

#### **Category: Food Microbiology**









# Prevalence of *Escherichia coli* O157:H7 in Clinical and Food Samples: A Cross-sectional Study

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#### **ABSTRACT**

Background and Aim: Escherichia (E.) coli O157:H7 is a virulent bacterial strain known for producing a toxin called Shiga toxin, which can damage intestinal and kidney cells leading to bloody diarrhea and kidney failure. Food poisoning is a significant public health issue worldwide. This study aimed to investigate the prevalence of E. coli O157:H7 in clinical and food samples.

Materials and Methods: In this cross-sectional study, 62 food samples, including raw chicken meat, milk, and beef were randomly collected from several supermarkets in different areas of Hamadan City, Iran. Additionally, 62 *E. coli* strains were collected from various clinical specimens, including wounds, urine, blood, and cerebrospinal fluid, from Besat Hospital in Hamadan, Iran, and analyzed for *E. coli* O157:H7 contamination. All samples were cultured on specific media, and any suspicious colonies were subjected to PCR for verification of *E. coli* O157 H7 strain using two target genes.

Results: In total, 2 clinical and 8 food samples were positive for *E. coli* O157:H7 phenotypically. All isolates were positive for the presence of target genes using PCR. The highest frequency was related to contaminated food, particularly beef, with a frequency of 12.9%.

**Conclusion:** The results indicated high prevalence of *E. coli* O157:H7, especially in beef that was higher than the global average. Therefore, it is necessary to implement more precise controls in production and maintenance processes of food samples.

Keywords: Clinical, Escherichia coli O157: H7, Food Samples, PCR, Shiga toxin

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#### 1. Introduction

ood poisoning is a major public health issue globally. Extensive research in this area has highlighted the significant role of *Escherichia* (E.) coli bacteria in different types of food. Escherichia coli O157:H7 can cause severe foodborne illness in humans. It is a highly pathogenic strain of E. coli recognized for its production of Shiga toxin, which has potential to harm intestinal cells and result in symptoms including bloody diarrhea and, in some cases, kidney failure (1, 2).

Escherichia coli O157:H7 is primarily transmitted through contaminated food, particularly undercooked beef, vegetables, and raw milk. It may also be transmitted through direct contact between individuals and by consuming contaminated water (3, 4). The infectious dose of *E. coli* O157:H7 is minimal, indicating that even a small quantity entering the human system can lead to illness. The transfer of this bacterium to humans is promoted by key factors, including the ingestion of unpasteurized or inadequately cooked food, along with exposure to animal feces (5, 6).

Preventing *E. coli* O157:H7 infection involves practicing good hygiene, such as thoroughly washing hands, avoiding cross-contamination between raw and cooked foods, and drinking safe water (7, 8). *Escherichia coli* O157:H7 is causing acute or chronic diarrhea in individuals through many mechanisms (9, 10). These mechanisms include the production of enterotoxin in enterotoxigenic strains, adherence to the mucosal cells of small intestine, and induction of epidemic and endemic diarrhea in infants caused by enteropathogenic strains (11).

Additionally, enteroinvasive strains can cause dysenteric diarrhea through invasion and subsequent inflammation, while enterohemorrhagic strains produce cytotoxins that result in hemorrhagic colitis without fever and the presence of leukocytes in fecal matter (12, 13). Focus of this research was to examine *E. coli* O157:H7 in clinical and food samples through the use of culture techniques and polymerase chain reaction (PCR).

#### 2. Materials and Methods

# 2.1 Bacterial Strains

In this cross-sectional investigation, a total of 62 food samples, comprising raw chicken meat, milk, and beef, were randomly obtained from various butchers and supermarkets across different regions of Hamadan city, west of Iran. The study was performed from March to August 2024 utilizing an electronic random number generator

(https://www.randomresult.com). Furthermore, 62 *E. coli* strains were isolated from a range of clinical specimens, including wound, urine, cerebrospinal fluid (CSF), and blood, sourced from Besat Hospital in Hamadan, Iran.

The patients provided a signed agreement for this study, and the protocol was approved by the local ethics committee of the Hamadan University of Medical Science (IR.UMSHA.REC.1397.444). For more information, read section "6.2 Ethical Considerations".

Food and clinical specimens were inoculated onto MacConkey agar plates (Merck, Germany) and incubated at 37°C for a duration of 24 hr. Following this incubation period, the colonies of interest were identified through biochemical testing. Sorbitol MacConkey agar (Merck, Germany) and serotyping with anti-O157 sera (Mast, UK) were employed for identification of *E. coli* O157 serotypes. Ultimately, these strains were preserved in a 10% glycerol broth (w/v) at -70°C for subsequent analyses (14).

#### 2.2 Disk Diffusion

The Kirby-Bauer disk diffusion method was conducted in accordance with the Clinical and Laboratory Standards Institute (CLSI 2024) sets standards for various aspects of laboratory medicine. The following antibiotic discs were utilized: gentamicin (10  $\mu$ g), ciprofloxacin (5  $\mu$ g), ceftazidime (30  $\mu$ g), piperacillin/tazobactam (110  $\mu$ g), nitrofurantoin (50  $\mu$ g), ceftriaxone (30  $\mu$ g), imipenem (10  $\mu$ g), ampicillin/sulbactam (10, 10  $\mu$ g), and trimethoprim-sulfamethoxazole (23.75, 1.25  $\mu$ g) (Mast, England).

### 2.3 DNA Extraction

A sweep of *E. coli* colonies on MacConkey agar was inoculated in LB broth and incubated overnight at  $37^{\circ}$ C. The genomic DNA of the colonies was extracted by the boiling method (15). For final purification of DNA,  $100~\mu$ l of tris solution was added to the precipitate and centrifuged at 1400~rpm for 5~min. The supernatant, which contained pure DNA, was collected. The concentration and absorbance of the purified DNA at 260/280~nm wavelengths were determined by spectrometer (NanoDrop Technologies, Inc., Wilmington, DE, USA), and the templates were preserved at  $-20^{\circ}$ C (16).

# 2.4 PCR

The PCR mixture was prepared in a total volume of 25  $\mu$ l, consisting of the following components: 12.5  $\mu$ l of 2x Taq premix Master mix (Ampliqon UK), 7.5  $\mu$ l of sterile double distilled water, 1  $\mu$ l of each forward and reverse primer (10 pmol/ $\mu$ l), and 3  $\mu$ l of the DNA

sample (200 - 600  $\mu$ g/ml). The PCR mixtures including a quality positive control (*E. coli* O157:H7 strain ATCC 700728) underwent amplification for the O157 gene target, beginning with an initial denaturation at 95°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 59.5°C for 30 sec, and 72°C for 45 sec, concluding with a final extension at 72°C for 5 min. For the H7 gene target, the initial denaturation was performed at 94°C for 5 min, followed by 35 cycles of 94°C for 45 sec, 65°C

for 45 sec, and 72°C for 1 min, with a final extension at 72°C for 5 min, all conducted in a Bio-Rad Thermal Cycler (Bio-Rad Laboratories, Inc., USA). The PCR products were detected by staining with SYBR Safe and gel electrophoresis on 1% agarose gel and finally, were visualized under UV light. The primers specifications utilized in this study are detailed in Table 1.

Table 1. The primers specifications.

Gen name		Primer Sequence (5'-3')	TM°C	length	reference
0157	F	AAGATTGCGCTGAAGCCTTTG	59.5	503bp	(17)
0157	R	CATTGCCATCGTGTGGACAG	39.3		
Н7	F	GCGCTGTCGAGTTCTATCGAGC	C.F.	625bp	(18)
Н7	R	CAACGGTGACTTTATCGCCATTGC	65		

#### 2.5 Statistical Analysis

The analysis of data was conducted utilizing the chisquare test and Fisher's exact test, with a significance threshold established at a P-value of less than 0.05 (SPSS version 19, Chicago, IL, USA).

#### 3. Results

In this study, 62 clinical samples and 62 food samples were collected from Besat Hospital and food markets,

respectively from Hamadan, Iran. All samples were examined and analyzed for contamination by *E. coli* O157:H7.

In total, 2 clinical samples and 8 food samples were tested positive for *E. coli* O157:H7 by culture.

All 10 isolates were positive for the presence of O157 and also H7 target genes using PCR (Figure 1-3). More details of the results are presented in Tables 2 and 3.



**Figure 1.** *E. coli* O157 H7 strain growth on Sorbitol MacConkey agar. Non-O157:H7 *E. coli* ferment sorbitol to produce yellow colony: *E. coli* O157:H7 cannot ferment sorbitol to produce pink colony.



Figure 2. E. coli O157 H7 strains by Anti-O157 sera, left: agglutination positive for O157, right: agglutination negative.

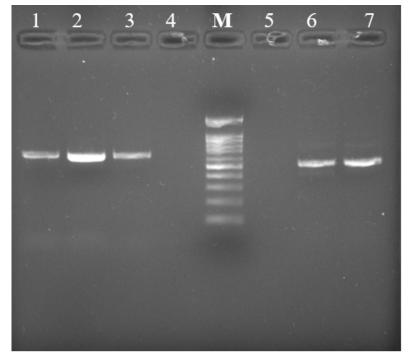


Figure 3. Agarose gel electrophoresis. M: Marker (100 bp); lane numbers 1–3: H7 gene (625bp); lane numbers 4 and 5 negative control, lane numbers 6–7: O157 gene (503bp).

Table 2. E. coli O157:H7 from food samples and its antibiotic profiles

Sample	Total number of samples collected N (%)	Total number of isolated by phenotypic method N (%)	Total number of isolated by molecular method N (%)	Antibiotic resistance and susceptibility profile
Milk	4 (6.45)	1 (1.62)	1 (1.62)	GM (S), IMP (S), CRO (S), CIP (S), SXT (R), PTZ (S), CAZ(I), SAM(R)
Chicken meat	28 (45.16)	2 (3.22)	2 (3.22)	GM (S), IMP (S), CRO (R), CIP (S), SXT (R), PTZ (S), CAZ(I), SAM(R)
				GM (I), IMP (S), CRO (R), CIP (S), SXT (R), PTZ (S), CAZ(R), SAM(R)
Beef	30 (48.39)	5 (8.06)	5 (8.06)	GM (S), IMP (S), CRO (S), CIP (S), SXT (R), PTZ (S), CAZ(S), SAM(R)
				GM (S), IMP (S), CRO (I), CIP (S), SXT (S), PTZ (S), CAZ(R), SAM(R)
				GM (S), IMP (S), CRO (I), CIP (S), SXT (R), PTZ (S), CAZ(R), SAM(S)
				GM (S), IMP (S), CRO (I), CIP (S), SXT (R), PTZ (S), CAZ(R), SAM(R)
				GM (S), IMP (S), CRO (I), CIP (S), SXT (R), PTZ (S), CAZ(R), SAM(R)
Total	62 (100)	8 (12.90)	8 (12.90)	

GM (gentamicin), CRO (ceftriaxone), SXT (trimethoprim/sulfamethoxazole), PTZ (piperacillin-tazobactam), CAZ (ceftazidime), SAM (Ampicillin / Sulbactam), CIP (Ciprofloxacin), IMP (imipenem), S (susceptible), I (intermediate), R (resistance)

Table 3. E. coli O157:H7 from clinical samples and its antibiotic profiles

Sample	Total number of samples collected N (%)	Total number of isolated by phenotypic method N (%)	Total number of isolated by molecular method N (%)	Antibiotic resistance and susceptibility profile
Outpatient	13 (20.97)	1 (1.62) (urine culture)	1 (1.62)	NIT (S), GM (I), IMP (S), CRO (R), CIP (R), SXT (S), PTZ (S)
Urology	18 (29.03)	0	0	
ICU	7 (11.30)	0	0	
Pediatric	4 (6.45)	0	0	
Neurology	9 (14.51)	0	0	
Internal Ward	11(17.74)	1 (1.62) (urine culture)	1 (1.62)	NIT (S), GM (S), IMP (S), CRO (R), CIP (R), SXT (R), PTZ (S)
Total	62 (100)	2(3.24)	2(3.24)	

GM (gentamicin), CRO (ceftriaxone), SXT (trimethoprim/sulfamethoxazole), PTZ (piperacillin-tazobactam), CAZ (ceftazidime), IMP (imipenem), CIP (Ciprofloxacin), NIT (nitrofurantoin), S (susceptible), I (intermediate), R (resistance).

#### 4. Discussion

Escherichia coli is a component of the normal microbiota found in gastrointestinal tracts of both humans and animals (19). Certain strains of this bacterium possess virulence factors that allow them to induce both intestinal and extra intestinal diseases (20, 21). One of the key strains -the O157:H7 serotypecan produce Vero toxin, which leads to hemolytic uremic syndrome and hemorrhagic colitis. Animals, especially cattle are considered as a primary reservoir for this serotype, but limited information is available regarding the origin of other Vero toxin serotypes (20, 22).

The prevalence of *E. coli* O157:H7 infection reported in this study (14%) aligns with the prevalence found in the previous research conducted in Bahir Dar town, Ethiopia (23). It is also comparable to 20% prevalence reported in a study conducted in Benin City, Nigeria (24). However, it is higher than the prevalence reported in a study conducted in central Ethiopia (25) and southern Ethiopia (26). It is also significantly higher than the findings from studies conducted in other African countries (27, 28). The discrepancy may be attributed to variations in sample size, differences in the study population source, and the methods used for sample collection.

Accurate identification and isolation of bacteria is very important in the management of diseases caused by pathogens. While traditional biochemical techniques are often time-consuming and errorprone (29, 30). However, the advent of molecular methods, such as PCR, has revolutionized this field by allowing the identification of pathogens at the strain and serotype levels, even at low concentrations. In a study in Mashhad, out of 7 samples that tested positive through biochemical tests, only 2 samples were confirmed positive by PCR assay (29, 31). Also in this study all samples were confirmed by PCR assay.

Escherichia coli O157:H7 is widely distributed across the United States and Canada, though its prevalence in the fecal shedding of adult cows remains consistently low (32).

In previous investigations carried out in England and America, *E. coli* O157:H7 in slaughtered beef was found to be 13.4% and 18% during 2000 and 2001, respectively. These rates were significantly higher than those observed in the current study (33, 34). The variation observed in the incidence of *E. coli* O157:H7 in beef carcasses examined in this study, as compared to other investigations, may be attributed to differences in the sampling methodology, the dimensions of the meat cuts, and the number of samples analyzed. Abebe et al (35) studied prevalence and antibiotic resistance of *E. coli* O157:H7 in milk and meat samples. They found that prevalence of this

strain was 6.5%, which was the highest recorded prevalence in the carcass of a cow (35). They also reported the presence of multiple resistance strains in E. coli O157:H7 (35). So that, E. coli O157:H7 exhibited complete sensitivity to ampicillin, sulfamethoxazole, trimethoprim, and ciprofloxacin. Conversely, all strains were found to be resistant to penicillin G, vancomycin, and oxacillin (35). Rahimi et al (36) suggest that survival of E. coli O157:H7 in food is dependent on acidity of the sample. In particular, the bacterium is eliminated when the sample pH level drops below 3.5 (36). The escalation of antibiotic resistance in pathogens obtained from food has become a growing concern. E. coli O157:H7 strains demonstrate a diverse array of antibiotic resistance patterns. The spread of antibiotic resistance among *E*. coli O157:H7 strains sourced from both humans and animals, along with the rise of multi-drug resistant variants, has emerged as a global concern (35). In Safaari et al (37) 214 samples were subjected to E. coli O157:H7 antiserum. The results indicated that 4 samples (1.87%) were identified as E. coli O157:H7, which is consistent with findings of the present study

In the latest U.S. survey, NAHMS Dairy '96, the prevalence of *E. coli* O157:H7 shedding in feces was recorded as follows: 0.9% among 3,600 dairy cows across 91 farms in 21 major dairy states, 2.8% among 600 cows scheduled for culling within seven days, and 1.8% among 2,200 culled dairy cows at 97 markets (38).

Another research during a diarrhea outbreak involved the analysis of 19 stool samples from the patients with acute diarrhea, 16 stool samples from restaurant workers, and 10 environmental samples from different age groups. The samples were subjected to biochemical and PCR tests, and the results revealed that only one strain of *E. coli* O157:H7 was present in the patient samples (39). In a study conducted by Kargar et al (40) out of 428 analyzed hamburger samples, 5 samples, representing 1.17%, were identified to contain *E. coli* O157:H7 (40). This discovery underscores the possible health hazards linked to the intake of animal products tainted with this bacterium.

Potential limitations of our study include the relatively small sample size, geographic confinement to Hamadan, and variability in sampling methods.

# 5. Conclusion

The occurrence of *E. coli* O157:H7 contamination in food was determined to be 12.90%. This statistic indicates that meat is a major and primary reservoir for

this pathogen, which may be transmitted to humans via consumption of beef and its derivatives. In the light of the seriousness of this matter, it is essential to pursue additional research on antibiotic resistance, transmission pathways, and strategies for mitigating the spread of this pathogen.

#### 6. Declarations

# 6.1 Acknowledgment

The authors would like to thank the staff of the Department of Microbiology, School of Medicine, Hamadan University of Medical Sciences, Hamadan, Iran.

#### 6.2 Ethical Considerations

This study was approved by the Institutional Review Board of Hamadan University of Medical Sciences (IR.UMSHA.REC.1397.444). The initial research on the pathogenicity of Escherichia coli O157 began in 2018 (see <a href="https://doi.org/10.1155/2021/3333240">https://doi.org/10.1155/2021/3333240</a>), involving initial sampling and analysis. Recently, a follow-up sampling was conducted to assess the

current prevalence and update the data, based on the original study and funding. No new ethical approval was required, as activities adhered to the original project framework and permits.

#### 6.3 Authors' Contributions

H.H, H.M, A.H.A and M.Y.A wrote the main manuscript text and A.M collected food sample. All authors reviewed the manuscript.

# 6.4 Conflict of Interests

The authors declare no competing interests.

# 6.5 Financial Support and Sponsorship

Not applicable.

# 6.6 Using Artificial Intelligence Tools (AI Tools)

None.

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