# DNA sequencing and phylogenetic analysis of Escherichia coli isolated from animal fecal samples by conventional and molecular methods

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# DNA sequencing and phylogenetic analysis of *Escherichia coli* isolated from animal fecal samples by conventional and molecular methods

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## Abstract

**Aim:** This study aims to study the presence of *E. coli* in different animal feces samples. In addition to investigate the relationships among these strains and previously isolated *E. coli* using phylogenetic analysis.

**Methods**: One hundred feces samples were collected from cows and sheep in Basrah city. Vitek and the PCR method using 16s RNA were used to identify the isolated bacterial colonies.

**Results:** The result shows that the percentage of *E coli* recovered from cows was 86%, followed by sheep at 82%. Based on 16S rRNA, all isolates showed a band of 1350bp when the gene was amplified. DNA sequencing revealed that one isolate belonged to *E. coli* O145:H28, and the rest belonged to *E coli* O175:H7. The phylogenetic analysis shows that they fall into three categories. The evolutionary history was inferred using the UPGMA method. Eight different DNA sequences were analyzed in this investigation. 1st+2nd+3rd+Noncoding locations of codons were included. There were a total of 525 unique locations in the whole data set.

**Conclusion:** *E coli* strains can be recovered with high numbers from animal feces. This lead to contamination of the water body and can transmitted to human and other animals.



**Keywords**: Polymerase Chain Reaction, Feces, *Escherichia coli,* cow, sheep


## INTRODUCTION

*E. coli* is a prevalent pathogen in both humans and animals' gastrointestinal tracts. There are *E. coli* strains that are common infections of people and animals, as well as others that are innocuous commensals of the gastrointestinal system. It is a natural element of the intestinal flora of warm-blooded animals, humans, and birds. Some strains, however, are pathogenic and cause various clinical disorders and diseases (Islam *et al.,* 2015). Additionally, *E. coli* may cause septicemia and diarrhea in a variety of hosts, including humans, birds, cattle, piglets, goats, lambs, and buffaloes, as well as children in underdeveloped nations (Baker *et al.,* 2019; Kaper, 2004).

As part of the natural gut flora, most *E. coli* strains are innocuous. They can benefit their hosts by assisting in food digestion, creating vitamin K and B-complex vitamins, and inhibiting the growth of dangerous bacteria inside the intestine. Humans, for example, require *E. coli* and other types of bacteria in the digestive system to be healthy. *E. coli* is not exclusively confined to the gastrointestinal tract. Its ability to last for brief durations outside the human body renders it a valuable indicator for assessing faecal contamination in many environmental contexts, such as water, soil, plants, and food items like vegetables and meat. Additionally, *E. coli* serotypes are

frequently host-specific, making it easy to identify whether the source of faecal contamination came from humans, other animals, or birds (Baker *et al*., 2019). Infection with any of these pathotypes has the potential to induce three overarching clinical syndromes: enteric/diarrheal sickness, urinary tract infections (UTIs), and sepsis/meningitis. There are six main classifications of intestinal pathogens, specifically enteropathogenic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent *E. coli* (DAEC). The many pathotypes of Escherichia coli are often clonal assemblages characterized by shared O (lipopolysaccharide, LPS) and H (flagellar) antigens, which establish serogroups (based solely on O antigen) or serotypes (based on both O and H antigens) (Nataro, & Kaper, 1998; Rodrigues *et al*., 1996).

## MATERIALS AND METHODS

### Sample collection

A total of one hundred faecal samples were obtained from cows and sheep residing in various locations within the city of Basrah. The samples were put in clean cases with screw-on lids and marked with their serial numbers and the time and date they were taken. Subsequently, the collected samples were carefully transferred to containers chilled with ice and promptly sent to the Laboratory of Microbiology at the College of Veterinary Medicine to undergo urgent examination.

### Bacterial Isolation

Standard techniques were used to isolate and identify the *E. coli* present in the samples. The faeces samples were introduced into tubes containing newly made Tryptone Soya Broth (TSP) supplemented with Vancomycin. The tubes were then placed in an aerobic incubator at 37°C for overnight incubation. Subsequently, the samples were transferred onto MacConkey agar plates and allowed to grow for 24 hours at the same temperature of 37°C. The colonies that underwent lactose fermentation were immediately transferred onto Eosin methylene blue (EMB) agar and incubated at 37°C for 24 to 48 hours. Colonies exhibiting a metallic appearance were collected thereafter (McFadden, 2000; Collee, *et al*., 1996).

### Bacterial Identification

Vitek-2 and the PCR method using 16s rRNA were used to identify the isolated bacterial colonies.

### Bacterial DNA Extraction and PCR Analysis.

| Primer | Sequence | Primer sequence | Tm (°C) | GC% | Size of Product (bp |
|---|---|---|---|---|---|
| *16s rRNA* | F | 5'- AGAGTTTGATCCTGGCTCAG- 3' | 54.3 | 50.0 | 1250 |
| | R | 5'- GGTTACCTTGTTACGACTT- 3' | 49.4 | 42.1 | |

| Animal | No of samples | No of *E coli* positive | Percentage % |
|---|---|---|---|
| **cow** | 50 | 43 | 86% |
| **sheep** | 50 | 41 | 82% |
| **Total** | 100 | 84 | 84% |

Table 1 lists the primers used in this investigation (Miller *et al*., 2013). The primers were dissolved in distilled water to get a stock solution with a final concentration of 100 pmol/$\mu$l. This stock solution was maintained at -20°C. To create a working primer suspension with a concentration of 10 pmol/$\mu$l, 10 $\mu$l of the stock solution was mixed with 90 $\mu$l of distilled water, resulting in a final volume of 100 $\mu$l. Table 2 shows the PCR conditions.

**Phylogenetic analysis**

Using the UPGMA method, the evolutionary past was inferred (Sneath and Sokal,1973).

Table 1. The sequence of primers that used this study.

Table 2. The optimum condition of detection.

## RESULTS AND DISCUSSION

### Bacterial isolation

The result of bacterial isolation and identification using culture characteristics and Vitek-2 identification, shows that the percentage of *E coli* recovered from cow was 86% followed by sheep 82%. The total percentage was 84%,Table 3.

Table 3. Number and percentage of *E. coli* recovered from cows and sheep.

| No. | Phase | Tm (°C) | Time | No. of cycle |
|---|---|---|---|---|
| 1 | Initial Denaturation | 95°C | 5 min | 1 cycle |
| 2 | Denaturation -2 | 95°C | 45sec | 35 cycle |
| 3 | Annealing | 56°C | 45sec | |
| 4 | Extension-1 | 72°C | 1min | |
| 5 | Extension -2 | 72°C | 5 min. | 1 cycle |

**Molecular identification**

The bacterial DNA was isolated using 16S rRNA (Fig. 1). All isolates showed a band of 1350bp when the gene was amplified using the primer mentioned above (Fig. 2).

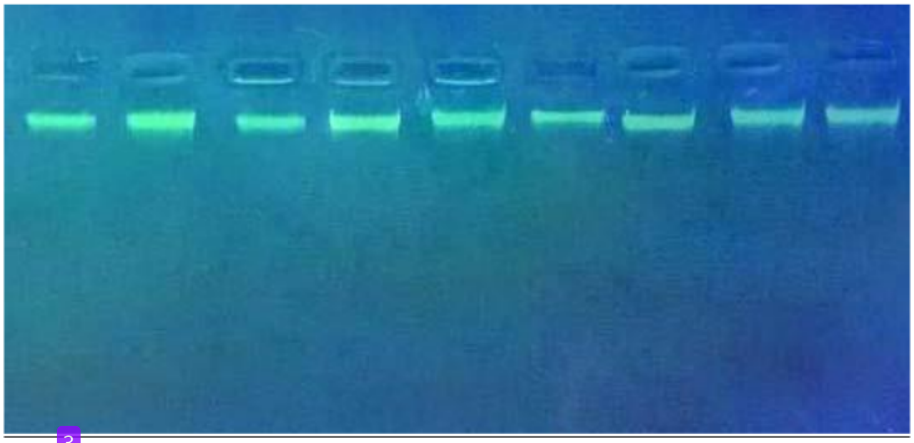

Figure 1. Gel electrophoresis of genomic DNA extraction from bacteria 1% agarose gel.

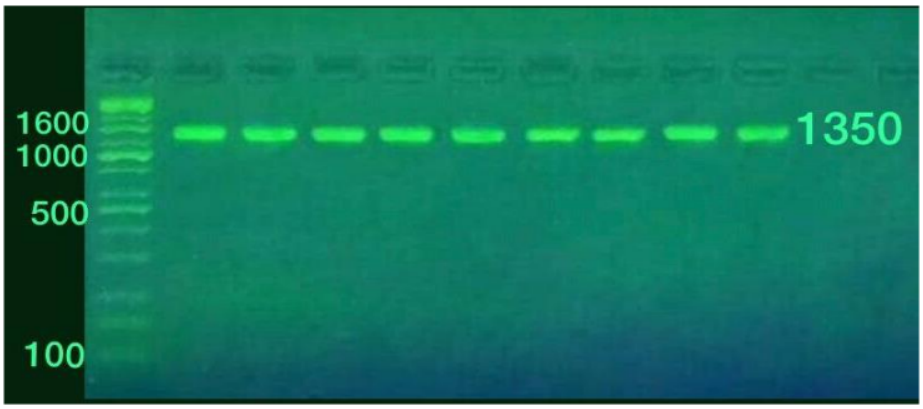


Figure 2. PCR product the band size 1350 . The product was electrophoresis on 1.5% agarose at 5 volt/cm$^2$. 1x TBE buffer for 1:30 hours. M: DNA ladder 100bp.

**DNA Sequencing of rRNA gene**

DNA sequencing revealed that one isolate belonged to *E. coli* O145:H28 and the rest to *E. coli* O175:H7. The results of mutation occurring in genes are listed in table 4. Most are identical to previously reported bacteria with 99% homology with a few transitions and transversion mutations.

Eight new sequences were submitted to NCBI data base, these are ON680705.1; ON680706.1; ON680707.1; ON680708.1; ON680709.1; ON680710.1; ON680711.1 and ON680712.1.

Table 4. Type of substitution of nucleotides in sequenced DNA.

| 16S ribosomal RNA gene | | | | | | | |
|---|---|---|---|---|---|---|---|
| **No.** | **Type of substitution** | **Location** | **Nucleotide** | **Sequence ID with compare** | **Sequence ID with Submissions** | **Source** | **Identities** |
| 1 | Transvertion | 228345 | A\T | ID: AP019703.1 | ID: ON680705.1 | E. coli O145:H28; Human | 99% |
| | Transition | 228569 | A\G | | | | |
| | Transvertion | 228798 | G\T | | | | |
| | Transvertion | 228825 | G\T | | | | |
| | Transition | 228840 | G\A | | | | |
| | Transvertion | 228869 | G\T | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 2 | Transvertion | 4422856 | T\A | ID: CP064383.1 | ID: ON680706.1 | E. coli O157:H7; Sheep | 99% |
| | Transition | 4422858 | C\T | | | | |
| | Transition | 4422860 | A\G | | | | |
| 3 | Transition | 594768 | G\A | ID: CP015832.1 | ID: ON680707.1 | E. coli O157; Human | 99% |
| | Transition | 594783 | C\A | | | | |
| 4 | Transvertion | 555482 | T\G | ID: CP038398.1 | ID: ON680708.1 | E. coli O157:H7; Human | 99% |
| | Transition | 555483 | C\T | | | | |
| | Transition | 555486 | T\C | | | | |
| | Transvertion | 556081 | G\C | | | | |
| 5 | Transvertion | 16418 | T\G | ID: CP051782.1 | ID: ON680709.1 | E. coli O157:H7; Cow | 99% |
| | Transition | 16485 | C\T | | | | |
| 6 | ---------- | -------- | ------ | ID: CP051782.1 | ID:ON680710.1 | E. coli O157:H7; Cow | 100% |
| 7 | Transition | 16195 | T\C | ID: CP051782.1 | ID: ON680711.1 | E. coli O157:H7; Cow | 99% |
| 8 | Transvertion | 4422259 | A\C | ID: CP064383.1 | ID: ON680712.1 | E. coli O157:H7; Sheep | 99% |
| | Transvertion | 4422429 | A\T | | | | |
| | Transvertion | 4422432 | A\T | | | | |

**The phylogenetic analysis**

The tree that is considered to be optimal is characterized by a cumulative branch length of 14.44764665. The tree has been subjected to scaling, wherein the lengths of its branches are measured using the same units as the evolutionary distances employed for estimating the phylogenetic tree. The study used a total of nine nucleotide sequences. The codon sites considered in the study encompassed 1st+2nd+3rd+Noncoding. All positions that included missing or partial information were removed. The ultimate dataset had a total of 525 locations. The software MEGA6 was utilized for conducting evolutionary analysis.

The study included 9 nucleotide sequences. The following codon locations were included: 1st+2nd+3rd+Noncoding. All positions with missing or incomplete information were eliminated. The final dataset contained 525 positions in total. MEGA6 was used to perform evolutionary analyses. According to the phylogenetic study, they are classified into four groups (Fig 3, 4).

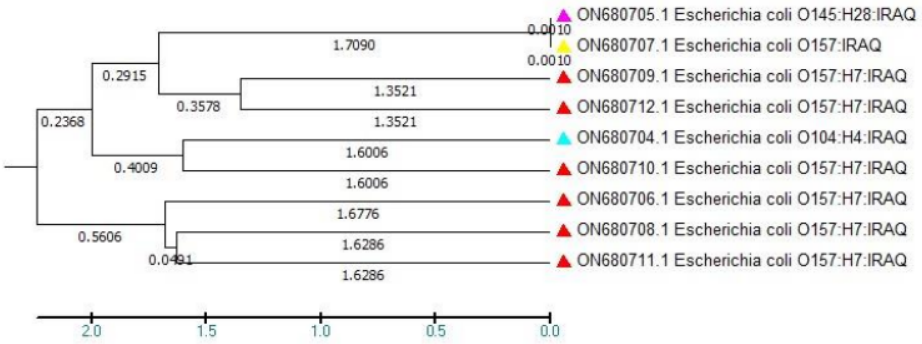


Figure 3. Evolutionary relationships of the taxa.

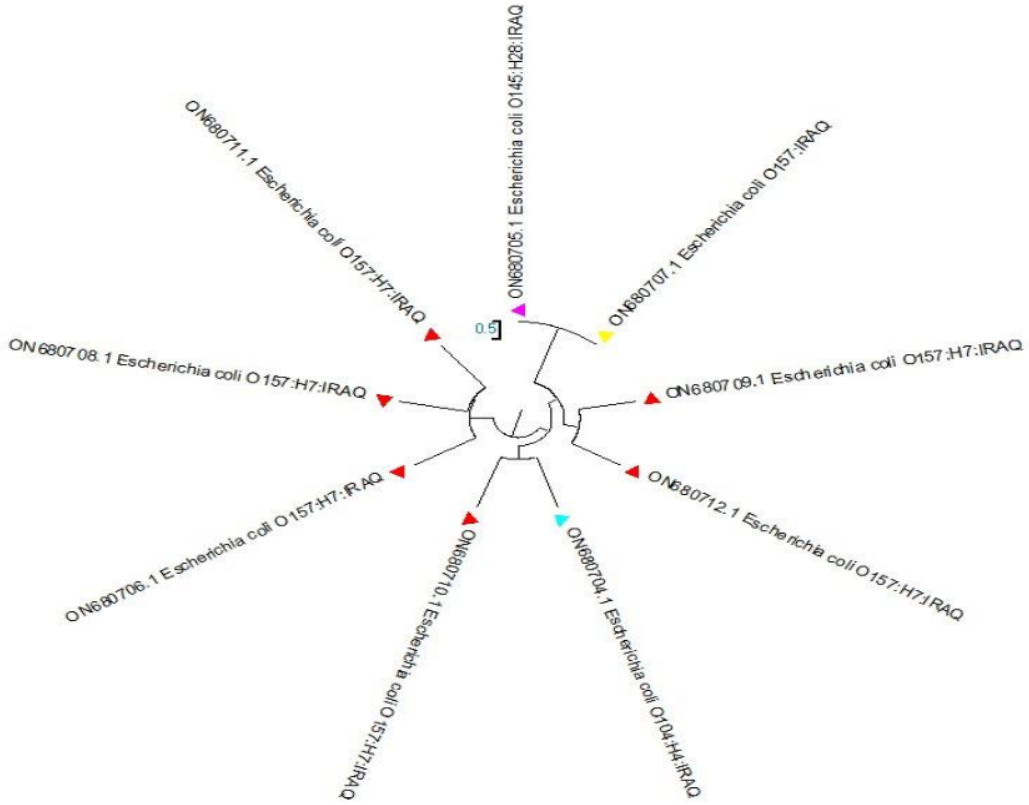


Figure 4. Evolutionary relationships of the isolated strains of *E. coli*.

*E. coli* is found in human and animal digestive systems; it is discharged into the environment via faeces and is thus utilized to indicate faecal pollution (Ishii and Sadowsky, 2008). *E. coli* is also a source of genes for antibiotic resistance (Bailey *et al.*, 2013). In 1935, a strain of the bacterium was linked to an outbreak of diarrhea among infants (Todar, 2008). Surveillance data from a number of hospitals in the United States revealed that E. coli was the most commonly

reported nosocomial infection by Jarvis and Martone (1992). Human infections caused by pathogenic genotypes of E. coli include urinary tract infections, neonatal meningitis, and intestinal diseases (Todar, 2008).

Biochemical characteristics of a specific clade of Escherichia. Strains were classified based on their indole, methyl-red, Voges–Proskauer, and citrate reaction profiles (IMViC test).

There has been a suggestion that the sensitivity of *E. coli* O157:H7 isolation might be enhanced by implementing an enrichment step prior to plating on selective agar, as opposed to directly plating test samples on selective agar. The CT-SMAC agar medium has been characterized as producing the greatest results for the selective culture of *E. coli* O157:H7 (Jarvis and Martone, 1992; Sanderson. 1995). As an alternative to immunological assays, it might be possible to identify *E. coli* O157:H7 using the selective medium and specific antisera employed in this study.

Molecular methods provide a good way for bacterial identification. It always gives an accurate result for such bacteria (17-20). DNA sequencing revealed that one isolate belonged to *E. coli* O145:H28, and the rest to *E. coli* O175:H7. The phylogenetic analysis shows that they fall in to four categories. The phylogenetic tree was constructed using evolutionary distances and scaled to match branch lengths. Maximum Composite Likelihood calculated evolutionary distances (base substitutions per site (Tamura and Kumar ,2004; Tamura et al 2013).

The results of mutation occurring in genes are listed in table 4. Most are identical to previously reported bacteria with 99% homology with a few transitions and transversion mutations. The present results showed a higher percentage than previous studies. There were no differences in isolation percentage between cows and sheep. It is related to the type of feed which always take place out of the farm in this area (Khudaier, et al 2012; Abbas *et al*., 2012; Abbas et al. 2013; Abbas et al. 2014).

## CONCLUSION

*Escherichia coli* strains can be recovered with high numbers from animal feces. This lead to contamination of the water body and can transmitted to human and other animals. Some strains are related to *E. coli* O157:H7 which recognized as most virulence strain. Phylogenetic analysis lead to conclude that strains belong to different origin since they have at least four phylogenetic clads.

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