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Original Article

Genomic fingerprints of uropathogenic *Escherichia coli* isolated from patients in Babylon province

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Abstract:

The 95isolates obtained from patient were confirmed as *E. coli* by 16S rRNA gene the REP-PCR was used to examine (22) *E. coli* strains that were obtained from patients yielded genomic profiles with five to sixteen bands and many DNA fragments with sizes ranging from 150 bp to 10,000 bp, as appeared, the genetic profiles in respect to the reference strain *E. coli* the phylogenetic tree that was produced shows the relationships between the various strains under study, the vertical straight line at 88% on the dendrogram indicates the strains' family tie is positively correlated. The phylogenetic tree analysis reveals that the strains under study are diverse. Twenty-two groupings were therefore produced. The genetic profiles with respect to the reference strain *E. coli*; the phylogenetic tree that was produced shows the relationships between the various strains under study. The vertical straight line at 88% on the dendrogram indicates that the strains' family tie is positively correlated.

Key words: REP-PCR, Genomic fingerprints, phylogenetic tree.

Introduction:

Enterobacteriaceae family of Gram-negative bacteria; *E. coli* can cause intestinal or extraintestinal infections, including serious invasive diseases like sepsis and bacteremia, or it can colonize the human gut in a harmless manner. Along with being a major cause of meningitis in newborns, it is the most prevalent cause of bacteremia in high-income nations, surpassing even the most common causes of bacteremia [1].

The ability of strains to differ in causing disease is due to differences in the genes that encode virulence factors, which are primarily acquired through horizontal transmission. There are many sites in the body that can be infected with this bacteria, like the urinary tract, brain tissue, biliary tract, peritoneal membrane, lungs, skin, and soft tissues. Can get infected with *E. coli* [2].

The most problematic mechanisms in *E. coli* pathogenicity are the gene-acquired pathways that code for carbapenemases, which confer resistance to carbapenems; the plasmid-mediated quinolone resistance feature, which permits resistance to broad-spectrum antibiotics; 16S rRNA methylases, which confer pan-resistance to aminoglycosides; and mcr genes, which confer resistance to polymyxins. *E. coli* is inherently susceptible to nearly all therapeutically relevant antibiotics that contribute to its survival. [3].

much virulence, with *E. coli* pathogenicity, ranging from activities linked to bacterial colonization to virulence-related activities. Usually encoded on plasmids, pathogenicity islands (PAIs), and other

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mobile genetic elements, they include adhesins, toxins, iron acquisition factors, LPS, PS capsules, and invasion factors [4].

Bacterial cell surface and the ability to express virulence factors are the two major categories that enable *E. coli* to have wide resistance and survival. Both fimbrial types (1 and P) represented the most common forms of bacterial surface virulence factors. These fimbriae facilitate the formation of biofilms as one of the methods of camouflage, the activation of cytokines, adherence to the host cell surface, and tissue invasion, all of which are critical to the pathophysiology of UPEC that causes UTIs. The outer membrane proteins, capsular lipopolysaccharide, and flagellum are further components of bacterial cell surface virulence factors [5].

In order for bacteria to sustain these specialized curli fimbriae, Type-I, and cellulose, these are encoded in EPEC species by the multi-genes csgA, fimA, and bcsA in succession; during uropathogenic biofilm formation, these structures are required [6].

Repetitive Intergenic Consensus in uropathogenic Enterobacteria has repeating and conserved sequences that are present in bacteria. Different species can be recognized from one another by these sequences, which are often found throughout the entire chromosome in the inter-genome of random distribution of intergenic sections. This indicates that the number of repetitive sequences varies amongst microorganisms [7].

Rep-PCR, or repetitive sequence-based PCR, is a promising epidemiological method that may quickly supplement high-throughput genotype fingerprints of multi-strain studies of studied bacteria. According to earlier research, rep-PCR primers may not be very selective for *E. coli* since they are based on the nucleotide sequences of Gram-negative bacterial types [8]. Furthermore, it proved difficult to ensure the study's continued advancement after the commercial rep-PCR kit was taken off the market. Repeated sequence-based PCR (rep-PCR), variable number tandem repeats (VNTR), and other molecular methods. Thus, there is a need for thorough information, including SNP data that can be utilized to classify strains and determine allelic variation. A specific method for data analysis is needed for this WGS and the interpretation of sequencing data [9,10].

Material and methods

Four major hospitals in the province of Babylon provided a total of 95 clinical samples for the cross-sectional study. Samples of urine from patients suspected of having urinary tract infections and vaginal discharge, Males and females of all ages participated in the study.

E.coli isolation and identification were performed using sterile cups, tubes, and disposable, sterilized transport swabs; the clinical isolates were obtained from patients in hospitals. Each specimen was immediately inoculated into MacConkey, EMB agar, and blood agar, and the incubation period lasted for 24 hours at 37°C. The laboratory diagnostic procedure followed the steps recommended by [9].

DNA Extraction: Every UPEC isolate under the current investigation was separated and grown in Luria-Bertani (LB) broth. The Presto™ Mini gDNA Bacteria Kit (Geneaid company, USA) was used to extract the genomic DNA of the examined UPEC strains after a whole day at 35 °C. For every PCR-based test, the isolated chromosomal DNA served as the DNA template. This assay was carried out according to the manufacturer's information.

PCR amplification:

Molecular identification of UPEC strains was done according to Sabat et al. 2000, where 16S rRNA-specific primers (Forward 5’ GGAAGAAGCTTGCTTCTTTGCTGAC-3′, Reverse 5’ AGCCCGGGGATTTCACATCTGACTTA-3′) were used to produce a 544 bp product. DNA was amplified by PCR using a thermal cycler in a final

mixture volume of 25 µl. After the ethidium bromide staining step, PCR products were examined using a 1.5% agarose gel for electrophoresis and photographed under a UV transilluminator. PCR mixtures and conditions of this assay were summarized in Table (1).

REP-PCR:

These reactions were performed according to Borges at al., 2003 with minor modifications in 25 µl volumes containing 1 µl of each primer (REP1R (5′-III ICG ICG ICA TCI GGC-3′) and REP2I (5′-ICG ICT TAT CIG GCC TAC-3′)), 12.5 µl of the master mix (GoTaq® G2 Green Master Mix, Promega, USA), 3 µl of DNA template, and 7.5 µl of deionized water. Following an initial denaturation at 95°C for 7 minutes, the ERIC-PCR conditions were carried out according to the standard method. The subsequent 35 cycles included a denaturation phase at a known temperature for one minute, annealing at 41°C for less than one minute (about 45 sec.), an extension at 65°C for 3 minutes, and a final extension at 65°C for 16 minutes. A 2% agarose (Merck) gel electrophoresis was used for the assay results, which were electrophoresed at 75 V for 75 minutes, followed by UV visualization in a gel documentation analyzer. The BIONUMERICS programmed system version 8.0 was used to examine gel images, and the unweighted pair method with arithmetic mean was used to create a phylogenetic tree [10].

Table 1: Uniplex PCR mixtures and conditions for identification of 16S rRNA gene.

PCR mixtures		PCR conditions		
Contents	**Volume**	**Type of cycle**	**Condition**	**No. of cycles**
Master Mix	12.5 µl	Initialization	95 °C for 5 min	1
Forward Primer	2.5 µl	Denaturation	94 °C for 1 min	30
Reverse Primer	2.5 µl	Annealing	60 °C for 1 min	
Template DNA	3 µl	Extension	72 °C for 1 min	
Nuclase-Free Water	4.5 µl	Final Extension	72 °C for 10 min	1

Results:

The 95 isolates obtained from the Babylon province sampling sites were confirmed as *E. coli* by 16S rRNA gene. (Figure 1-1). All isolates had their genomic DNA effectively extracted, and spectrophotometric analysis was used to measure the amount of extracted DNA and evaluate its purity to ensure the extraction was OK.

Twenty-two *E. coli* were isolated from 95 urinary tract samples, indicating high UTI prevalence. The PCR fingerprinting approach was based on analysing the genetic diversity of 95 E. coli isolates. All of them exhibit complex fingerprint patterns. A typical fingerprint produced by PCR for a few isolates of E. coli has been displayed in (Fig. 1 -2)

For the standard strain with code "ATCC 25922," when based on it, REP-PCR fingerprinting's repeatability was perfect for amplifying bands up to 3 kb. (five repetitions).

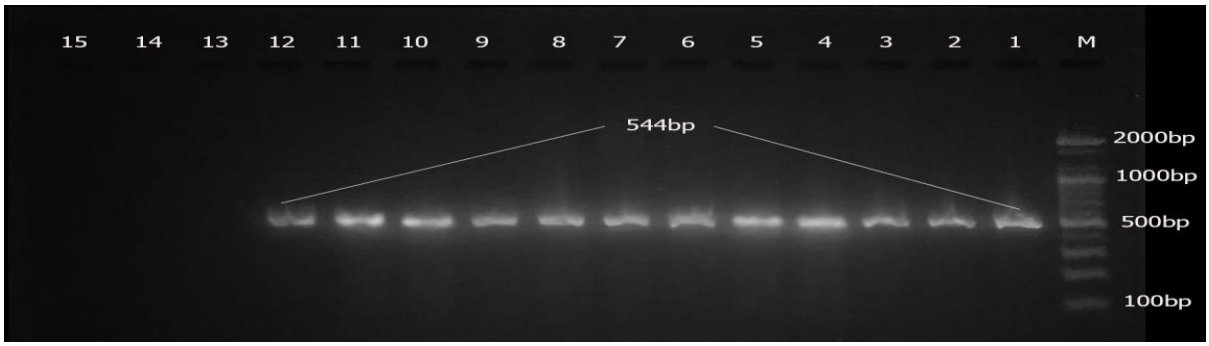

Fig. (1-1): Agarose gel electrophoresis of PCR product obtained with Uropathogenic *E. coli* strains -specific primers that generated 544bp amplicon of 16S rRNA gene. Lanes (1-12); positive, Lanes (13, 14, 15); negative, Lane M represent 100bp DNA ladder.

Distribution of Uropathogenic *E. coli* strains isolated from patients with UTI diseases in Babylon Province.

Results	No.	%	P value
Positve	22	23.15	<0.0001*
Negaive	73	76.49	
Total	95	100	

* Significant difference at P<0.05.

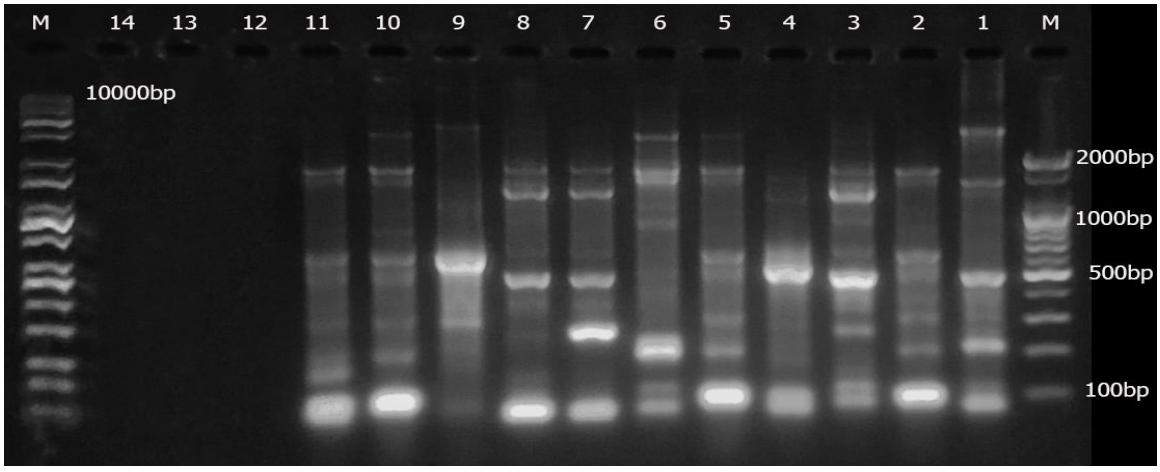

Fig. (1-2): Genomic fingerprints -derived profile by Random Amplified Polymorphic DNA (Rep)-PCR data for the studied Uropathogenic *E. coli* strains. M-Lane (on left), 100bp universal DNA ladder; Lane M (on right), 100bp DNA ladder; lanes1-11, EC1-EC11 UPEC strain

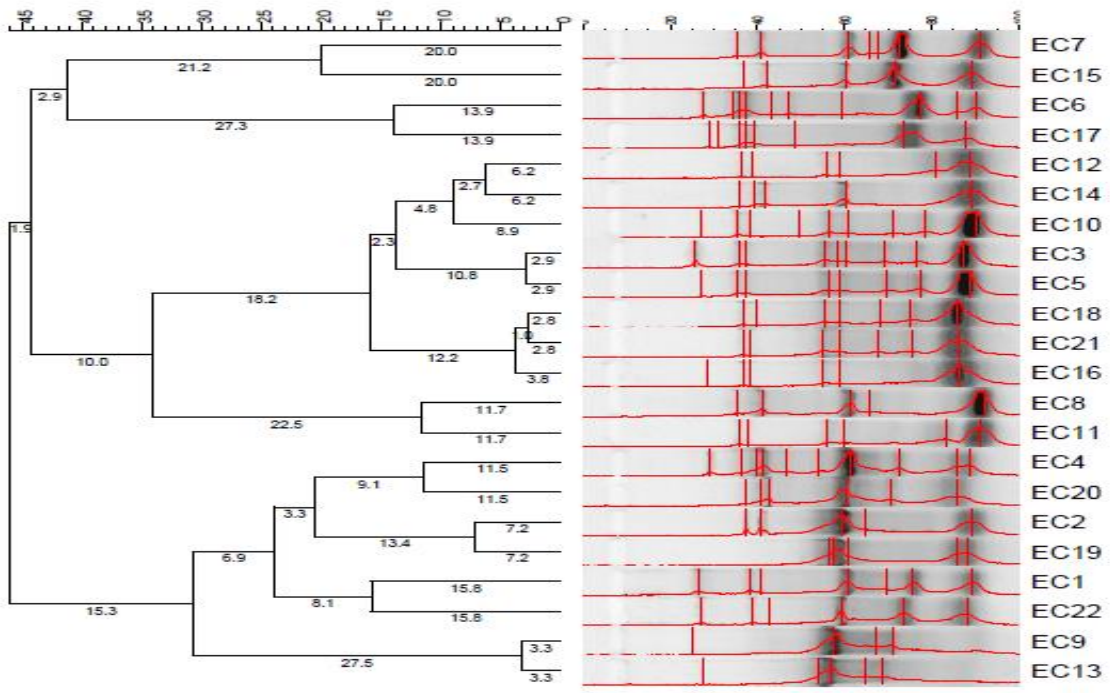

Figure 1-3:

Heat map of SNP differences between *E. coli* strains. SNP rates (SNPs/aligned base pairs) between different sequenced strains were interpreted as a distance matrix. Hierarchical clustering was done on this symmetric matrix of SNP rates. colour in the heat map represents SNP rate as shown in the legend at the top left. A tree based on Euclidean distance is shown to the left of the heat map. The colors of the strains indicate their patient of origin.

Discussion

These were the primary phylogenetic groupings that appeared among the isolates from various regions of the nation that were examined in the current study, and they can represent the phylogenetic groups globally *E. coli* strains have been identified as a significant contributor to UTI morbidity and mortality, pathogenic strains of *Escherichia coli* strains have been linked to a wide range of infectious diseases, and urinary tract infections urinary tract infections can be caused by many bacterial pathogens [10,11].

The REP-PCR was used to examine (22) *E. coli* strains that were obtained from patients (count=41) (Figure 1). The REP PCR yielded genomic profiles with five to sixteen bands and many DNA fragments with sizes ranging from 150 bp to 10,000 bp, as shown in Figure 1. The dendrogram was used to determine the strains' genetic relationships. To find out if they are absent, as stated in UPEC isolates, a more thorough UPEC investigation would be required [12-14].

Based on distinct REP-PCR profiles and sample origins obtained from various individuals, 73 varied E. coli strains were chosen for additional characterization. The outcome confirms by [13], that the various REP-PCR patterns discovered were grouped into numerous clusters and four genetic groups. For fingerprint pattern comparisons in the current study, a 1.5% optimization option was found to offer the best similarity recognition between the strains. Cluster analysis of the genomic fingerprints of the 108 strains of E. coli acquired by REP-PCR produced 53 clusters (C1-C22) with a similarity coefficient of 85% (closely related) and 66 distinct profiles (arbitrarily labeled as REP-01 to REP-27)

(Figure 1). The distinct strains studied were confirmed [15].

In the present study, gain of Complex fingerprint patterns was observed for isolated strains of *E. coli* based on the REP PCR analysis. Because samples that were taken within the same area had genetically comparable (F ≥ 85%) *E. coli* strains, the dendrogram suggested that bacterial transmission may have occurred. Finding strains that are genetically and epidemiologically linked is typically the primary objective of a strain typing investigation. For this reason, understanding how this information is yielded is essential to understanding and stopping the spread of infectious diseases [16].

Clermont used a novel quadruplex PCR technique to classify *E. coli* strains into eight phylogenetic categories. Finding *E. coli* phylogenetic groups was the study's main goal [14]. The majority of *E. coli* strains from the same individual had similar REP-PCR patterns, suggesting that the strains were genetically identical. If the number of bands in a bacterial strain's genetic fingerprinting pattern matches the size of the corresponding bands, the strains are considered genetically identical [17].

According to a prior study, bacterial strains of specific species exhibited 2-3 band variance whether they were recovered from the same patient or grown again over time [16]. When a single genetic change, such as a point mutation or the insertion or deletion of DNA, occurs in a single manner, bacterial strains are thought to be closely linked and can cause differences in fingerprinting patterns. Two- to three-band deviations are typically the outcome of such events, suggesting that the strains were genetically identical [18].

The locality of the strains can be shown by *E. coli* strains that have the same genotype fingerprinting patterns, which varied slightly but were often consistent for samples taken from the same individual. While some strains from the same area had (>85%) genetic similarity, the majority of strains from that area showed 85% similarity, making them closely related strains with modifications [19].

When a single genetic modification occurs, such as a point mutation or the insertion or deletion of DNA, bacterial strains are regarded as closely related depending on tiny changes. can cause differences in fingerprinting patterns. Two to three band deviations are typically the outcome of such modifications. According to a prior study, strains of specific bacterial species exhibited two to three band variance whether they were recovered from the same patient or grown again over time [18].

Shared similarity when occurring at less than 85% similarity between bacterial strains indicated that they were not connected genetically, and the reason for the four to six band variances could have been caused by at least two separate genetic processes [20].

Phylogenetic affiliation: The genetic profiles with respect to the reference strain *E. coli*; the phylogenetic tree that was produced shows the relationships between the various strains under study. The vertical straight line at 88% on the dendrogram indicates that the strains' family tie is positively correlated [21]. The standard strain *E. coli* ATCC 25922 reference and strain *E. coli* [22] are shown to be in the same position by the dendrogram. The phylogenetic tree analysis reveals that the strains under study are diverse. Twenty-two groupings were therefore produced. The strains under study have a poor phylogenetic link (with percent >88% similarity, Fig. 3). The same technique was employed by researchers to classify *E. coli* strains [8, 22].

genetic profiles being compared with the reference strain, the phylogenetic tree that was created shows the link between the many strains under study. The dendrogram's vertical straight line at 88% indicates the strains' family tie. The strain *E. coli* and the reference strain are positioned in the same spot, as can be seen in the dendrogram. Phylogenetic tree

analysis demonstrates this [23]. The strains under investigation exhibit variety. Twenty-two groupings were therefore produced. This demonstrates that the strains under study have a weak phylogenetic link (similarity rate < 88%, Fig. 3). The same approach was used by [24].

Microbial genome evolution can be investigated via Rep-PCR fingerprinting [25]. Fifty-three strains of *E. coli* may be studied thanks to Rep-PCR. PCR electrophoresis results can produce profiles of each strain's genetic fingerprints. These profiles show four distinctive bands at base pairs (200, 300, 500, and 1000) (Fig. 2), which are typical of the reference strains utilized in this investigation, such *as E. coli* ATCC 25,922. [26]. By examining the genetic profiles produced by rep-PCR, twenty-two groups were identified (Figs. 1 and 2). The group with the distinctive bands at bps (200, 300, 400, and 500) was the most diverse and represented about 15% of bacterial species. *E. coli* exhibited two to six distinctive bands. In conclusion, the fingerprint profiles generated by the strains under investigation were different from those reported in research on *E. coli* strains with a band count between 15 and 24 [27].

Environmental factors and the strains' genetic evolution are responsible for this heterogeneity [28, 29]. Of the *E. coli* strains examined, 75.47% displayed (4-5) characteristic bands, whereas 45.28% displayed four diagnostic characteristic bands similar to the reference strain, with the sole difference being in their position. *E. coli* fingerprints from the same animal are comparable but not usually identical, according to certain research [30]. Only 3.77% of the strains exhibit two distinctive bands [31].

These were the primary phylogenetic groups found among the investigated isolates from various regions of the nation and can represent phylogenetic groups worldwide; the importance of this study is evident. Pathogenic strains of *E. coli* have been connected to a variety of infectious diseases; urinary tract infections can be caused by numerous bacterial pathogens, and *E. coli* strains have been identified as a major contributor to UTI morbidity and mortality [32]. Genetic evolution is responsible for this heterogeneity [33]. Of the *E. coli* strains examined, 75.47% displayed (4-5) characteristic bands [34].

Conclusion:

When genetic profiles are compared with the reference strain, the phylogenetic tree that was created shows the link between the many strains under study. The dendrogram's vertical straight line at 88% indicates the strains' family tie. The strain *E. coli* and the reference strain are positioned in the same spot, as can be seen in the dendrogram. The locality of the strains can be shown by *E. coli* strains that have the same genotype fingerprinting patterns varied slightly, they were often consistent for samples taken from the same individual. While some strains from the same area had (>85%) genetic similarity,

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