Molecular characterization of enterohemorrhagic *E. coli* O157 and O153 isolated from tissue camel and human stool samples in Al-Diwaniyah, Iraq

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Abstract

The present study aimed to describe the genetic relationships of zoonotic characterization of *Escherichia coli* isolated from human and livestock camel clinical infection. The study includes collected (50) meat of camel and (50) stool human samples. These samples were foreword to traditional bacterial isolation and identification using enrichment culture method and biochemical tests, then confirmed by PCR technique based on *Gyr B* gene Escherichia *coli* and DNA sequencing was done on some positive isolates. The results show that *Escherichia coli* were isolated from animals at 42 (84%) and 39 (78%) from human infection. The PCR technique was show highly sensitive and specific confirmative detection of *Escherichia coli* the positive results into 40 (95%) meat sample of camel, and 35 (89.7%) stool sample of a human. To evaluate of Virulence *E.coli*,we used specific virulence hlyA gene from NCBI-GenBank, published sequence of *E. coli hly A* gene (Genbank code: X94129.1) and the results show high of presence of virulence gene *hly* A in camel in percentage (19) 45% than of virulence gene in human (15) 38%. DNA sequencing of a partial sequence of *GyrB* gene was shown highly homology sequence identity with NCBI-Blast *Escherichia coli* strain O157H7 isolates from human and O153H3 from the camel. The phylogenetic analysis was shown there is clear genetic similarity at between human and animal's *E. coli* isolates and then the gene sequence deposited into NCBI-Genbank accession numbers (MG560867.1, MG560866.1). Also, study design for detection of some virulence gene *hly A Escherichia coli*. In conclusion, there prevalence *E. coli* in humans and camel. Therefore, it is essential to define the role of animals as an important source for the distribution of pathogen related to public health. Our study found gyrB gene sequence could be used for identification and making a phylogenetic analysis of *gyrB* nucleotide.

Keyword: *E. coli* PCR O153H2, O157H7, *gyr B, hly A*, sequencing and phylogenetic tree
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التوصيف الجزيئي لبكتريا الايشريشيا القولونية النزفية O157 وO153 المعزولة من لحوم الجمال وعينات براز الإنسان في الديوانية، العراق

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الخلاصة

هدفت الدراسة الحالية إلى وصف العلاقة الوراثية للتوصيف الحيواني المنشأ بين الاشيركيا القولونية المعزولة من العدوى السريرية للإنسان والماشية. تضمنت الدراسة جمع ٥٠ عينة من لحوم الجمال و ٥٠ عينة براز من الإنسان. تم زرع العينات على أوساط أغنائية وتم تفريقها وتشخيصها بالاختبارات البايوكميائية، ثم أكدت بتقنية سلسلة تفاعل البلمرة على أساس جين الجايريز صنف (ب) للاشريشيا القولونية وتم أجراء تسلسل الحامض النووي على بعض العزلات الايجابية. تشير النتائج أن نسبة العزل للاشرشيا القولونية من لحوم الجمال ٤٢ (٨٤%) بينما كانت النسبة من الإنسان ٣٩ (٧٨%). أظهرت تقنية التفاعل البولمريز المتسلسل حساسية عالية اذ كانت النتائج

الايجابية في لحوم الجمال ٤٠ عينة (٩٥%) بينما في براز الإنسان كانت النسبة ٣٥ عينة (٨٩,٧%). في هذه الدراسة استخدمنا تقنية تفاعل سلسلة البلمرة للكشف عن وجود جين الذيفان الهيمولايسين hlyA كعامل ضراوة ينتج من قبل البكتريا (EHEC) المعزولة من حالات إسهال من الإنسان ولحوم الجمال. تم تصميم البادئات لجين hlyA حسب برنامج من قبل بنك الجينات NCBI (Genbank code:X94129.1). تظهر النتائج وجود عالي لجين الفوعة hlyA في الجمال بنسبة ١٩ (٤٥%) وفي الإنسان ١٥ (٣٨%). تم إظهار تسلسل الحامض النووي من التسلسل الجزيئي من جين Gyr B كهوية تسلسل لتسجيل العزلات في NCBI للاشركيا القولونية حيث كانت O157H7 في الانسان وO153H3 من عزلات الجمال. واظهر التحليل للجينات بوجود تشابه جيني واضح بين عزلات البشرية والحيوانية ومن ثم تسلسل الجينات المودعة في أرقام انضمام NCBI MG560866.1،MG560867.1 . أيضا هدفت الدراسة للكشف عن بعض جينات الفوعة hlyA وتبن أن هناك انتشار للاشريشيا القولونية في البشر والجمال وعليه من الضروري تحديد دور الحيوانات كمصدر مهم لنقل العامل الممرض المتعلق بالصحة العامة. وجدت الدراسة أن التتابع الجيني Gyr B يعمل على تحديد هوية وعلاقته بالتطور النوعي وتحليل الأحماض الأمينية.

Introduction

Extra intestinal diseases can be caused by varied strains of *E. coli* which have the capacity to lysis of RBC of different animals (hemolysis). Camel and other small ruminants are considered the main natural reservoir of *E. coli* and other Enterobacteriaceae pathogens. *E. coli* have several types of hemolysin related to different pathogroups (1), ὰ-hemolysin is the best being (2). The four linked genes hly C, A, B, D products are required for the production of ὰ-hemolysin inactive form (3).

Hly C protein plays an important role in activation of inactive ὰ-hemolysin polypeptides by catalysis add of the fatty acid group, hemolysin of *E. coli* is the main virulence factor for some strains of the pathogenic bacteria involved in human and animals extraintestinal diseases like urinary tract infections, peritonitis, meningitis and septicemia (4). ὰ- hemolysin secretion is signal peptide-independent and interpose by determinant membranous translocated protocol encoded by hly B, D (5). Enterohemolysin is another hemolysin closely related with *E. coli* serotype O157, O26, O 111. *E. coli* O 157:H7 strains caused several types of diseases as hemorrhagic colitis. Cytolysin has activity in several cells of the blood and renal tubular cell (6). Hemolysin owes its name to the fact that it causes hemolysis or lysis of red blood cells, and this is indeed the basis for its most commonly used assay. (The prefix a, that is often omitted nowadays, indicated the extracellular form of the toxin originally). In spite of its name, ὰ-hemolysin is widely believed to act mainly by attacking the immune system cells of the host, usually without inducing cell lysis, yet severely impairing their function (7).

HlyA gene composed of a long chain of polypeptides (1024 amino acids) with 31 and 32 is some carbohydrates which are associated. The polypeptide activity damage membrane directly, since changes as mutations as one or two amino acids changes in specific positions render the inactive of protein (8). The gyr B gene is a coding protein called DNA gyrase, a type II DNA topoisomerase. The protein has a great role in DNA multiplication in the bacteria,Sequences of the gyrB gene used in phylogenetic reports of *Salmonella spp, Shigella and E. coli* (9). Our study found gyrB gene sequence could be used for identification and making a phylogenetic analysis of gyrB nucleotide, this study aimed to isolate and characterize EHEC strains from animal tissue and stool samples to elucidate the extent of food contaminated by camel tissue in Iraq.

Materials and methods

Samples collection

One hundred samples divided into fifty tissues (meat) of camel were collected from slaughterhouse of Al-Diwaniyah city, and fifty samples of human suffered from diarrhea in different sites of Al- Qadisyah province. These samples were placed in a cold container and transported to bacteriology Lab, in Veterinary Medicine College for isolationi and definition of *E. coli*.

Isolation and identification of *E. coli*

Bacterial isolation

E. coli was isolated from stool samples and many of the tissues by culture on Brain Heart Infusion Broth (BHIB) agar for overnight at 37°C. then the bacterial growth were cultured on MacConkey media, Eosin methylene blue (EMB) media, blood agar, and orientation- CHROM agar for overnight for preparation pure *E. coli* colony. Biochemical identification tests (IMViC and TSI) were also used for more detection of *Escherichia coli* isolates agreement with (10).

DNA extraction of bacteria genome

E. coli genome was extracted by using a special kit called (PrestoTM Mini gDNA Bacteria Kit. Gene aid. the USA) however, the extraction included 1ml of the new bacterial colony from nutrient broth media and put it in 1.5 ml microcentrifuge tubes. The centrifuge used to run the tubes for one minute at (10000). After centrifuge finished, the supernatant is produced then it removed. The Nanodrop spectrophotometer was used to testing the concentration DNA and keep in the freezer at -20°C.

Polymerase chain reaction (PCR)

The specific primer was used in PCR assay to detect *E. coli* the housekeeping gene *gyrB, F* (GAA GTC ATC ATG ACC GTT CTG CA) and R (AGC AGG GTA CGG ATG TGC GAG CC) (11). With product size 1256 bp and specific virulence gene of hemolysin toxin (hlyA). *E. coli* hlyA-F forward primer (GGAGTTAGTGCAGCCTCCAG) and hlyA-R reverse primer (ACCACTCTGACTGCGATCAG) 360bp. In NCBI-GenBank website used for primers designing under code (Genbank code: X94129.1) through use primer 3 plus design online. Bioneer Company provided the primers at a 360 bp. PCR premix tube consist of Taq DNA polymerase 1U, dNTPs 250 µM, Trisi-HCl (pH 9.0) 10mM, KCl 30 mM, MgCl2 1.5 mM, stabilizer, and tracking dye). PCR reaction was acted by added 5µl of purified genomic DNA and 1.5 µl of 10pmole of forwarding primer and 1.5 µl of 10 pmole of reverse primer to PCR premix tube and complete the volume into the 20 µl total volume by deionizer PCR water according to kit instructions. Briefly, it mixed by Exispini vortex centrifuge (Bioneer. Korea). The PCR thermocycler apparatus (Mygene Bioneer. Korea) used to make a reaction. The reaction consists of 30 cycles. The reaction consists of four stages included the first stage is DNA denaturation for one minute at 94°C, the second stage is primer annealing for one minute at 60°C, and the third stage is primer extension for one minute at 72°C. The last stage is extension stage for ten minutes at 72 °C for gre gene. While in *hly A* gene was 95°C in the initial denaturation for five minutes, next 30 cycles at 95°C as denaturation for 30 second, annealing for thirteen second at 58°C, and extension stage at 72°C for one minute and finallyi extensioni for ten minute at 72°C. The last step, the electrophoresis used to testing PCR products band at a concentration an agarose gel 1.5%, then stained by ethidiumi bromide stain and watch underi UV light.

DNA sequencing

The sequencing was performed for final diagnosis of *E.coli*, one isolate was taken from human and one from camel based on *Gyr B* gene, the PCR product was purified from agarose gel by using (EZ EZ-10 Spin Columni DNA Gel Extraction Kit, Biobasic. Canada). Then purifiedi *Gyr B* gene was sent to Korea in the Bioneer Company for testing and confirm DNA sequence of the *Gyr B* gene. The final genomic sequences were submitted in GenBank-NCBI website then alignment was made by search Tool (BLAST) for showing phylogenetic analysis and tree construction by MEGA program.

Results

Cultural characters of *E.coli* growth on MacConkey agar pink colonies while some strains show zone β-hemolysis on blood agar, the metallic green sheen on Eosin-methylene blue agar and Orientation -CHROM agar showed a metallic pink colour (Figure 1).

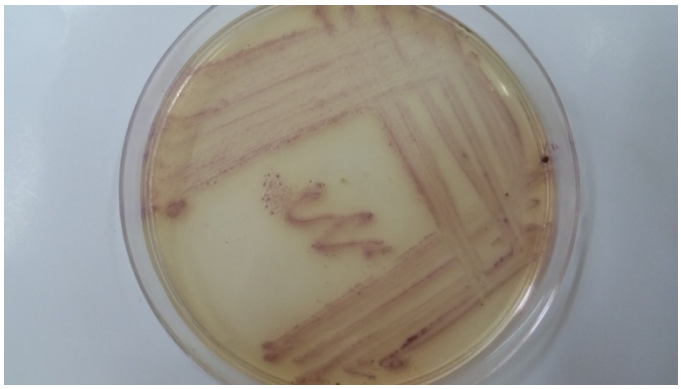

Figure 1: Orientation CHROM agar showed colonies have metallic pink colour.

The results showed that 42 (84%) samples were positive for *Escherichia coli* from a tissue sample of a camel while positive culture was 39 (78%). Obtain from the stool of human samples (Table 1).

Table 1: *E. coli* isolates numbers and percentages from the both samples

Types of samples	Positive (%)	Negative (%)	Total
Tissue of camel	42 (84) %	8 (16) %	50 (100) %
Stool of human	39 (78) %	11 (22) %	50 (100) %
Total	81 (81) %	19 (19) %	100 (100) %

The results of PCR

The results of PCR technique show 75 (92.5%) positive samples of *Gyr B* gene, divided into 40 (95%) tissue sample of camel, and 35 (89.7%) stool sample of a human (Table 2).

Table 2: Isolates number of *Escherichia coli* which have *Gyr B* gene

Types of samples	No. of *E.coli*	gyr B PCR	Percentage %
Tissue of camel	42	40	95%
Stool of human	39	35	89.7%
Total	81	75	92.5%

The results of PCR technique show 34 (41.9%) positive samples of hemolysin toxin gene, divided into19 (45%) tissue sample of camel, and 15 (38%) stool sample of a human (Table 3).

Table 3: Isolates number of *Escherichia coli* which have hemolysin toxin gene (hlyA)

Types of samples	No. of *E.coli*	Result of hlyA PCR	Percentage hlyA gene
Tissue of camel	42	19	45%
Stool of human	39	15	38%
Total	81	34	41.9%

PCR assay results were dependent on the extracted bacterial genomic DNA by using (PrestoTM Mini gDNA Bacteria Kit. Geneaid. USA) this kit was used spin column-based nucleic acid purification its solid phase extraction method to quickly purify nucleic acids. This method relies on the fact that nucleic acid will bind to the solid phase of silica under certain conditions. Therefore this kit was appeared rapid in 1hours extraction and simple method for purification of *Escherichia coli* bacterial genomic DNA from overnight bacterial growth on enrichment BHI broth media at 37°C and the agarose gel electrophoresis was appeared, there were sharp *gyr B* bands without nucleic acid lysis (Figure 2).

PCR amplification of hlyA gene in positive samples was shown clear PCR product bands on agarose gel electrophoresis at 360 bp PCR product (Figure 3).

Two isolates, one from camel while the second isolation from human sequence sent for sequencing were after that submission in NCBI-GenBank database to get accession number codes (MG560867.1, MG560866.1) frequently. DNA sequencing method was performed for phylogenetic confirmative of *E-coli on Gyr B* detection, phylogenetic analysis, and zoonotic importance (Figure 4 and 5).

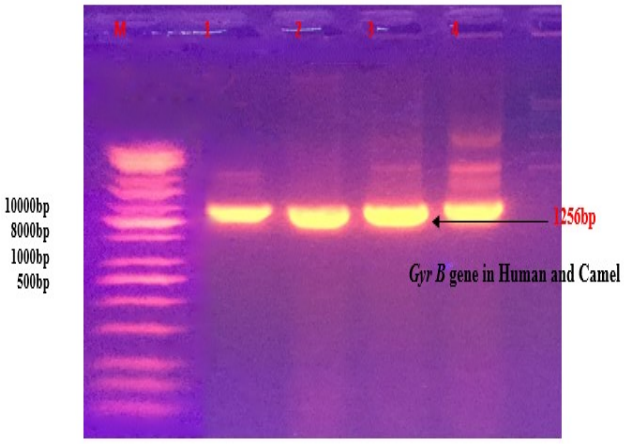

Figure 2: The image shows the PCR product band on gel electrophoresis represent of *gyr B* gene in *E. coli* isolates.

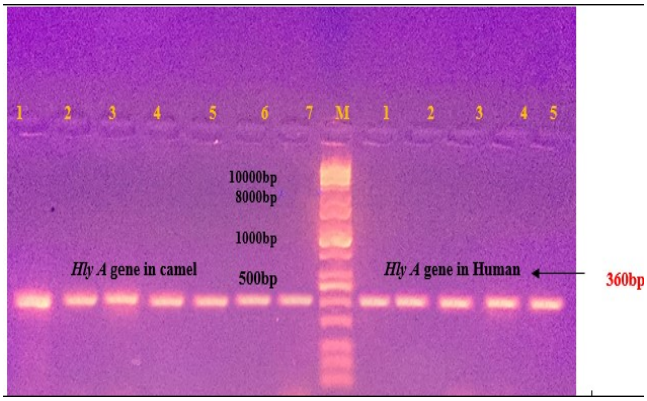

Figure 3: The image was shown the PCR product band that represent a product band of the of *Hly* A gene *in E. coli* isolates. Sequencing and phylogenetic analysis.

Discussion

Enterohemorrhagic *Escherichia coli (*EHEC) is an important emerging zoonotic foodborne pathogen that can cause gastroenteritis in humans as well as in animal (11,12). The Food contaminated with EHEC is a principal source of infection in humans (13,14).

The results of this study had shown the isolation of *E. coli hly A* gene at percentage 19 (45%) from tissue camel and,15 (38%) stool from human isolates. The high prevalence of EHEC in Camel is dependent on various factors, including the hygienic conditions of the farms and abattoirs. Also, surrounding environments can impact the distribution of EHEC in animal faeces, food products, and water samples respectively so that these results appeared the main role of *E.coli;* our results showed that the proportion of EHEC was higher in faeces (15-17). The pathogenesis of EHEC O157 is associated with several virulence factors, such enterohemolysin (encoded by hlyA) (18).

Used PCR methods by using multiplex PCR technique for detection some genes in *E. coli* in stool samples from many of animals such as sheep, goat, pigs, and cattle. The hemolysin is virulence factor used for cell detachment in vitro (19). The *E. coli* isolate can production of the hemolysins. Ehx gene or hly gene is very similar to *Hly*A gene in the function that including calcium ion dependency in the cell (20).

In the phylogenetic-tree analysis of gyrB, *E.coli* isolated from human and from camel The phylogenetic tree shows high identity of human isolates is similar to O157H7 which consider virulence strain and zoonotic impotence that may transmitted to animal as well as the *E. coli* isolates show O153H3 high similarity with 99% with AB083866.1,the strain that coded MG560866.1, a Japan with human resource that mean of zoonotic strain and importance in

infection to human, while isolate that coded MG560867.1 and recorded as O157H7 high similarity with 99% with CP014314.1 O157H7 an USA isolated from camel that mean of zoonotic importance and high virulence in transmission disease.

The percentage of changing of the gene *gyrB* is higher than the 16S rRNA region, and all bacterial carry *the gyrB* gene. We think gyrB gene have a high capability for identifying microbe generally and bacteria especially. The method is characterized by rapid and high accuracy for identification most genus of the bacteria (21), using of the *gyrB* gene is considered a useful method for identification and confirmation species of the bacteria (22,23).

In the end, the PCR is technique have rapidly in the detection of (*hlyA)* gene. Enterohemorrhagic *E. coli have hlyA* that important virulence factor wherever isolated from human and camel. The PCR has high specific and high sensitive very useful for detection pathogen.

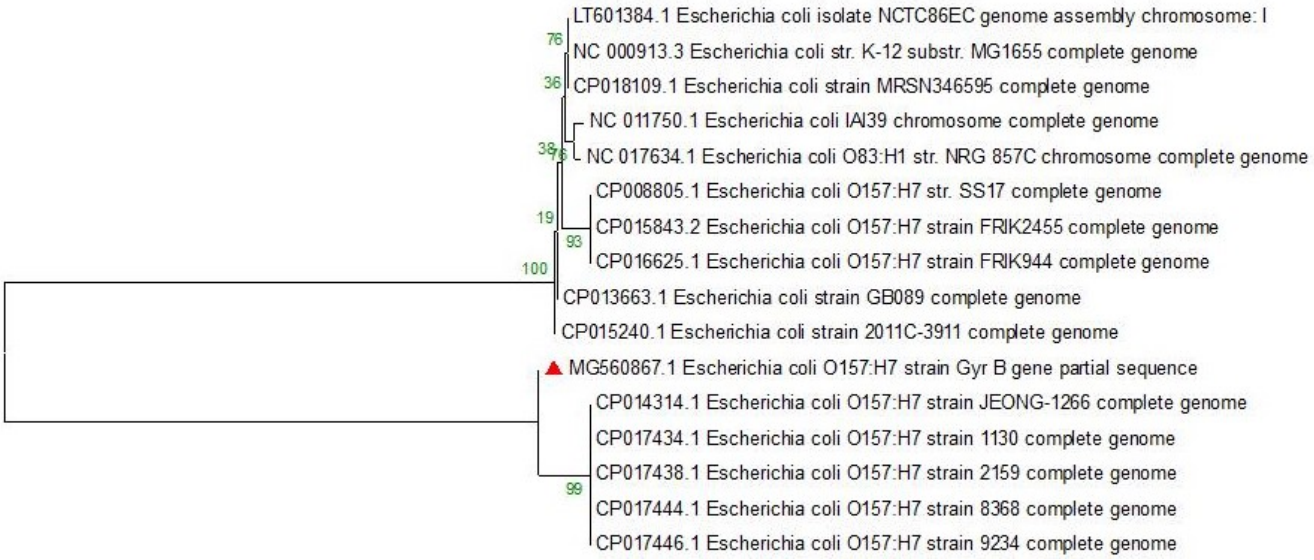

Figure 4: Phylogenetic tree of *E.coli* strain O157H7which isolated from human.

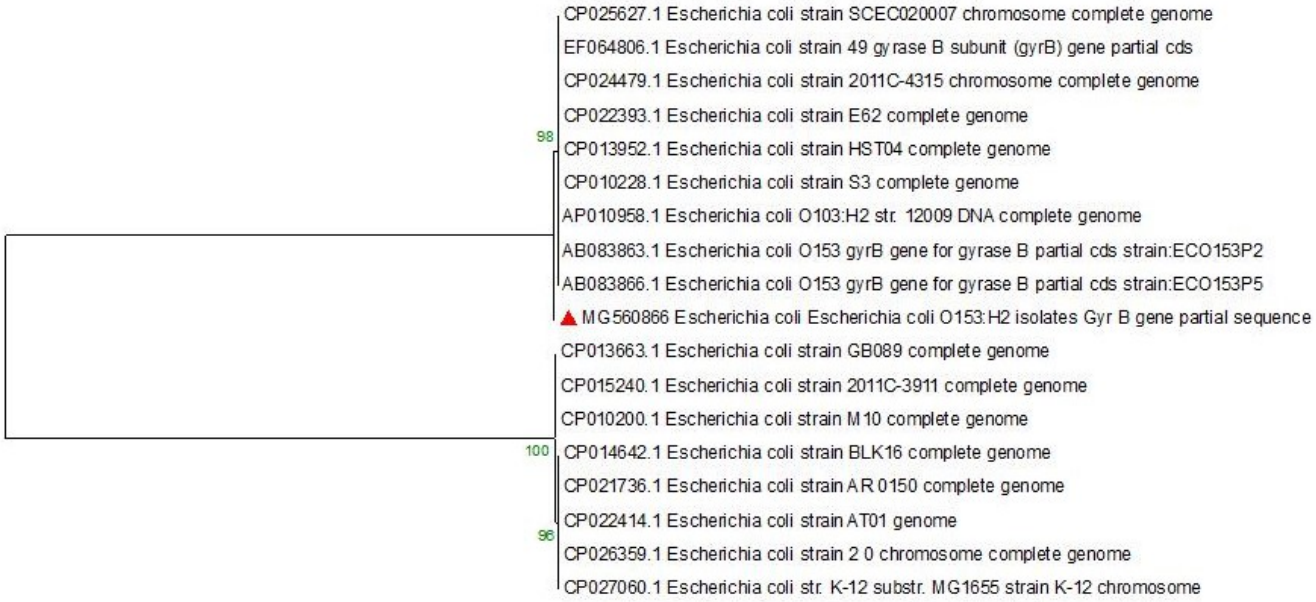

Figure 5: Phylogenetic tree *E. coli* strain O153H2 which isolated from camel.

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