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# Genetic diversity of *Escherichia coli* harboring virulence gene *Stx1* and *Stx2* isolated from common carp fish in Nineveh Governorate using ERIC-PCR

**N.A. Alttai, R.A. Alsanjary and O.H. Sheet**

Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Iraq

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***Correspondence:***
O.H. Sheet
omar.sheet@uomosul.edu.iq

**Abstract**

The goal of this study is to discover the genetic diversity of twelve *Escherichia coli* isolates harbor virulence genes (*Stx1* and *Stx2*) isolated from farm fish and local market fish in different locations of Nineveh Governorate using Enterobacterial Repetitive Intergenic Consensus (ERIC-PCR) technique in the time span November, 2021 - January, 2022. The source of contamination was found depending on the fingerprint analysis of the repetitive polymerase chain reaction among the genes of the intestinal micro-organism ERIC-PCR for every isolate. The results show that there are similarities among the isolates and it ranged about 51%-100%. The isolates are divided into 7 genotypes, based primarily on a 90% similarity threshold 1 to 7, with the most offspring being inside genotype 1, since genotype 1 used to be the biggest team containing four isolates. Then, two isolates are grouped under genotypes 3 and 5. In contrast, genotype 2, 4, 6 and 7 have only one isolate. By virtue of genetic variations in the location, there was once an excessive clonal dispersion of most *E. coli* strains. In addition, the study shows genetic differences in *E. coli* in 83.3%, (10/12) of the isolates, in the different geographical locations of Mosul city. The *E. coli* isolates were classified into some groups: first, different genetic grouping, different geographical locations, same genotype; second, different genetic grouping, same geographical locations, different genotypes.

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## Introduction

Enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR), also regarded as intergenic repeating units, was first recorded in the Enterobacteria, *E. coli*, and the *Salmonella typhimurium* enterovirus serotype. However, these sequences are recently described in most bacterial species, such as Enterobacter and skillfully in cholera micro-organism (1,2). *E. coli* consists of a massive crew of lines, with an amazing variety in its genetic material, including some traces inflicting serious diseases, such as the urinary tract infections (3). Incomplete symmetric sequences are typically detected inside transcribed areas in association with intergenic consensus. Furthermore, there are different numbers of copies of the ERIC sequence amongst bacterial species (4). Interestingly, there is a first-rate variety in replica numbers among different isolates of *E. coli*. This range raises the strategies of evolution between bacterial traces inside a unique species, such as *E. coli* (5). This is a simple, sharp and most economical genotyping method to distinguish among unique kinds of isolates. Indeed, miniature inverted transposable factors (MITE) in combination with DNA are recognized as molecules with ERICs (6). Significant genetic variations are no longer unique in all the traditional microbiological and the biochemical tests, depending on a single identification approach when publishing can lead to misidentification of many microbial species (7). Therefore, as an alternative of the ordinary analysis and different tests, the utility of the current superior techniques, such as molecular diagnostic

equipment and molecular fingerprinting, may additionally be an appropriate choice in the molecular epidemiological investigations (8, 9). Through specific fingerprint patterns. ERIC-PCR appears to be a good approach for molecular printing of *Escherichia coli* strains isolated from different animal sources. ERIC-PCR is recommended for identification of different strains related to bacterial species as a simple tool and the results can be processed by different types of software as GelClust to generate tree diagrams beneficial as a precious methodology involving the classification of a range of bacterial lines, such as *E. coli* (10). Through the emergence of these molecular genetic strategies and their purposes in the subject of microbial ecology, solely a small proportion of the natural microbial variety is observed due to the fact that the microbial variety have species with the operational taxonomic units (OTUs), which particularly are based on 16S ribosomal RNA sequences, and these possess insufficient genetic resolution for the reliable binning of microbes into species (11). However, detailed information on the molecular genetics of previously uncharacterized microorganisms may shed more light on the evolution and function of bacteria of a particular species within their own habitat or in an exotic environment, and this biological technique can be used to assess the clonal variation of many bacterial isolates, such as *E. coli* (12,13). The aim of this study is to find out the genetic diversity of *Escherichia coli* carrying virulence genes isolated from common carp fish, using ERIC-PCR.

## Materials and methods

### Ethical approve

University of Mosul, College of Veterinary Medicine, the approval issue number and date are 1650 at 21/11/2021.

### Samples collection

In a previous study, a total of 46 isolates of *Escherichia coli* were isolated from 153 fish samples, in different regions of Nineveh Governorate, in November 2021 to January 2022 (14). The work was carried out in the laboratories of Department of Veterinary Public Health, College of Veterinary Medicine, University of Mosul, Iraq. Isolation and all molecular strategies together with the detection of virulence genes of *E. coli* micro-organism the usage of ERIC-PCR (15,16), were therein conducted.

### Detection of isolates and confirmation

For this study, these forty-six isolates were previously diagnosed as *E. coli* with the useful resource of phenotypic techniques, as correct as biochemical techniques. They were also examined via molecular strategies to enlarge the target genes to utilize of the *uidA* gene. The total is 24 isolates of *E. coli* from local markets and 13 isolates from fish farms harboring *Stx1* and *Stx2* genes, respectively. The affiliation of these isolates to their authentic ancestors and genetic variety is confirmed through abiding by exclusive sources of fish sampling places.

### Amplification of DNA by using PCR-ERIC

Twelve of these isolates harbor the two genes of (*Stx1* and *Stx2*) had been subjected to ERIC-PCR to make sure of comparable isolates and to represent exclusive isolates using primers (ERIC1: 5'ATGTAAGCTCCTGGGGATTCAC-3') and (ERIC2: 5'AAAGTAAGTG ACTGGGTGAGCG-3'), which are described, thanks to Versalovic *et al.* (17). The mix reaction of this technique was performed with a total volume of 25 µl, each reaction consisting of 1 µl of each PADI at concentration (10 pmole), 12 µl of the prepared reaction mix from (Genedirex, Taiwan), and 2 µL of DNA extracted from *E. coli* isolates at concentration (30-100 ng/µL), supplemented with sterile, deionized, nuclease-free water (9 µL), (supplied from Qiagen, Germany) to a volume of 25 µL (18). The polymerase chain reaction was performed using a GeneAmp Applied Biosystem (9700), according to the PCR program used by Bakhshi and colleagues (19). The initial denaturation was for 5 min at 94°C, thereafter 35 cycles of repetitions for each of them. Then, denatured at 94°C for 1-minute, annealing temperature at 54°C for 1 minute, and elongation at 72°C for 5 minutes. A cycle was extended for 10 min at temperature 72°C.

### Electrophoresis and UV-trans illumination

The amplified PCR product was loaded into a 2% agarose gel prepared from 2 g agarose,1X Tris-acetate-EDTA (TAE) solution and stained with Safe Red (GeNetBio, Korea). as well as loading 100-bp DNA as a marker in gel electrophoresis apparatus (Biometra) Germany, as a standard for the molecular size of the resulting bands. Then, the agarose gel was placed on the UV-trans illuminator device to take the image for data analysis using a digital camera to check the presence or absence of the replication DNA bands in the gel, which were obtained by the ERIC-PCR.

### Analysis by GelJ software version 2.0

finally analyzed using GelJ software version 2.0 available at https://sourceforge.net/. To construct a dendritic diagram based on Heras and coworkers (20), isolate grouping was performed based on the method of unweighted pairs group, with UPGMA analysis and similarity coefficient of variance of about 1%. Isolates with a similarity coefficient equal to or greater than 90% were grouped as the same genotype (18). Also, isolates were grouped according to their genetic differences with the same and different isolate source (in situ).

## Results

The evaluation of the effects of ERIC-PCR on Enterobacteria, was based totally on the number and size of the resulting packet sequence variations depending on the

ERIC-PCR fingerprint evaluation for each isolate. The results showed that the similarity of the isolates was between 51% - 100 %. The isolates were divided into 7 genotypes based primarily on the 90% similarity restriction (from 1 to 7), with the offspring being the most widely wide-spread inside genotype 1. Genotype 1 was the biggest team, containing four progenies. Two isolates have been then grouped into genotypes 3 and 5. In contrast, genotype 2, 4, 6, and 7 included only one strain.

According to the genetic differences in terms of site, *E. coli* isolates showed that the genetic difference is 83.3%, (10/12) in different geographical locations. Also, the results found out that there are genetically different isolates from different geographical locations for the same genotype, and likewise the assembly of genetically different isolates from the same geographical site for the different genotypes. For example, clustering in genotype 1 for strains No. 30, 28, 27, and 25 from Al-Nabi Yunus market, Al-Baladiyat market and Al-Midan market, respectively. Moreover, clustering in genotype 5 for both strains, no. 17 (from Al-Hamdaniya region) and no. 16 (from Al-Baladiyat market). In addition, strain no. 23 (from Al-Hamdaniya region) clustered in genotype 2, and strain no. 5 (from Hawi Al-Kaneesah) in genotype 4, strain no. 26 (from Al Maidan market) in genotype 6 and finally strain no. 3 (from Hawi Al-Kaneesah) in genotype 7. In contrast, only 2 strains isolated from the same geographic location were clustered in a single genotype, which are strains no. 12 and no. 6 (from Wanah Sub-district) in genotype 3 (Figure 1).

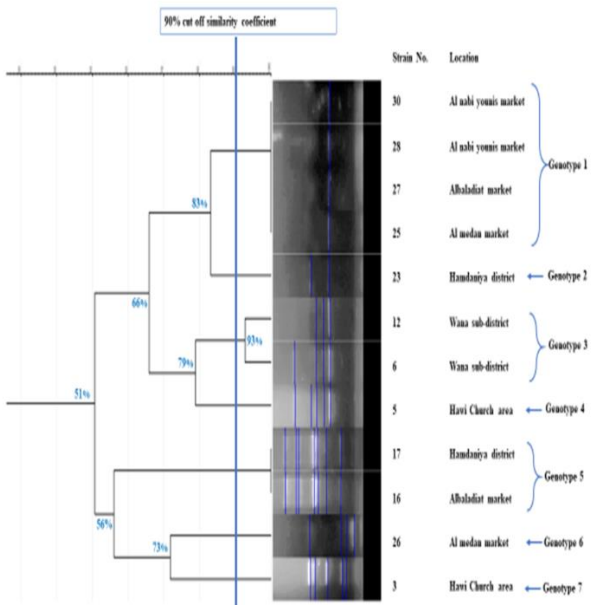


Figure 1: Dendrogram obtained from ERIC-PCR shows the banding pattern of 12 *E. coli* strains harboring (*Stx1* and *Stx2*) genes, isolated from farm fish and local markets fish in Mosul city.

## Discussion

Discussing the analysis of the repetitive polymerase chain reaction among Enterobacteria genes, the results of this study show the presence of an excessive clonal dispersal (high degree of genetic heterogeneity) in most isolates from exclusive geographical locations (21). This variability is an indication that there are many clones of *E. coli* circulating in certain geographic areas in Nineveh Governorate, which can come from different geographic regions (multiple sources and ecosystems). Not only for fish or seafood, this can be attributed to different animals, which are by means imported from exclusive areas or through importing food products (which are manufactured from animal meat from extraordinary sources) from different locations (including local neighborhoods and imported animals from neighboring provinces) (22).

Sellyei *et al.* (23) noted that the entry of animals from exclusive areas may additionally have supported the dissemination of a giant variety of bacterial genotypes, and some other motive for this variety may additionally be that there is little hazard of distribution of these micro-organisms amongst these geographical location sources.

In general, bacterial genetic diversity is established by point mutations or insertions or deletions of precise DNA sequences which will lead to pressure variations in microbial communities (24, 25). This can happen after passing through many positions or ecosystems (26), which is an appropriate clarification for why there is such genetic variety in the *E. coli* isolates definitely considered in this study.

On the other hand, there were two considered isolates of the same geographic place grouped into a single genotype 3, strains no. 12 and no. 6 (from Wanah Sub-region), which indicates that there is one supply of illness in these traces (27,28). In terms of awareness, Keesing *et al.* (29) emphasised that preserving wholesome ecosystems and their related endemic biodiversity might also minimize the incidence of bacterial transmission, leading henceforth to decreased bacterial exchanges among different sites. Loss of biodiversity can have a significant impact on the spread of bacterial diseases into other ecosystems.

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## Conflict of interest

The researchers appeared that there is no conflict of interest.

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# التنوع الجيني لجينات سموم الشيكا *Stx1* و *Stx2* في جرثومة الإيشريكيا القولونية المعزولة من أسماك الكارب الشائعة في محافظة نينوى باستخدام تقنية تفاعل البلمرة المتسلسل التكراري بين الجينات للجراثيم المعوية

**نور عبد الجبار الطائي، رعد عبد الغني السنجري**
**و عمر هاشم شيت**

فرع الصحة العامة البيطرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

**الخلاصة**

إن الهدف الرئيسي من هذه الدراسة هو العثور على التنوع الجيني لاثنتي عشرة عزلة من جراثيم الإيشريكيا القولونية التي تحتوي على جينات سموم الشيكا *Stx1* و *Stx2* المعزولة من أسماك المزارع وأسماك السوق المحلية في مواقع مختلفة من محافظة نينوى باستخدام تقنية تفاعل البلمرة المتسلسل التكراري بين الجينات للجراثيم المعوية للفترة الممتدة من تشرين الثاني ٢٠٢١ إلى كانون الثاني ٢٠٢٢. عُثر على مصدر التلوث بالاعتماد على تحليل بصمات العزلات باستخدام تقنية تفاعل البلمرة المتسلسل التكراري بين الجينات للجراثيم المعوية. تُظهر النتائج وجود تشابه بين العزلات، حيث تراوحت ما بين ٥١ -١٠٠٪. قُسمت العزلات إلى ٧ طرز وراثية، على أساس عتبة تشابه تبلغ ٩٠٪ (١ إلى ٧)، مع وجود العزلات الأكثر انتشارا ضمن النمط الجيني ١. شكل النمط الجيني ١ المجموعة الأكبر باحتوائه على ٤ عزلات. واحتوت الأنماط الجينية ٣ و ٥ على عزلتين لكل منهما، في حين انضوى تحت كل من الأنماط الجينية ٢ و ٤ و ٦ و ٧ عزلة واحدة فقط. وفيما يتعلق بالاختلافات ما بين العزلات في الموقع الجغرافي الواحد، كان هناك تشتت نسيلي مرتفع لمعظم عزلات جراثيم الإيشريكيا القولونية. علاوة على ذلك، تُظهر نتائج الدراسة أن الاختلافات الجينية تبلغ نسبة ٨٣,٣٪، أي ١٢/١٠ من العزلات من المواقع الجغرافية المختلفة. هذا وقد صُنفت العزلات الى مجموعتين: الأولى، عزلات مختلفة وراثيا من مواقع جغرافية مختلفة لنفس النمط الجيني؛ والثاني، عزلات مختلفة وراثيا من نفس الموقع الجغرافي في أنماط وراثية مختلفة.