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مجلة علمية محكمة تصدر عن كلية التربية

جامعة المرقب

العدد التاسع عشر

يوليو 2021م

هيئـــة تحريـر
مجلة التربوي

- **المجلة ترحب بما يرد عليها من أبحاث وعلى استعداد لنشرها بعد التحكيم .**
- **المجلة تحترم كل الاحترام آراء المحكمين وتعمل بمقتضاها .**
- **كافة الآراء والأفكار المنشورة تعبر عن آراء أصحابها ولا تتحمل المجلة تبعاتها .**
- **يتحمل الباحث مسؤولية الأمانة العلمية وهو المسؤول عما ينشر له .**
- **البحوث المقدمة للنشر لا ترد لأصحابها نشرت أو لم تنشر .**

(حقوق الطبع محفوظة للكلية)

ضوابط النشر:

يشترط في البحوث العلمية المقدمة للنشر أن يراعى فيها ما يأتي :

- أصول البحث العلمي وقواعده .
- ألا تكون المادة العلمية قد سبق نشرها أو كانت جزءا من رسالة علمية .
- يرفق بالبحث تزكية لغوية وفق أنموذج معد .
- تعدل البحوث المقبولة وتصحح وفق ما يراه المحكمون .
- التزام الباحث بالضوابط التي وضعتها المجلة من عدد الصفحات ، ونوع الخط ورقمه ، والفترات الزمنية الممنوحة للتعديل ، وما يستجد من ضوابط تضعها المجلة مستقبلا .

تنبيهات :

- للمجلة الحق في تعديل البحث أو طلب تعديله أو رفضه .
- يخضع البحث في النشر لأولويات المجلة وسياستها .
- البحوث المنشورة تعبر عن وجهة نظر أصحابها ، ولا تعبر عن وجهة نظر المجلة .

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Analysis of Genetic Diversity of *Escherichia Coli* Isolates Using RAPD PCR Technique

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Abstract

RAPD-Polymerase chain reaction technique was used to analyze genetic variety for nine isolates of *Escherichia coli*, causing Urinary Tract Infection in pregnant women. Fifty-three urine samples were collected from inpatients and identified fourteen isolates of *E. coli* using biochemical analysis. *E. coli* isolates were subjected to phylogenetic analysis using fifteen random ten nucleotide primers. DNA amplification with each of the fifteen primers resulted in generation of different DNA fingerprinting profiles with a varied number of bands. The results of the reactions of RAPD technique for the nine selected isolates revealed differences of the number of amplified bands and in the molecular size of the produced bands. The total number of polymorphic bands were 122. The highest number of bands (13 bands) were produced by primer A14, while the primer O20 gave the lowest number of polymorphic bands(5 bands). Dendrogram based generation of clustering of *E. coli* isolates showed three major clusters, members in one of which showed the most close genetic profile and biochemical properties as well. Application of Random Amplification of Polymorphic DNA as a new alternative approach in molecular characterization of *E. coli* infections is remarkable to trace genetic relatedness in a short duration, hence dramatically increasing its clinical relevance over existing biochemical or biogramatic methods in studying epidemiological patterns of pathogen sources and distribution manners.

Keywords: Genetic diversity Escherichia coli, RAPD-PCR technique.

Introduction:

Escherichia coli is one of the most common and important microorganism in the intestine microflora [(1,2)]. Nevertheless, members of this species can give rise to severe infections, including urinary tract infection (UTI), diarrhea, sepsis and neonatal meningitis. *E.*

coli is a major cause of UTI in pregnancy which accounts for 80-90 % of UTI infections in pregnant women [3,4].

Efficient methods for *E. coli* strains identification are required to study the epidemiology and to screen for possible spread of *E. coli* strains in hospital wards. *E. coli* isolates are typically characterized by serotyping which is based on the use of specific antisera and the detection of H- antigens and somatic O- antigens expressed by the bacteria. However, a minority of strains do not serotype satisfactorily [5].

In general, Molecular Characterization is considered to be more discriminatory than phenotypic methods and is increasingly being used in diagnostic laboratories. It has been suggested that comparing data obtained by different typing methods will give an optimal insight into strain relatedness [6].

Different molecular typing methods have evolved in recent years and have demonstrated their utility in examining genotypic differences among a group of closely related organisms., however, there is no technique as powerful or has been used as extensively as polymerase chain reaction (PCR). Classical PCR and its many methods have been used in the diagnosis and characterization of a wide variety of organisms based on the analysis of total genomic DNA from the bacteria. One method is randomly amplified polymorphic DNA (RAPD) PCR introduced by Welsh and McClelland in 1990 [7,8].

RAPD-PCR has been described as a simple and rapid method that able to offer detailed fingerprinting of the genomic composition of the organism, and the advantage of this method is due to the fact that no prior sequence information about the target genes is needed [9].The principle of RAPD lies in that a short 10-bp oligonucleotides were chosen as primers due to greater possibility that these small sequences are reiterated many times within the bacterial genome. The genetic composition of each bacterial isolate ultimately dictates the position and number of times that the primer anneals to several locations on the chromosome. The distinctive DNA patterns generated by RAPD technique for each bacterial strain is a reflection of the genetic variety present within a species [10,11].

The present work was aimed to investigate the genetic correlation between *E. coli* strains isolated from pregnant women having symptoms of UTI by PCR analysis using randomly amplified genome segments through the RAPD technique.

MAREIALS AND METHODS

Bacterial Isolates

A total of 53 midstream urine samples were collected from patients having symptoms of UTI, at Khoms Hospital, without prior administration of antibiotic treatment. The urine samples were streaked on plates of MacConkey agar and EMB for 24 hours, and incubated at 37C°. the *E. coli* isolates were identified based on colony morphology on MacConkey agar, Gram staining, and confirmed using the biochemical tests included the API-20E. A total of 9 *E. coli* stains were identified and stored as glycerol stock culture at -20C°.

Bacterial DNA extraction

The bacterial isolates were cultured in nutrient broth over-night, and the DNA was extracted using the Phenolchloroform extraction method.. To ensure extraction efficiency, 5 µl of extracted DNA samples were electrophoresed on 0.7% agarose gel to detect related bands. Also, to obtain the concentration and DNA purity, optical density of the samples $OD_{260/280}$ wavelengths was measured using a nanodrop system (Thermo, USA) [(12)].

Agarose gel electrophoresis

The quality of the Genomic DNA was determined by using gel electrophoresis which stained by ethidium bromide, the samples were stored at -20 °C until further.

RAPD-PCR amplification of genomic DNA

Fifteen random ten mer primers (OP) having GC content 60–70 % were used to amplify the genomic DNA of the nine E. coli isolates. The sequence of primers used (OP Technology, USA) along with amplified band size range and GC content is provided in **Table 1** [(12)].

The DNA banding patterns were developed electrophoraticaly using the same steps as used to examine the genomic DNA quality with agarose concentration of 1.2%.

Scoring and RAPD data analysis

The bands were manually scored ‘1’ for the presence and ‘0’ for the absence and the binary data were used for statistical analysis. The scored band data (presence or absence) was subjected to cluster analysis using STATISTICA. The dendrogram was constructed by

Ward's method of clustering using minimum variance algorithm through SPSS computer program. The dissimilarity matrix was developed using Squared Euclidean Distance (SED), which estimated all the pair wise differences in the amplification product.

Table 1. Sequence of primers used in the RAPD-PCR

No.	**Primer**	**Sequence (5'to 3')**	**MW**
1	OPA-12	TCGGCGATAG	3059
2	OPA-13	CAGCACCCAC	2933
3	OPA-14	TCTGTGCTGG	3041
4	OPA-15	TTCCGAACCC	2939
5	OPA-17	GACCGCTTGT	3010
6	OPA-18	AGGTGACCGT	3059
7	OPA-19	CAAACGTCGG	3028
8	OPA-20	GTTGCGATCC	3010
9	OPO-11	GACAGGAGGT	3108
10	OPO-13	GTCAGAGTCC	3019
11	OPO-14	AGCATGGCTC	3019
12	OPO-15	TGGCGTCCTT	3001
13	OPO-17	GGCTTATGCC	3010
14	OPO-19	GGTGCACGTT	3050
15	OPO-20	ACACACGCTG	2988

RESULTS AND DISCUSSION

RAPD profile

Fifteen RAPD primers were applied to differentiate nine strains of *E. coli*. **Table 2** showed that different primers generated variable numbers of fragments with different lengths of DNA amplified products. It showed that screening of genetic variation by fifteen primers generated a various numbers of bands ranged from 5 to 13 bands. The highest number of bands (more than 10 bands) were amplified with both OPA-14 (13 bands), OPA-15 (11 bands). A total number of 122 bands were produced using the fifteen selected primers, of which 122 (100%) was found to be polymorphic with an average of 8.13 polymorphic bands per primer.. There was no correlation between the G + C content of the primer and their ability to detect polymorphisms.

Table 2. Numbers of different DNA-segment bands produced by the fifteen primers applied to the nine selected *E. coli* isolates in RAPD Analysis.

Primer No.	Isolate No.									No. of different bands
	1	2	3	4	5	6	7	8	9	
A12	6	6	8	7	7	7	7	8	8	9
A13	5	2	6	6	6	6	6	6	5	7
A14	10	10	6	9	7	10	7	9	4	13
A15	7	7	8	7	8	9	9	8	8	11
A17	4	5	5	5	7	6	6	4	6	8
A18	5	6	3	6	1	6	6	6	4	7
A19	8	7	8	9	4	8	8	6	6	10
A20	5	5	6	6	6	5	5	6	5	6
O11	7	7	8	8	8	10	9	8	8	10
O13	6	5	7	6	7	4	3	6	7	8
O14	4	3	2	3	6	6	6	4	7	8
O15	3	4	4	5	5	4	3	4	5	6
O17	4	5	5	5	5	3	3	3	4	7
O19	4	4	4	4	4	2	4	2	3	7
O20	4	4	4	4	4	3	3	4	4	5
Total Bands	82	80	84	90	85	89	85	84	84	122

RAPD profiles using primer A18

Primer A18 produced different banding patterns. It produced a total number of seven bands with lengths ranged from 668 bp to 2629bp. As shown in **figure 1** the primer produced a variable number of bands. It produced 5, 6, 3, 6, 1, 6, 6, 6 and 4 bands in the nine isolates, respectively. Only one common band with length of 668bp was produced using primer A18, indicating kind of similarity in the genetic background of the nine selected *E. coli* isolates. The variability in the banding patterns of the other bands between the nine isolates indicated a kind of variation. The produced band with length of 1021bp was absent only in isolate No. 5 but it was present in all the other isolates. It is possible to use this band as a marker to differentiate this isolate.

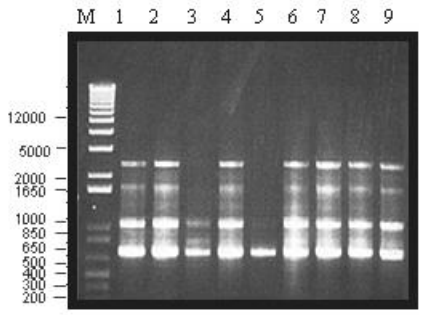

Figure 1. RAPD patterns of E. coli isolate using primer A18. from left to right: DNA marker, Lane1 -9 are banding patterns of the nine E. coli isolates.

RAPD profiles using primer O15

Results of primer O15 with the nine *E. coli* selected isolates as shown in **figure 3** revealed a total number of six bands were produced. The primer produced 3, 4, 4, 5, 5, 4, 3, 4 and 5 bands in isolates 1, 2, 3, 4, 5, 6, 7, 8 and 9, respectively. Bands No. 4, 5 and 6 were common in the nine isolates. This primer resulted in a marker for isolate No. 6, where it produced the band No. 2, while it was absent in the other isolates, it is possible to use this band as a marker to discriminate isolate No. 6 from the other isolates. The other bands were variable overall the isolates indicating a kind of variability in the genetic background of these isolates.

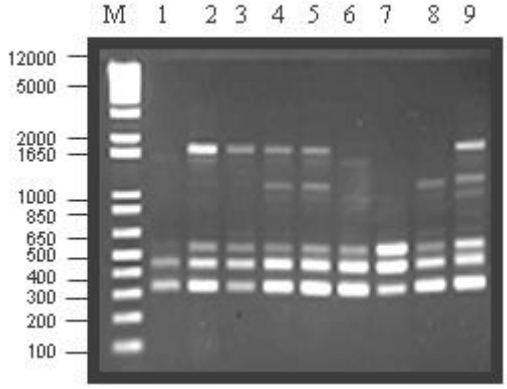

Figure 2. . RAPD patterns of E. coli isolate using primer O15. from left to right: DNA marker, Lane1 -9 are banding patterns of the nine E. coli isolates.

It could be concluded that over all the fifteen different used primers and the nine E. coli isolates, some bands represent a full similarity and other showed some variation. Similarity index was calculated using SPSS computer program, on the base of the banding patterns obtained from the RAPD PCR patterns. The genetic similarities and genetic differences were given in **Table 3**.

	VAR01	VAR02	VAR03	VAR04	VAR05	VAR06	VAR07	VAR08	VAR09
VAR01	***	0.911	0.87	0.867	0.739	0.887	0.817	0.797	0.793
VAR02	0.911	***	0.832	0.881	0.734	0.852	0.797	0.81	0.789
VAR03	0.87	0.832	***	0.876	0.839	0.864	0.843	0.807	0.839
VAR04	0.867	0.881	0.876	***	0.838	0.923	0.873	0.855	0.786
VAR05	0.739	0.734	0.839	0.838	***	0.843	0.821	0.748	0.759
VAR06	0.887	0.852	0.864	0.923	0.843	***	0.923	0.844	0.81
VAR07	0.817	0.797	0.843	0.873	0.821	0.923	***	0.855	0.855
VAR08	0.797	0.81	0.807	0.855	0.748	0.844	0.855	***	0.852
VAR09	0.793	0.789	0.839	0.786	0.759	0.81	0.855	0.852	***

Table 3. Similarity index among the nine different genotypes of *E. coli* according their RAPD profiles

The two most similar isolates were No. 4 and No. 6 with the highest genetic similarity value of 0.923. As well, isolates No. 6 and No. 7 have the same similarity value. On the other hand, the two most varied *E. coli* isolates were isolates No. 2 and No. 5, with the lowest genetic similarity value of 0.734, followed by isolate No. 1 and No. 5, with similarity index value of 0.739. The similarity index table shows a variable value of similarities between each couple of isolates reflecting kind of similarity or variability in RAPD banding patterns of these strains.

Dendrogram Analysis

The genetic relatedness among *E. coli* isolates was illustrated through a tree-like structure that is generally known as dendrogram. Dendrogram generated using 10-base primers is presented in **Figure 3**. This dendrogram was developed on the base of the collected data of (0, 1) which obtained from the banding patterns of these isolates and the used of the fifteen RAPD primes. Data were analyzed statistically using SPSS computer program. *E. coli* strains were divided into three main clusters. , the first cluster included isolates 6, 7 and 4 in a sub cluster and 3, 1 and 2 in the other sub cluster. The second main cluster included isolates No. 8 and 9. The third cluster included only isolate No. 5. The dendrogram results indicate that the most two closely related isolates were isolate No. 6 and isolate No. 7, and the two most distantly related isolates were isolate No. 2 and No. 5. Dendrogram outline is in agreement with that of similarity index, where both of the two analyses indicate a closely relation genetic bake up between isolate 6 and 7.

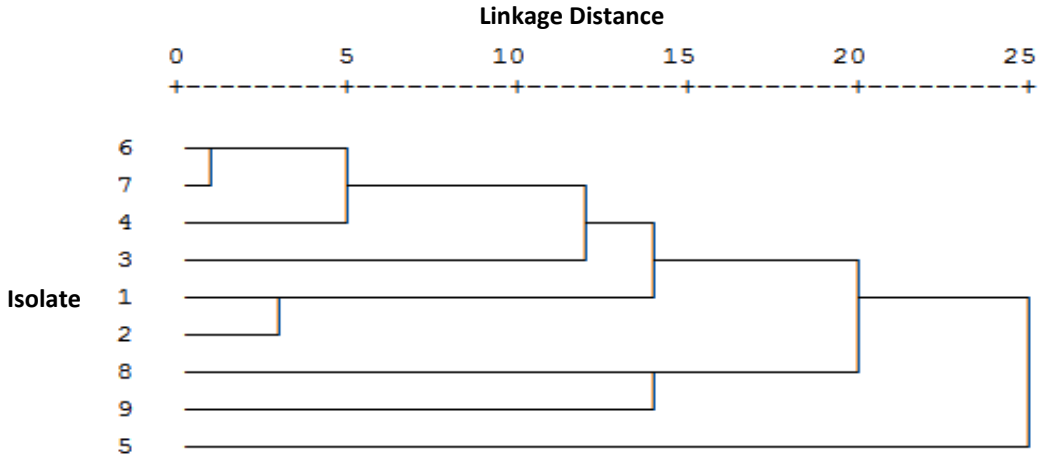

Figure 3. Dendrogram classifying the nine different genotypes of *E. coli* into groups according their RAPD profiles similarities

dendrogram based analysis of the RAPD profiles of various bacteria is a suitable tool in understanding the genetic relationship between isolates grouped into several clusters. These phylogenetic studies successfully showed the predominance of a single epidemic strain that was transmitted between hosts and its persistence over a period of time [13,14,15].

In the present study, the genetic diversity of *nine E.coli* strains isolated from pregnant women having symptoms of UTI was investigated. Biochemical characterization was first applied to identify the *E coli* isolates. Evaluation of genetic diversity at the genomic level was analyzed by RAPD PCR technique. Fifteen arbitrary primers have been selected based on their success of producing variable bands from other studies . The selected primers were used to differentiate the bacterial isolates according to their genetic relatedness.

Primer O11 has the heaviest molecular weight of 3108 and yielded 10 different bands. The primer notably has the highest G ratio among all used primers. Nonetheless, number of different bands can not be a consequence of molar ratio of the primer. Kumar et al. (2017) observed that oligonucleotides with high GC content (>60%) resulted in a greater and better reproducible number of bands in *E. coli* [16].our study did not observe influence of GC content of the primer on the number of polymorphic bands.

Primers A14 and A15 generated most number of entirely different banding patterns for each genotype and could differentiate all the genotype from one another. Hence, these

primers are suggested for molecular typing of *E. coli* isolates as they disclosed inter genotype variations.

The RAPD profiles were distinct for each *E.coli* strain revealed the existence of main clusters. Basically, the nine *E coli* isolates were divided into three main clusters and the main cluster consisted of about 30% of the total isolates . A phylogenetic tree was generated from the diverse *E. coli* RAPD patterns obtained in this study. These dendrogram generated from different primers was branched, suggestive of a genetically diverse population of uropathogenic *E. coli* causing UTI in Khoms area.

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