

# Molecular detection of *Escherichia coli* O157:H7 isolates from stool samples in Khartoum State, Sudan

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## ABSTRACT

**Background:** *E. coli* O157:H7 is a causative agent of food-borne illness. This study aimed to detect the presence of *E. coli* O157:H7 in stool samples from Khartoum by using a molecular technique. This was a laboratory-based study. A total of 100 stool samples were collected from different types of hospitals in Khartoum State; 54 (54%) male and 46 (46%) female samples were cultured on MacConkey agar media at 37°C for 24 hours overnight, and colony morphology, Gram's staining technique and different biochemical tests were subsequently performed for identification. **Results:** The results revealed that *E. coli* was present in 70% of the samples analyzed. DNA was extracted via the boiling method, and primers for *E. coli* O157:H7 were used to detect the presence of genes via the polymerase chain reaction (PCR) technique. Molecular detection via PCR was performed on 70 isolates of *E. coli* to detect the target gene O157:H7, and the results revealed that a band appeared in 1 (1.4%) *E. coli* O157:H7 sample and that no band was detected in 69 (98.6%) samples. **Conclusions:** The study concluded that the isolation of *E. coli* O157:H7 from patients should direct the attention of physicians to the possibility of complications among young and elderly patients. **Key words:** PCR, MacConkey agar, Foodborne illness, Gene

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## BACKGROUND

*Escherichia coli* are gram-negative bacilli, catalase test positive, oxidase test negative, facultative anaerobic, some strains capsulated, flagellated, citrate test negative and indole test positive. It is normally found in the gastrointestinal tract of humans and animals. Some of these diseases cause diarrhea and a range of extraintestinal diseases<sup>1</sup>.

*Escherichia coli* has a broad spectrum of lifestyles and phenotypes and is a well-suited model organism for studying bacterial evolution and adaptation to different growth conditions<sup>2</sup>. A harmless commensal needs only to acquire a combination of mobile genetic elements to become a high pathogen capable of causing a range of diseases<sup>3</sup>.

Enteric *Escherichia coli* is both a normal flora and a pathogen that causes morbidity and mortality worldwide. The strains that cause gastroenteritis are traditionally divided into 6 pathotypes on the basis of their virulence properties, mechanisms of pathogenicity, clinical syndromes and O:H serogroups: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and enteroaggregative *E. coli* (EAEC) enterohemorrhagic *E. coli* (EHEC)<sup>4</sup>.

Infectious *E. coli* bacteria can be spread from humans and animals through the consumption of uncooked

meat, the consumption of contaminated fruits and vegetables, the consumption of unpasteurized milk, and contact with infected animals; anyone has the potential to develop *E. coli* infection if they are exposed to the bacteria, and symptoms may begin 1 to 10 days after exposure<sup>5</sup>.

Most *E. coli* infections are opportunistic infections. Special strains of *E. coli* can cause diarrhea, one of which is verotoxigenic *E. coli*<sup>6</sup>.

The entero-hemorrhagic *E. coli* (EHEC) serotype O157:H7 is the most commonly identified member of the Shiga toxin-producing *E. coli* (STEC) family; it is the most notorious emerging pathogen and is considered prototypical for the current paradigm of foodborne disease in the United States<sup>7</sup>. It is a human pathogen responsible for bloody diarrhea, causing sporadic and epidemic outbreaks and severe life-threatening hemolytic uremic syndrome (HUS) worldwide<sup>8</sup>. Previously isolated as a foodborne pathogen in 1982 in the United States (U.S.), a rare strain of *E. coli* was investigated and isolated from stool and food samples. Serotyping confirmed the presence of the somatic O157:flagellar H7<sup>9</sup>. *E. coli* O157:H7 is a cause of intestinal disorders, ranging from mild infection to severe bloody diarrhea. Most isolates have been shown to produce Shiga toxins

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(Stx1, Stx2), and most clinical laboratories now routinely screen stools for *E. coli* O157:H7 from patients with bloody and nonbloody diarrhea<sup>10</sup>. *E. coli* O157:H7 infects the alimentary tract, induces abdominal cramps with hemorrhagic diarrhea and causes a wide range of clinical illnesses, including nonbloody diarrhea, hemorrhagic colitis, hemolytic uremic syndrome and death. The infection is initiated by the ingestion of a small inoculum of 10–100 CFUs<sup>11</sup>.

*Escherichia coli* O157:H7 is a causative agent of foodborne illness through the consumption of contaminated milk and uncooked beef and may be present after swimming in or drinking water contaminated with *E. coli* O157:H7.

Infection with these bacteria causes hemorrhagic diarrhea and kidney failure, leading to hemolytic uremic syndrome (HUS) according to the Central Disease Control (CDC) (3–5%), and individuals who develop hemolytic uremic syndrome (HUS) may die from this complication.

This study aimed to detect *E. coli* O157:H7 in stool samples from Khartoum State, Sudan

## METHODS

### Study Design:

This laboratory-based study was performed in different hospitals in Khartoum state (Al-Amal National Hospital, Jabal Awliya Hospital, Bahri Aleaskarii Hospital). The sample size included all samples from hospitals during the study period. The inclusion criterion included all patients with gastrointestinal distress, whereas the exclusion criterion did not include patients without gastrointestinal distress.

### Sample collection:

Stool samples were collected from a total of 100 diarrheic patients in wide-mouth containers from different hospitals.

### Direct wet preparation:

One drop of normal saline was placed on a clean slide. - A small portion of each stool sample was collected via a wound stick and mixed well with a drop of normal saline.

The glass was covered and then examined under a microscope with 10x and 40x objectives<sup>12</sup>.

### Culture technique:

Diarrheal samples were cultured on MacConkey agar and incubated for 24 hours at 37°C aerobically<sup>13</sup>.

### Identification of bacteria:

The isolated organisms were identified via standard laboratory procedures via colony morphology, the Gram's staining technique and different biochemical tests.

### Colonial morphology:

The colonies on MacConkey agar were lactose fermented, medium-sized, round, smooth, slightly convex and pink in color. Yellow colonies (nonlactose ferment) were excluded<sup>14</sup>. The Gram staining technique was performed according to a known protocol in the literature<sup>15</sup>.

### Biochemical tests:

These tests include the use of indole in tryptone water<sup>16</sup>, citrate utilization tests<sup>17</sup>, urease tests, Kligler iron agar (KIA)<sup>18</sup>, and motility tests (nonbiochemical tests)<sup>19,20</sup>.

### Molecular technique

Genomic DNA was extracted from whole cells via the heat-shock method in this study. Twenty-four-hour colonies were grown on nutrient media, and the culture media were harvested in 2 ml of sterile distilled water with a sterile loop. One millimeter aliquot of the cell suspension was transferred to a 1.5 ml microcentrifuge tube, and the cell mixture was boiled in a water bath for 10 minutes, followed by sudden freezing for 10 minutes. This step was repeated three times. The cell debris was pelleted by centrifugation at high speed (1300 rpm at 4°C for 10 minutes), and the supernatant was transferred to a new 1.5 ml tube and then used immediately as a template for PCR amplification or kept at 4°C for up to one month (Santa Cruz Biotechnology Dallas Company, TX, USA)<sup>21</sup>.

### Polymerase chain reaction (PCR):

The following steps were adopted to carry out the process of DNA amplification. Five microliters of the extracted DNA was added to the Maxime PCR premix kit (i-Taq) mixture, and 1 µL of each forward or reverse primer mixture (1 bp) plus 13 µL of distilled water was added. Thermal cycling for 35 cycles was performed at 94°C for 1 min, 60°C for 1 min and 72°C for 5 min. The final extension step was performed for 5 min at 72°C (Santa Cruz Biotechnology Dallas Company, TX, USA).

DNA inhibitors were removed as described previously, and the manufacturer's guidelines were precisely followed by the use of master mix for PCR primers (Santa Cruz Biotechnology Dallas Company, TX, USA)<sup>22</sup>.

**Primers:**

A 10% agarose gel<sup>23</sup> was prepared, and gel electrophoresis was performed via a documentation system (Bio-Rad, USA) with a run time of 6 hours<sup>24,25</sup>.

**Data analysis:**

The data were analyzed via the Statistical Package for Social Sciences (SPSS) version 20.

**Results**

The present study addresses a significant public health concern: the detection of *E. coli* O157, a notorious food-borne pathogen, in stool samples from Khartoum State. The use of molecular techniques, specifically polymerase chain reaction (PCR), aims to assess the prevalence of this pathogen among patients and draw attention to its potential health implications. Given the relevance of food safety and disease prevention in the context of increasing global health threats, this study provides important insights.

A total of 100 diarrheal samples were collected from people of different ages in Khartoum state, ranging from 9 months to 70 years old, who can be male or female during the study period (Tables 2 and 3).

All the samples were cultured on MacConkey agar, and colony morphology, Gram's staining technique and different biochemical tests were performed. The results revealed that *E. coli* accounted for 70 (70%) samples, whereas *E. coli* accounted for 30 (30%) samples (Table 4).

The distribution between the gender and test results revealed that 38 (38%) males and 32 (32%) females were positive for *E. coli* (Table 5).

Molecular detection via PCR was performed on 70 isolates of *E. coli* for detection of the target gene O157:H7, and the results revealed that a band appeared in 1 (1.4%) *E. coli* O157:H7 sample and that no band was detected in 69 (98.6%) samples, which are considered other than *E. coli* O157:H7 (Table 6).

The associations between sociodemographic factors (gender, age) and test results revealed that 1 (1.4%) male was positive for *E. coli* O157:H7 via the PCR technique, and the other 37 (52.9%) females and 45 males (45.7%) were negative for *E. coli* O157:H7 (P value = 0.488) (Tables 7 and 8).

**DISCUSSION**

One of the most commendable aspects of this study is its focus on a region that may be underrepresented in the literature regarding food-borne pathogens. By investigating the presence of *E. coli* O157 in Khartoum, the present study addresses a crucial gap in understanding how this pathogen affects local populations. The choice of using a molecular technique such

as PCR for detection is also a notable strength, as it offers higher sensitivity and specificity than traditional culture methods do.

The methodological approach is well structured, beginning with the collection of stool samples from a variety of hospitals. This diversity in sampling can enhance the generalizability of the findings. The use of different microbiological techniques—including culture on MacConkey agar, Gram staining, and biochemical tests—demonstrates a thorough approach to isolate and identify *E. coli*, ensuring that the results are reliable.

Additionally, the study's results highlight a critical concern: while 70% of the samples contained *E. coli*, only 1.4% of the samples tested positive for the more virulent strain O157. This disparity raises important questions about the prevalence of pathogenic strains versus nonpathogenic strains, emphasizing the need for ongoing surveillance and monitoring of food-borne pathogens.

Verocytotoxin-producing *E. coli* O157:H7 emerged as a major food-borne pathogen from the 1980s–1990s, and the disease is often associated with significant mortality in young and elderly patients<sup>26</sup>. It causes severe life-threatening hemolytic uremic syndrome (HUS) worldwide<sup>27–29</sup>.

One hundred samples were collected from 54 (54%) males and 46 (46%) females; 70 isolates (70%) were *E. coli*. This result is in agreement with previous reports that revealed that 70% of bacteria are positive for *E. coli*, although this result disagrees with other studies performed for the isolation and detection of *E. coli* O157:H7, which detected (78%) *E. coli* isolates from stool cultures<sup>29,30</sup>.

All *E. coli* isolates in this study were tested via conventional PCR to identify the O157:H7 gene, and the percentage of *E. coli* O157:H7 was 1.4%. This result is in agreement with previous findings that reported *E. coli* O157:H7 (1.14%). This finding was similar to that of other previous studies in which *E. coli* O157:H7 was detected in 1 (0.77%) diarrheic patient. This proportion was lower than that reported in a previous study, which revealed that *E. coli* O157:H7 was detected in 5 isolates (2.3%) that were positive in 210 samples. However, the highest percentage of *E. coli* O157:H7 (6%) was detected<sup>29–32</sup>.

Wang *et al.*, Getaneh *et al.*, Peroutka-Bigus *et al.*, and Jenkins *et al.*<sup>33–36</sup> agreed with our results and support our findings.

Future studies including larger sample sizes could increase the statistical power and reliability of the results. Furthermore, a more detailed demographic breakdown of the patients, including their age, health

**Table 1: Sequences of Primers used for detection of the *E. coli* O157:H7 gene via PCR.**

Gene detected	Primer name	Primer sequence	Product size(bp)	Reference
O157:H7	E. coli F	GAGCGAAATAATTTATGTG	518	Toma et al(2003)
	O157:H7 R	TGATGATGGCAATTCAGTAT		

**Table 2: Distribution of samples according to sex**

Gender	Frequency	Percent
Male	54	54.0%
Female	46	46.0%
Total	100	100.0%

**Table 3: Distribution of age**

Age (years)	Frequency	Percent
< 20 years	40	40.0%
20 - 40 years	32	32.0%
> 40 years	28	28.0%
Total	100	100.0%

**Table 4: Distribution of *E. coli* culture results**

		Frequency	Percent
Culture for <i>E. coli</i>	Positive	70	70.0%
	Negative	30	30.0%
	Total	100	100.0%

**Table 5: Association between gender and test results**

		Gender		Total	P. value
		Male	Female		
Culture for <i>E. coli</i>	Positive	38 (38.0%)	32 (32.0%)	70 (70.0%)	0.930
	Negative	16 (16.0%)	14 (14.0%)	30 (30.0%)	

**Table 6: Distribution of *E. coli* O157:H7 results**

		Frequency	Percent
<i>E. coli</i> O157:H7	Positive	1	1.4%
	Negative	69	98.6%
	Total	70	100.0%

**Table 7: Association between gender and test results**

		Gender		Total	P. value
		Male	Female		
<i>E. coli</i> O157:H7	Positive	1 (1.4%)	0 (0.0%)	1 (1.4%)	0.335
	Negative	37 (52.9%)	45.7 (45.7%)	69 (98.6%)	

**Table 8: Association between age and test results**

			Age			Total	P. value
			< 20 years	20 - 40 years	> 40 years		
E. coli O157:H7	Positive		1 (1.4%)	0 (0.0%)	0 (0.0%)	1 (1.4%)	0.488
	Negative		28 (40.0%)	24 (34.3%)	17 (24.3%)	69 (98.6%)	

status, and dietary habits, could provide valuable insights into the risk factors associated with *E. coli* O157 infection.

Further details about the PCR process—such as the specific primers used, amplification conditions, and controls employed—would increase the reproducibility of the study. Clearer documentation of these methodological steps would allow other researchers to replicate the study and validate its findings.

Further research including the implications of the findings more thoroughly would benefit from a deeper exploration of these complications and how they may differ among various demographic groups. Providing context about the health impacts of this pathogen, particularly in vulnerable populations such as children and elderly individuals, would strengthen the public health message. Moreover, further studies could address potential environmental and socioeconomic factors contributing to the prevalence of *E. coli* O157 in the region. Understanding the broader context of food safety practices, sanitation, and public health infrastructure in Khartoum would provide a more holistic view of the issue. Finally, the study should consider recommendations for public health interventions on the basis of its findings. Suggestions for improving food safety practices, enhancing community awareness about food-borne illnesses, and implementing regular surveillance programs.

**Study limitations**

The study’s limitations include its focus on patients from Khartoum State, potentially limiting its generalizability across Sudan. Additionally, the sample size is small.

**CONCLUSIONS**

In conclusion, this study contributes to the understanding of *E. coli* O157 in Khartoum State by employing robust molecular techniques to detect this significant pathogen. Its strengths lie in addressing a regional gap in research and utilizing effective identification methods. However, there are several areas for improvement, including the need for a larger sample size, more detailed methodology, and a comprehensive discussion of the findings’ implications. Fu-

ture research can better inform public health strategies aimed at preventing food-borne illnesses and protecting vulnerable populations in the region.

**LIST OF ABBREVIATIONS**

- CDC: Central Disease Control
- E. coli* : *Escherichia coli*
- EAEC: Enteraggregative *E. coli*
- EHEC: enterohemorrhagic *E. coli* ()
- EIEC: Enteroinvasive *E. coli*
- EPEC: Enteropathogenic *E. coli*
- ETEC: Enterotoxigenic *E. coli*
- HUS: Hemolytic uremic syndrome
- KIA: Kligler iron agar
- PCR: Polymerase chain reaction
- SPSS: Statistical Packages for Social Sciences
- STEC: Shiga toxin-producing *E. coli*

**DECLARATIONS**

**Ethics approval and consent to participate**

Ethical approval was obtained from the Ministry of Health Ethical Research Committee in accordance with the Declaration of Helsinki Principles, and the agreement was taken from the Dentistry Hospital Administration before sample and data collection. The patient’s information was highly secure and not used for purposes other than scientific inquiry. The aims and objectives of the study, along with its procedure, methods, risks and benefits, were explained to each participant in easily understandable local language, and written informed consent was obtained from each patient .

**Ethical clearance code number**

MH-RES/17-022-11

**Date:** 22/12/2022

**CONSENT FOR PUBLICATION**

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data sets used and/or analyzed during the current study are available from the corresponding author upon reasonable request .

## COMPETING INTERESTS

The authors declare that they have no competing interests .

## FUNDING

Not applicable.

## AUTHORS' CONTRIBUTIONS

SAM and SMS conceived the design and carried out the experiments. AAI obtained, analyzed and interpreted the data. RAA, SAM and SMS wrote and revised the manuscript. AAI and RAA provided financial support for all the experiments. All the authors critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript .

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## REFERENCES

- Liu C, Hauk G, Yan Q, Berger JM. Structure of Escherichia coli exonuclease VII. *Proc Natl Acad Sci U S A*. 2024 Jan 30;121(5): e2319644121;PMID: 38271335. Available from: <https://doi.org/10.1073/pnas.2319644121>.
- Oliveira Alves N, Dalmasso G, Nikitina D, Vaysse A, Ruez R, Ledoux L, Pedron T, Bergsten E, Boulard O, Autier L, Allam S, Motreff L, Sauvanet P, Letourneur D, Kashyap P, Gagnière J, Pezet D, Godfraind C, Salzet M, Lemichez E, Bonnet M, Najjar I, Malabat C, Monot M, Mestivier D, Barnich N, Yadav P, Fournier I, Kennedy S, Mettouchi A, Bonnet R, Sobhani I, Chamailard M. The colibactin-producing Escherichia coli alters the tumor microenvironment to immunosuppressive lipid overload facilitating colorectal cancer progression and chemoresistance. *Gut Microbes*. 2024 Jan-Dec;16(1):2320291;PMID: 38417029. Available from: <https://doi.org/10.1080/19490976.2024.2320291>.
- Tian R, Rehm FBH, Czernecki D, Gu Y, Zürcher JF, Liu KC, Chin JW. Establishing a synthetic orthogonal replication system enables accelerated evolution in E. coli. *Science*. 2024 Jan 26;383(6681):421-426;PMID: 38271510. Available from: <https://doi.org/10.1126/science.adk1281>.
- L A LA, Waturangi DE. Application of BI-EHEC and BI-EPEC bacteriophages to control enterohemorrhagic and enteropathogenic escherichia coli on various food surfaces. *BMC Res Notes*. 2023 Jun 13;16(1):102;PMID: 37312167. Available from: <https://doi.org/10.1186/s13104-023-06371-6>.
- Riley LW. Distinguishing Pathovars from Nonpathovars: Escherichia coli. *Microbiol Spectr*. 2020 Dec;8(4): 10.1128/microbiolspec.ame-0014-2020;PMID: 33385193. Available from: <https://doi.org/10.1128/microbiolspec.AME-0014-2020>.
- Liu B, Furevi A, Perepelov AV, Guo X, Cao H, Wang Q, Reeves PR, Knirel YA, Wang L, Widmalm G. Structure and genetics of Escherichia coli O antigens. *FEMS Microbiol Rev*. 2020 Nov 24;44(6):655-683;PMID: 31778182. Available from: <https://doi.org/10.1093/femsre/fuz028>.
- Gelalcha BD, Brown SM, Crocker HE, Agga GE, Kerro Deogo O. Regulatory Mechanisms of Virulence Genes in Enterohemorrhagic Escherichia coli. *Foodborne Pathog Dis*. 2022 Sep;19(9):598-612;PMID: 35921067. Available from: <https://doi.org/10.1089/fpd.2021.0103>.
- Penzel A, Schützler K, Dröge J, Mellmann A, Ehrlich R, Engelmann I, Braun SD, Schleenvoigt BT, Löffler B, Rödel J. Rapid culture-based identification of Shiga toxin-producing Escherichia coli and Shigella spp./Enteroinvasive E. coli using the eazyplex® EHEC complete assay. *Eur J Clin Microbiol Infect Dis*. 2020 Jan;39(1):151-158;PMID: 31529306. Available from: <https://doi.org/10.1007/s10096-019-03704-x>.
- Graf F, Zehentner B, Fellner L, Scherer S, Neuhaus K. Three Novel Antisense Overlapping Genes in E. coli O157:H7 EDL933. *Microbiol Spectr*. 2023 Feb 14;11(1): e0235122;PMID: 36533921. Available from: <https://doi.org/10.1128/spectrum.02351-22>.
- Oliveira Souza SM, de Alencar ER, Ribeiro JL, de Aguiar Ferreira M. Inactivation of Escherichia coli O157:H7 by ozone in different substrates. *Braz J Microbiol*. 2019 Jan;50(1):247-253;PMID: 30637637. Available from: <https://doi.org/10.1007/s42770-018-0025-2>.
- Li Y, Liu H, Huang H, Deng J, Fang L, Luo J, Zhang S, Huang J, Liang W, Zheng J. A sensitive electrochemical strategy via multiple amplification reactions for the detection of E. coli O157: H7. *Biosens Bioelectron*. 2020 Jan 1; 147:111752;PMID: 31630033. Available from: <https://doi.org/10.1016/j.bios.2019.111752>.
- Sheele JM, Mead-Harvey C, Hodgson N. Clue Cells on Vaginal Wet Preparation Are Not Associated with Urinary Tract Infections or Positive Urine Cultures. *West J Emerg Med*. 2022 Jun 18;23(4):468-472;PMID: 35980405. Available from: <https://doi.org/10.5811/westjem.2022.2.55000>.
- Hasan MR, Suleiman M, Ilagan E, Crouch N, Lopez AP, Thomas E, Tang P. Growth of Clinically Important Gram-Negative Bacteria on MacConkey Agar under Aerobic versus CO2-Enriched Environment. *J Clin Microbiol*. 2019 Nov 22;57(12): e01441-19;PMID: 31597747. Available from: <https://doi.org/10.1128/JCM.01441-19>.
- Jacob ME, Keelara S, Aidara-Kane A, Matheu Alvarez JR, Fedorka-Cray PJ. Optimizing a Screening Protocol for Potential Extended-Spectrum  $\beta$ -Lactamase Escherichia coli on MacConkey Agar for Use in a Global Surveillance Program. *J Clin Microbiol*. 2020 Aug 24;58(9): e01039-19;PMID: 32434784. Available from: <https://doi.org/10.1128/JCM.01039-19>.
- Li H, Li L, Chi Y, Tian Q, Zhou T, Han C, Zhu Y, Zhou Y. Development of a standardized Gram stain procedure for bacteria and inflammatory cells using an automated staining instrument. *Microbiologyopen*. 2020 Sep;9(9): e1099;PMID: 32592452. Available from: <https://doi.org/10.1002/mbo3.1099>.
- Wu T, Wilhelm MJ, Li Y, Ma J, Dai HL. Indole Facilitates Antimicrobial Uptake in Bacteria. *ACS Infect Dis*. 2022 Jun 10;8(6):1124-1133;PMID: 35297612. Available from: <https://doi.org/10.1021/acscinf.1c00618>.
- Eckl DB, Landgraf N, Hoffmann AK, Eichner A, Huber H, Bäuml W. Photodynamic Inactivation of Bacteria in Ionic Environments Using the Photosensitizer SAPYR and the Chelator Citrate. *Photochem Photobiol*. 2023 Mar;99(2):716-731;PMID: 36004389. Available from: <https://doi.org/10.1111/php.13701>.
- Ryvchin R, Dubinsky V, Rabinowitz K, Wasserberg N, Dotan I, Gophna U. Alteration in Urease-producing Bacteria in the Gut Microbiomes of Patients with Inflammatory Bowel Diseases. *J Crohns Colitis*. 2021 Dec 18;15(12):2066-2077;PMID: 34111242. Available from: <https://doi.org/10.1093/ecco-ijc/jjab101>.
- Partridge JD, Harshey RM. Swarming Motility Assays in Salmonella. *Methods Mol Biol*. 2023; 2646:147-158;PMID: 36842113. Available from: [https://doi.org/10.1007/978-1-0716-3060-0\\_13](https://doi.org/10.1007/978-1-0716-3060-0_13).

20. Nakamura S. [Mechanism of bacterial motility]. *Nihon Saikingaku Zasshi*. 2019;74(2):157-165. Japanese; PMID: 31474648. Available from: <https://doi.org/10.3412/jsb.74.157>.
21. Cheema AS, Stinson LF, Lai CT, Geddes DT, Payne MS. DNA extraction method influences human milk bacterial profiles. *J Appl Microbiol*. 2021 Jan;130(1):142-156; PMID: 32654260. Available from: <https://doi.org/10.1111/jam.14780>.
22. Olcu M, Atalay MA, Percin Renders D. Development of multiplex PCR panel for detection of anaerobic bacteria in clinical samples. *Anaerobe*. 2022 Aug; 76:102611; PMID: 35820595. Available from: <https://doi.org/10.1016/j.anaerobe.2022.102611>.
23. Dai J, Huang C, Zhang H, Samuel R, Li Y, Jayaraman A, de Figueiredo P, Han A. Microfluidic Dielectrophoretic Method Enables On-Demand Spatial Arrangement of Bacteria-Encapsulated Agarose Gel Microparticles. *Anal Chem*. 2022 Sep 27;94(38):13197-13204; PMID: 36108268. Available from: <https://doi.org/10.1021/acs.analchem.2c02724>.
24. Neoh HM, Tan XE, Sapri HF, Tan TL. Pulsed-field gel electrophoresis (PFGE): A review of the "gold standard" for bacteria typing and current alternatives. *Infect Genet Evol*. 2019 Oct; 74:103935; PMID: 31233781. Available from: <https://doi.org/10.1016/j.meegid.2019.103935>.
25. Obradovic M, Wilson HL. Two-Dimensional Electrophoresis Coupled with Western Blot as a Method to Detect Potential Neutralizing Antibody Targets from Gram-Negative Intracellular Bacteria. *Methods Mol Biol*. 2022; 2414:63-73; PMID: 34784032. Available from: [https://doi.org/10.1007/978-1-0716-1900-1\\_5](https://doi.org/10.1007/978-1-0716-1900-1_5).
26. Wasiewska LA, Juska VB, Seymour I, Burgess CM, Duffy G, O'Riordan A. Electrochemical nucleic acid-based sensors for detection of Escherichia coli and Shiga toxin-producing E. Coli-Review of the recent developments. *Compr Rev Food Sci Food Saf*. 2023 May;22(3):1839-1863; PMID: 36871164. Available from: <https://doi.org/10.1111/1541-4337.13132>.
27. Liu Y, Thaker H, Wang C, Xu Z, Dong M. Diagnosis and Treatment for Shiga Toxin-Producing Escherichia coli Associated Hemolytic Uremic Syndrome. *Toxins (Basel)*. 2022 Dec 23;15(1):10; PMID: 36668830. Available from: <https://doi.org/10.3390/toxins15010010>.
28. Böckenhauer J, Schild R, Kemper MJ, Henne T, Stein MV, Oh J, Loos S. Volume expansion mitigates Shiga toxin-producing E. coli-hemolytic uremic syndrome in children. *Pediatr Nephrol*. 2024 Jun;39(6):1901-1907; PMID: 38240870. Available from: <https://doi.org/10.1007/s00467-023-06276-3>.
29. Pugh HL, Connor C, Siasat P, McNally A, Blair JMA. E. coli ST11 (O157:H7) does not encode a functional AcrF efflux pump. *Microbiology (Reading)*. 2023 Apr;169(4):001324; PMID: 37074150. Available from: <https://doi.org/10.1099/mic.0.001324>.
30. Safarirad M, Shahdadi M, Berizi E, Mazloomi SM, Hosseinzadeh S, Montaseri M, Derakhshan Z. A systematic review and modeling of the effect of bacteriophages on E. coli O157:H7 reduction in vegetables. *Heliyon*. 2023 Nov 28;9(12): e22961; PMID: 38058426. Available from: <https://doi.org/10.1016/j.heliyon.2023.e22961>.
31. Abebe E, Gugsu G, Ahmed M, Awol N, Tefera Y, Abegaz S, Sisay T. Occurrence and antimicrobial resistance pattern of E. coli O157:H7 isolated from foods of Bovine origin in Dessie and Kombolcha towns, Ethiopia. *PLoS Negl Trop Dis*. 2023 Jan 27;17(1): e0010706; PMID: 36706075. Available from: <https://doi.org/10.1371/journal.pntd.0010706>.
32. Abianeh HS, Nazarian S, Sadeghi D, Razgi ASH, Samarini MZ. PLGA nanoparticles containing Intimin-Flagellin fusion protein for E. coli O157:H7 nanovaccine. *J Immunol Methods*. 2023 Sep; 520:113517; PMID: 37385434. Available from: <https://doi.org/10.1016/j.jim.2023.113517>.
33. Wang F, Sun H, Kang C, Yan J, Chen J, Feng X, Yang B. Genomic island-encoded regulatory proteins in enterohemorrhagic Escherichia coli O157:H7. *Virulence*. 2024 Dec;15(1):2313407; PMID: 38357901. Available from: <https://doi.org/10.1080/21505594.2024.2313407>.
34. Getaneh DK, Hordofa LO, Ayana DA, Tessema TS, Regassa LD. Prevalence of Escherichia coli O157:H7 and associated factors in underfive children in Eastern Ethiopia. *PLoS One*. 2021 Jan 28;16(1): e0246024; PMID: 33508023. Available from: <https://doi.org/10.1371/journal.pone.0246024>.
35. Peroutka-Bigus N, Nielsen DW, Trachsel J, Mou KT, Sharma VK, Kudva IT, Loving CL. Phenotypic and genomic comparison of three human outbreak and one cattle-associated Shiga toxin-producing Escherichia coli O157:H7. *Microbiol Spectr*. 2024 Oct 3;12(10): e0414023; PMID: 39254337. Available from: <https://doi.org/10.1128/spectrum.04140-23>.
36. Jenkins C, Dallman TJ, Grant KA. Impact of whole-genome sequencing on the investigation of food-borne outbreaks of Shiga toxin-producing Escherichia coli serogroup O157:H7, England, 2013 to 2017. *Euro Surveill*. 2019 Jan;24(4):1800346; Available from: <https://doi.org/10.2807/1560-7917.ES.2019.24.4.1800346>.