sequences were recorded that this gene from Escherichia bacteria (**Figs. 3** and **4**). For classification of each microorganism like bacteria used the phylogeny method (Babbar et al. 2017) and the last scheme displayed the evolutionary history through the neighbor-joining manner. The finest tree with the summation of branch-length was shown,

Fig. 4. Multiple sequence alignment investigation display G>A, T>C and C>T; polymorphism in isolate 15 for 16SrRNAgene in E. coli

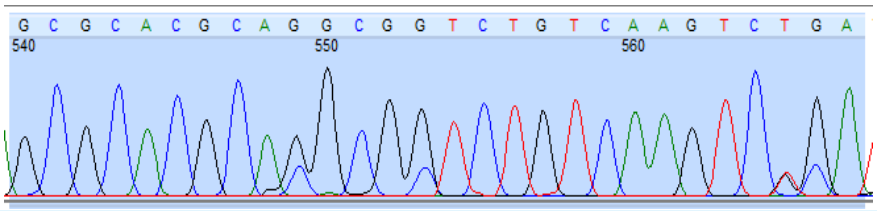

Fig. 5. Multiple sequence alignment study illustration C>T polymorphism in isolates 1, 2, 5, 6 and 7, also in sample 5 has T>G for 16SrRNA gene of *E. coli*

while Maximum-Composite-Likelihood-procedure was done to calculate the evolutionary distances and detection base substitutions of every site numerically (Haquea et al. 2019, Kumar et al. 2016, Mahdieh et al. 2020).

CONCLUSION

The present study showed that some E. coli isolates demonstrated 100% homology toward the sequence genome of many E. coli strains by sequencing, ID: MN559989.1 and CP022229.3, MK778502.1 while another strains established 99% homology with world strains by sequencing, these strains under ID that found in NCBI and including: ID: MN208081., MN173390.1 and CP041581.

The results of Multiple-sequence-alignment investigation, exhibited G>A, C>T and C>T polymorphism in fifteen isolate of 16SrRNA gene, shown in **Fig. 4**. While noted C>T polymorphism in samples 1,2,5,6 and 7 in different position, also in sample 5 has T>G for 16SrRNA gene of E. coli (**Fig. 5**). The change in nitrogen base must be linked with differentiation of the product of present gene (16SrRNA) that might be associated with identification of this germ which had this gene.

The sequencing results, the sequencing resulted in higher identity percentages (98-100%) towards the existing genome of E.coli strains. These results highlighted that 16SrRNA sequencing by short sequence could provide sufficient identification amongst E.coli strain. The current primer has been used before to detect 16S rRNA genes of E.coli isolates from different samples (Didelot et al. 2012).

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