Science Journal of University of Zakho
DOI: https://doi.org/10.25271/sjuoz.2026.14.3.1642

Original Article

IDENTIFICATION AND MOLECULAR CHARACTERIZATION OF *Escherichia Coli* O157:H7 ISOLATED FROM THE GREAT ZAB RIVER IN ERBIL AND INHIBITORY EFFECT OF SILVER NANOPARTICLES

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Received
June 22, 2025

Accepted
August 21, 2025

Published
July 2, 2026

ABSTRACT

Microbial pathogens may be introduced into surface water sources through agricultural runoff, sewage discharge, or stormwater, and such contamination poses a significant threat to public health. *Escherichia coli* is a bacterial species that inhabits several environments, including the gastrointestinal tract of humans and warm-blooded animals. *E. coli*, especially the O157:H7 serotype, is an important indicator species for fecal contamination and possible pathogenicity. The objective of this study was to recover *E. coli* O157:H7 from the Great Zab River in Erbil and to investigate its virulence factors and the susceptibility of isolates to silver nanoparticles (AgNPs). 150 water samples, from different sources, were bacteriologically analyzed using Sorbitol MacConkey agar. The presumptive isolates were identified by means of biochemical tests and the VITEK 2 system. Three (2%) *E. coli* O157:H7 were isolated from the samples. Sensitivity of isolates to various AgNP concentrations was demonstrated by antimicrobial assays. One isolate was confirmed by sequencing 16S rRNA gene. PCR detected the presence of the *eae* gene in all isolates, the absence of shiga toxins, and in one isolate, the *fliC* gene was observed. These results demonstrate the pathogenic E. coli O157:H7 in the river and the potential application of AgNPs as an antimicrobial in such water bodies.

Keywords: Virulence gene, *Escherichia coli* O157:H7, AgNP, Shiga toxin, 16S rRNA

1. INTRODUCTION

Water used for recreation and irrigation is now increasingly exposed to drinking water sources, which poses a risk to public health. Fecal-borne pathogens are commonly found in rivers, and the fecal contamination is effectively checked by the marker *Escherichia coli* (*E. coli*) as a major biological indicator (Korajkic *et al.*, 2018). The Great Zab River (GZR) is the major northern tributary of the Tigris River; it originates in its basin in Turkey and flows into the Kurdistan Region of Iraq. It is one of the chief sources of water for irrigation and domestic purposes, but unfortunately, the microbiological quality of this source of water has been less studied (Sharef & Dara, 2021). Surface water may contain fecal waste from different sources, such as domestic/wild animal feces, stormwater runoff, livestock waste, and untreated sewage (Menéndez-Serra *et al.*, 2019).

The commonly accepted fecal indicator is the *E. coli* bacterium, one of the Enterobacteriaceae family. It is a Gram-negative, rod-shaped bacterium that is non-sporulating and maybe motile or non-motile, since it is part of the intestinal tract of warm-blooded mammals and can indicate recent fecal pollution (Erjavec, 2019; Hasan *et al.*, 2020). That is why, *E. coli* was used as the target subject in the study. Whereas most strains of *E. coli* are commensal, and some are even beneficial, like those that synthesize vitamins (Gambushe *et al.*, 2022), pathogenic strains, in particular Shiga toxin-producing *E. coli* (STEC), cause serious illnesses such as hemorrhagic colitis and hemolytic uremic syndrome (HUS) (Saeed & Ibrahim, 2013; Paletta *et al.*, 2020; Garrine *et al.*, 2020).STEC pathogenicity is associated with several virulence factors, including Shiga toxins (*stx*), intimin (encoded by the eae gene), and flagellin (*fliC*) (Beata *et al.*, 2021; Yang *et al.*, 2018). Additionally, other factors contribute to bacterial motility, host adhesion, colonization, and invasion. Shiga toxins act on endothelial cells and can cause severe consequences like thrombotic thrombocytopenic purpura (TTP) (Oluwarinde *et al.*, 2023).

Expression of the *eae* gene in the locus of enterocyte effacement (LEE) is essential for pathogen induction of attaching and effacing (A/E) lesions on host epithelial cells (Beata *et al.*, 2021). Alongside virulence, antimicrobial resistance (AMR) of pathogenic *E. coli* has raised worldwide attention. Chromosomal and conjugable plasmid-carried resistance genes are easily transferable between bacterial species. This also facilitates the spread of resistance in water environments, as antibiotic resistance from some organisms gets transferred to others. It exhibits horizontal gene transfer and does not permit an antibiotic response for any specific pathogen (Basavaraju & Gunashree, 2022).

In this regard, nanotechnology is one of the most promising strategies to reduce AMR. In all nanomaterials, the antibacterial effect of silver nanoparticles (AgNPs) is the most powerful at very low concentrations. Thanks to their high surface area and volume and the capacity to disturb the bacterial structures (Oves *et al.*, 2018; Urnukhsaikhan *et al.*, 2021). These NPs (15,000–20,000 atoms of silver) are spherical and have a size commonly of 100 nm. That is an effective and low-cost large-scale technology for water treatment (Abdelghany *et al.*, 2018; Samuel *et al.*, 2020).

The objectives of the study were to find out the isolation and molecular identification of *E. coli* O157:H7 isolated from the Great Zab River, and to detect the virulent genes (*stx*, *eae*, *fliC*), as well as to evaluate the effects of silver nanoparticles (AgNPs) against the isolated strain.

Access this article online

DOI:https://doi.org/10.25271/sjuoz.2026.14.3.1642

Science Journal of University of Zakho
Vol. 14, No. 03, pp. 396-402, July -2026

Printed ISSN 2663-628X;
Electronic ISSN 2663-6298

2. MATERIALS AND METHODS

Water Sample and Collection Area:

For microbiological contamination, 150 samples of water were obtained from the Great Zab River from the Qandil water treatment plant to Kalak town. Samples were collected at intervals of 500 meters to 1 kilometer from September 2024 to April 2025. Samples were collected in 0.5-liter sterile glass vials and thereafter dispatched in a chilled box to the Department of Biology Research Laboratory at Salahaddin University within 1-2 hours.

Isolation and Identification of *E. coli* O157:H7:

The preliminary Most Probable Number (MPN) method was first applied to the processed water samples to estimate bacterial numbers. The presence–absence test was conducted based on serial dilutions on MacConkey broth (Microxpress, India) and incubated at 37°C for 24–48 hours (Lu *et al.*, 2024). Turbid tubes were streaked on MacConkey agar and Eosin Methylene Blue (EMB) agar (Scharlau, Spain; Neogen, UK) and incubated at 37°C for 24 hours. Colonies that were pink on MacConkey or that possessed a green metallic sheen on EMB were presumptive *E. coli*. Colonies were confirmed by biochemical tests, including oxidase and slants of triple sugar iron (TSI). Then, the colonies were subcultured in Sorbitol MacConkey agar (SMAC) (Neogen, USA) and incubated at 37°C for 24 hours. Colonies that did not ferment sorbitol (i.e., colorless or pale) and had a smooth, round appearance were tentatively identified as *E. coli* O157:H7. Definitive identification was performed using the VITEK 2 system with the gram-negative ID cards following the manufacturer's instructions (BioMérieux, France).

Bacterial Genomic DNA Extraction:

The *E. coli* O157:H7 strain was initially cultivated in nutrient broth (LabM, UK) and incubated at 37°C for 24 hours. Genomic DNA was subsequently isolated using a commercial extraction kit (Jena Bioscience, Germany), adhering to the manufacturer's specifications. The extracted DNA concentration and purity were measured using a NanoDrop spectrophotometer (Implen, Germany). DNA concentrations varied from 175.56 ng/μl to 568.12 ng/μl, with purity ratios (A260/A280) varying from 1.88 to 2.01.

PCR Amplification of 16S rRNA Gene for *E. coli* O157:H7 Identification:

The traditional PCR was used to confirm bacterial identification, including *E. coli*. The PCR premix for the 16S rRNA gene consisted of a total volume of 25μl, 12.5 μl Gotaq Green Master Mix (Promega/USA), 3μl of genomic DNA, 1 μl of each primer (forward primer 5'-AGAGTTTGATCCTGGCTCAG-3'; reverse primer 5'-TACGGTTACCTTGTTACGACTT-3') and the volume completed with 7.5μl nuclease free water. The primers were a modification of Lane (1991). The amplifications were performed in an automatic thermocycler as follows: an initial denaturation of 5 minutes at 95°C; 30 cycles of denaturation, 30 seconds at 95°C; annealing at 60°C, 30 seconds; extension at 72°C, 1 minute; and final extension for 7 minutes at 72°C. The target size of PCR products was confirmed by using 2% agarose gel electrophoresis in 1XTBE buffer (Promega/USA), and PCR amplicons of isolates were sent to Macrogen/South Korea for sequencing. Sequence alignment was performed for the targeted region. The sequence analysis was done using MEGA 11 and NCBI-BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The PCR products were sequenced on an ABI 3730 DNA apparatus (Applied Biosystems).

PCR Detection of *stx*1, *stx*2, *eae*, and *fliC* Virulence Genes:

To detect the virulence genes, for *stx*1 and *stx*2, we used a multiplex PCR. Master mix was prepared in a total volume of 25 μl, 12.5 μl Gotaq Green Master Mix, 3μl of genomic DNA, 1.25 μl each forward (*stx*1 and *stx*2) and reverse (*stx*1 and *stx*2) primers (Table 1), and 4.5μl μl of free water. The PCR was done via program cycle. The initial denaturation occurs for 5 minute at 95 °C, followed by 35 PCR cycles that consist of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 35 seconds, extension at 72 °C for 1 minute, and final extension at 72 °C for 7 minute

The *eae* gene was amplified by Uniplex PCR. The premix consisted of a total volume of 25μl, 12.5 μl Gotaq Green Master Mix, 3μl of genomic DNA, 1.5 μl of each primer, and the volume was completed with 6.5μl μl of nuclease-free water.

The PCR profile file was performed as follows: Initial denaturation at 95°C for 5 minute, followed by 35 cycles of 95°C for 30 seconds, 59°C for 30 seconds, and 72°C for 1 minute; and a final extension at 72°C for 10 minute.

On the other hand, the target *fliC* was detected via PCR, the ready-to-use master was prepared by mixing 12.5 μl GoTaq Green Master Mix, 3μl of genomic DNA, 1.5 μl of each primer, and the volume was completed to 25 μl by adding 6.5μl μl of nuclease-free water. The targeted DNA was amplified using a PCR file, Initial denaturation at 95°C for 5 minute, followed by 30 cycles of 95°C for 30 seconds, 63°C for 30 seconds, and 72°C for 60 seconds; and a final extension at 72°C for 5 minute.

Agar Well Diffusion Technique:

The antibacterial efficacy of the AgNPs against *E. coli* O157:H7 was evaluated using the agar well diffusion assay technique. Semi-solid Mueller-Hinton agar (Scharlau, Spain) was dispensed onto the petri dishes (Citotest, China). The bacteria were cultivated in nutrient broth for 24 hours. Thereafter, they were transferred onto the surface of solid medium Mueller-Hinton agar using a sterile swab, and cultured with suspensions of test bacteria at a concentration of 1.5×10^6 CFU/mL. A concentration of 10 mg/mL of silver nanoparticles was introduced into wells created using sterile pipette tips and subsequently positioned on the surface of inoculated agar plates. Plates were incubated for 24 hours at 37 degrees Celsius. The diameter of the inhibitory zone in millimeters quantified the antimicrobial activity.

Table 1: Details for the primers that are used for the amplification of *stx1*, *stx2*, *eae*, and *fliC* genes in this study

Target gene	Primer Sequence	Amplicon Size(bp)	Reference
Stx1	F: 5'-CAACACTGGATGATCTCAG-3' R: 5'-CCCCCTCAACTGCTAATA-3'	349	(Hessain *et al.*, 2015)
Stx2	F: 5'-ATCAGTCGTCACTCACTGGT-3' R: 5'-CTGCTGCTGTCACAGTGACAAA-3'	110	(Hessain *et al.*, 2015)
eae	F: 5' GTTCACTGGACTTCTTATTACCG 3' R: 5' ATCGTCACCAGAGGAATC 3'	484	(Beneduce *et al.*, 2008)
fliC	F: 5' GCGCTGTCGAGTTCTATCGAGC 3' R: 5' CAACGGTGACTTTATCGCCATTCC 3'	625	(Kadhim & Kareem, 2021)

bp, base pair

Minimum Inhibitory Concentration Method:

This research utilized commercially accessible silver (Ag) nanoparticles/nanopowder sourced from Skyspring Nanomaterials (USA). The nanoparticles exhibited a purity of 99.9% (trace metal base) with an average particle size between 50 and 60 nanometers. The substance appeared as a dark grey powder. The particles demonstrated a spherical morphology, having a certain surface area of about 12 m^2/g. The bulk density was recorded at 0.35 g/cm^3, while the real density was 10.5 g/cm^3. Nanopowders dissolve in various solvents, including water, ethanol, and isopropanol, yielding practical suspensions (Abou El-Nour *et al.*, 2010; Dankovich & Gray, 2011). A 96-well polystyrene microtiter plate test was employed to assess the antibacterial efficacy of AgNPs. The stock solution of AgNPs was created by dissolving them in deionized distilled water (DDW) at a concentration of 50 mg/ml and sonicating for 15 minutes using an ultrasonic processor (Vevor, China) to ensure uniform dispersion. The liquid was then vortexed for an additional 10 minutes using equipment from Stuart Scientific (UK). Upon confirming complete homogenization, it was added to the suspension. A range of AgNP concentrations (50, 40, 30, 20, 10, 5, 2. 2.5, and 1. 25 mL) was prepared by employing the conventional dilution formula ($C_1V_1 = C_2V_2$), mixing the stock solution with nutrient broth (LabM, UK). Subsequently, 200 μL of each AgNP dilution was introduced into separate microtiter plate wells, subsequently supplemented by 10 μL of a bacterial suspension that had been previously subcultured in nutrient broth. The plates were subsequently incubated at 37 °C for 24 hours. The negative wells contained only nutrient broth for control purposes, while the positive controls included broth and microorganisms without AgNPs. To prevent evaporation during incubation, the plates were covered with lids. Before incubation, the absorbance of each well was quantified at 630 nm with an ELISA reader (BioTek, USA) to establish the baseline optical measurement of the AgNPs. Following the 24-hour incubation period, a subsequent absorbance measurement was conducted to determine any increase in turbidity attributable to bacterial proliferation. The net bacterial growth in each well was accurately estimated by subtracting the initial absorbance from the final measurement.

3. RESULTS

Isolation and Identification of *E. coli* O157:H7 in Raw Water:

One hundred fifty water samples were obtained from various locations along the Great Zab River, and only three O157:H7 serotypes of *E. coli* were isolated: one sample near Dalare village, and two samples near Gopal Bridge. The recovered bacteria were initially described according to their colony shape on agar media and biochemical testing. Nevertheless, owing to the resemblance of these characteristics across many Enterobacteriaceae, the VITEK 2 system methodologies were utilized for precise identification. On MacConkey agar, the colonies of *E. coli* grew as smooth, shiny, and pink in color due to the fermentation of lactose. *E. coli* serotype O157:H7, unlike other microbiota, the colonies are colorless colonies because they cannot break down sorbitol. Therefore, sorbitol MacConkey agar is used rather than lactose as a selected medium for the identification of *E. coli* serotype O157:H7. In biochemical tests, oxidase was negative, meaning it does not produce the cytochrome c enzyme. The Triple Sugar Iron (TSI) agar test showed acid/acid gas production (A/A, G), changing color from red to yellow, meaning fermentation of glucose, lactose, and bubbles or cracks on the bottom indicate gas production. The results of VITEK 2 confirmed *E. coli* serotype O157:H7 with high probability.

PCR confirmation results for O157:H7 strains:

A total of 3 samples (2%) were isolated from raw water, confirmed through the amplification of the 16s rRNA gene via universal primers (27F and 1492R), followed by Agarose Gel Electrophoresis of the PCR results for the bacterial identification, including *E. coli. Serotype* O157:H7. The target size of PCR products was confirmed by using 2% agarose gel electrophoresis in 1XTBE buffer (Promega/USA), and PCR products of isolates were sent to Macrogen/South Korea for sequencing. The sequence identified for one isolate was submitted to NCBI GenBank under accession number PV746487. A second sequence, identified as *E. coli*, was submitted under accession number PV746488 (Figure 1). The phylogenetic tree for the 16S rRNA gene sequences is presented in Figure 2. The sequence analysis was done using MEGA 11 and NCBI- BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The PCR products were sequenced on an ABI 3730 DNA apparatus (Applied Biosystems).

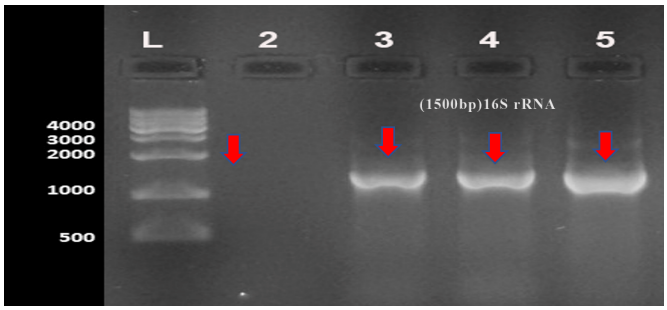

Figure 1: Agarose Gel Electrophoresis of the PCR products for 16Sr RNA. Lane 1: Ladder of 1kb (1 kilobase pair), Lane 2: Negative control, Lane 3-5 (1500bp)16Sr RNA for identification of E. coli

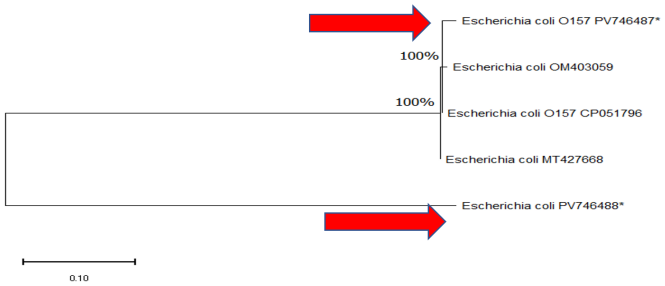

Figure 2: Agarose Gel Electrophoresis of the PCR products for *eae* gene. Lane 1 (L): Ladder of 100bp, Lane 2(N): Negative control, Lane 3-5 (484bp) *eae* gene in *E coli* .(B) Agarose Gel Electrophoresis of the PCR product for fliCh7 gene. Lane 1 (L): Ladder of 100bp, Lane 2(N): Negative control, Lane 3: Amplification of fliCh7 gene (625bp).

PCR Amplification of Virulence Genes:

All isolated samples lacked *stx1* and s*tx2*. In this current study, the identification and detection of two other genes revealed that the total isolated samples contained the *eae* gene, as shown in Figure 3(A). Additionally, one isolated sample possesses the *fliC* gene, as shown in Figure 3(B).

Minimum Inhibitory Concentration of Silver Nanoparticles:

The antibacterial effect of synthesized AgNPs was evaluated against *E. coli* serotype O157:H7 employing the broth microdilution technique to ascertain the MIC. After incubation, the MIC of AgNP against *E. coli* O157:H7 was 2.5-10 µg/mL exhibiting strong antibacterial activity.

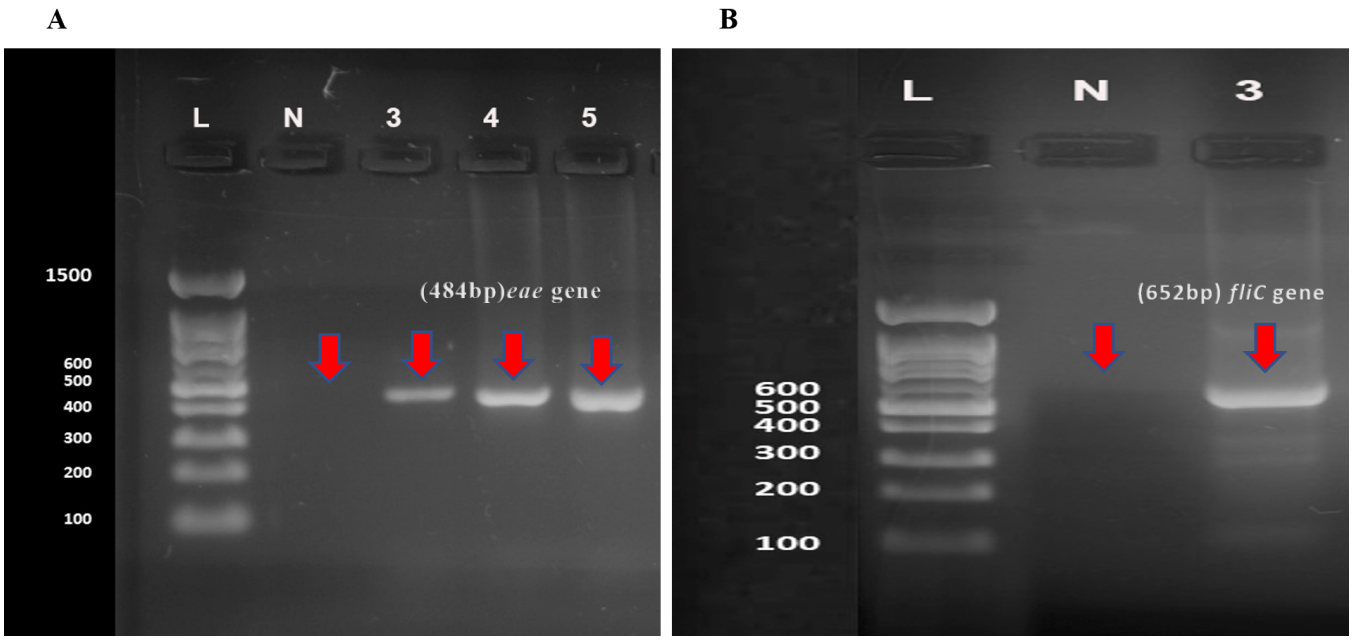

Figure 3: (A) Agarose Gel Electrophoresis of the PCR products for *eae* gene. Lane 1 (L): Ladder of 100bp, Lane 2(N): Negative control, Lane 3-5 (484bp) *eae* gene in *E coli*. (B) Agarose Gel Electrophoresis of the PCR product for fliCh7 gene. Lane 1 (L): Ladder of 100bp, Lane 2(N): Negative control, Lane 3: Amplification of fliCh7 gene (625bp).

Agar Well Diffusion Technique:

The results showed that silver nanoparticles have an antimicrobial effect against *E. coli* serotype O157:H7. A concentration of 10 mg/mL of silver nanoparticles resulted in an inhibition zone 3 to 6 mm in diameter, as indicated in Figure 4.

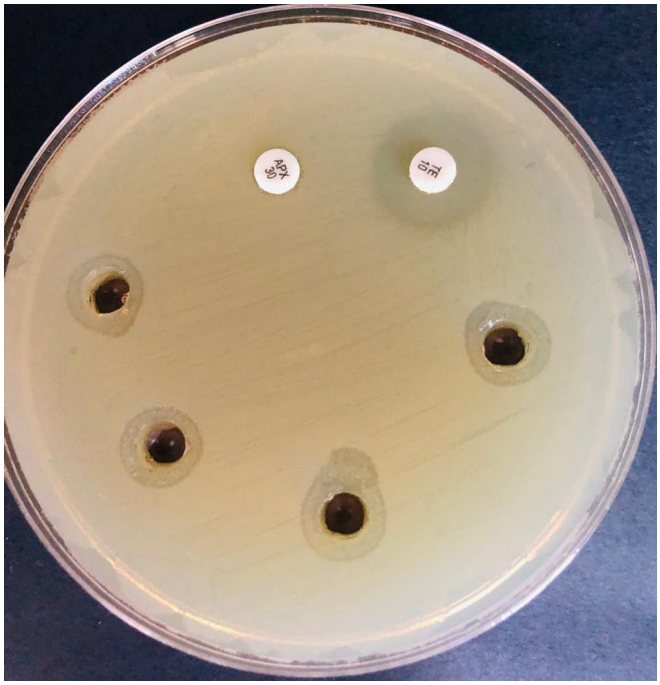

Figure 4: Antibacterial effect of AgNPs against E. coli serotype O157:H7 at a concentration of 10 mg/mL with a 3-6 mm inhibition zone and negative control, Ampicillin, and Tetracycline Antibiotic disc.

4. DISCUSSION

Rivers are critically important to communities all over the world. However, the uncontrolled pollution of water and the resulting decrease in the quality of freshwater also seriously threaten human well-being and sustainable development (Lu *et al*., 2015). The presence of *E. coli* O157:H7 in river water is a major public health issue, as this enteric pathogen is the leading pathogenic agent of waterborne diseases. The river water contamination commonly includes raw sewage, together with agricultural runoff and industrial waste discharge. The identification of *E. coli* O157:H7 in river water needs to be both fast and precise because it helps prevent outbreaks and protect drinking water sources (Razmi *et al.*, 2022).

In the current study, pooled water samples were enriched to reflect the true status of microbial contamination across the different locations of the Great Zab River. A final pool of 150 samples was gathered. Chigor *et al*. (2010) found that 2.1% of *E. coli* O157:H7 was present in river water. The researchers detected elevated fecal coliform levels during the dry season because water levels were low and the sun heated the water intensely, while pollution from septic tanks and abattoir effluents entered the water through seepage and runoff. The dry season temperatures reached high levels, which created a strong positive relationship with microbiological contamination, especially *E. coli* O157:H7.

Of all 150 samples tested in this study, VITEK 2 and conventional biochemical methods were used to detect *E. coli* O157:H7; only 3 (2.0%) samples were isolated. The finding are also intriguing, as the direct isolation of *E. coli* O157:H7 from river water in Iraq has not been frequently documented (Bisi-Johnson *et al*., 2023). For example, Alwash and Al-Rafyai (2019) isolated some *E. coli* strains from the Al-Hillah River in Iraq; however, the O157:H7 serotype was not reported.

One of the positive isolates was further identified by the 16S rRNA gene analysis. PCR products were sequenced (Macrogen, South Korea) and aligned with the reference sequences available in the NCBI database. It is a comprehensive and gene-based analysis like this that enables a more in-depth understanding of the pathogenicity of microorganisms and can provide important information for therapeutic and environmental health hazard research. Open access to such sequences may also help researchers to discover some diagnostic markers and therapeutic targets for bacterial infections.

Silver nanoparticles (AgNPs) have attracted significant research due to their antibacterial effects and ease of production (Fiorati *et al*., 2020). Their small size is an advantage for greater antimicrobial action than larger particles, since nanoparticles are large compared to microparticles, providing an increasing surface area and high Ag^{+} ion release (Reis *et al*., 2016; Fouda *et al*., 2019). The MICs for AgNPs in the present experiment varied from 2.5 to 10 mg/mL, and this is consistent with the report presented by Wang *et al*. (2022). AgNPs are probably effective due to bacterial cell wall penetration and subsequent interaction with intracellular structures.

*stx*1 and *stx*2 genes that encode Shiga toxins that cause HUS and hemorrhagic colitis are mostly associated with *E. coli* O157:H7, a widely recognized waterborne pathogenic group (El-Leithy *et al*., 2012). All isolates in our study do not contain *stx*1 or *stx*2 genes. The results are correlated with the findings of Hamner *et al*. (2007), who reported *E. coli* O157:H7 as negative for Shiga toxins in the water of the Ganges River. Similarly, Amézquita-López *et al*. (2024) found *E. coli*O157:H7 in river water from northwestern Mexico and were unable to detect *stx* genes. Variability in the detection rate of *stx* genes may be due to the spatial and seasonal components, as well as the sample size, variety of the matrix, and the detection method employed (Sthapit *et al*., 2022).

The *eae* gene, which codes for the intimin protein, was detected in all isolates in the current study. Intimin promotes bacterial adhesion to intestinal epithelial cells; it is also related to severe clinical manifestations like bloody diarrhea and HUS (Hua *et al*., 2020). A closer association is observed between certain *eae* serotypes and virulent *E. coli* serotypes such as O157:H7, O26:H11, O103:H2, O111:H8, and O145:H28 (Yang *et al*., 2020). As *eae* was constantly detected, these environmental isolates might be potentially pathogenic. Loukiadis *et al*. (2006) also detected stx-negative, *eae*-positive *E. coli* O157:H7 from French wastewater. Zhang *et al*. (2020) also presented such outcomes in the Mississippi River.

The flagellin is the structural protein of the H antigen, a component of flagella key for motility and host adhesion in some bacteria, which is encoded by the textitfliC gene. Motility is required for which has an important role in pathogenesis in the human intestines (Wolfson *et al*., 2020). Even the costly production of flagella is beneficial to bacteria that live within a host (Subramanian & Kearns, 2019). In the present study, only one isolate was found to be positive for the *fliC* gene. This result is consistent with El-Leithy *et al*. (2012), who were the first to find *fliC* in the Nile River in *E. coli* O157:H7 strains. *fliC* was also found by Olowe and Arogbodo (2022) in *E. coli* O157 isolates from different water sources.

5. CONCLUSION

The results of this investigation verify the existence of *E. coli* O157:H7 in the Great Zab River, which could represent a public health hazard due to water pollution. Even if the finding were only seen in a few isolates, the uniform presence of *eae* on all isolates indicates that they may have the capacity for intestinal colonization and virulence. None of the isolates harbored the *stx*1 and *stx*2 genes, and we may conclude that the strains studied were not Shiga-toxin producing.

A single isolate was found to harbor the *fliC* gene, suggesting diversity among virulence factors associated with the pathogen's motility. In addition, the tested *E. coli* O157:H7 strains were sensitive to silver nanoparticles, which suggests that they may be considered as a new, non-antibiotic intervention against water handling and food safety in the future. Nevertheless, additional research involving more samples, both in sample size and time season, is required to have a clearer picture of the prevalence and pathogenicity potential of *E. coli* O157:H7 in the region.

These results emphasize the need for water source surveillance and the application of molecular methods for detecting virulence markers in environmental isolates.

Acknowledgment:

The authors would like to express their sincere gratitude to Nanakaly Hospital for their support and cooperation during this study. Special thanks are extended to Prof. Dr. Akhtar Ahmad for his valuable guidance, support, and insightful contributions throughout the research.

Ethical Statment:

This work was implemented and accepted by the ethics committee of Science College at Salahaddin University – Erbil (No 45/287; date, August 5, 2024; Erbil, Iraq).

Conflict of Interests:

The authors declare no competing interests.

Funding:

This study was not supported by any outside sources.

Author Contributions:

A.J.A., and B.S.H. contributed equally to the conception and design of the study. A.J.A. performed sample collection. Both authors have read and agreed to the published version of the manuscript.

6. REFERENCES

Abdelghany, T. M., Al-Rajhi, A. M., Al Abboud, M. A., Alawlaqi, M. M., Ganash Magdah, A., Helmy, E. A., & Mabrouk, A. S. (2018). Recent advances in green synthesis of silver nanoparticles and their applications: about future directions. A review. *BioNanoScience*, 8, 5–16. DOI:https://doi.org/10.1007/s12668-017-0413-3

Abou El-Nour, K. M., Eftaiha, A. A., Al-Warthan, A., & Ammar, R. A. (2010). Synthesis and applications of silver nanoparticles. *Arabian Journal of Chemistry*, 3(3), 135–140. DOI:https://doi.org/10.1016/j.arabjc.2010.04.008

Alwash, M. S., & Al-Rafyai, H. M. (2019). Antibiotic resistance patterns of diverse Escherichia coli phylogenetic groups isolated from the Al-Hillah River in Babylon Province, Iraq. *The Scientific World Journal*, 2019, 5927059. DOI:https://doi.org/10.1155/2019/5927059

Amézquita-López, B. A., Soto-Beltrán, M., Lee, B. G., Bon-Haro, E. F., Lugo-Melchor, O. Y., & Quiñones, B. (2024). Virulence and antimicrobial resistance profiles of Shiga toxin-producing Escherichia coli from river water and farm animal feces near an agricultural region in northwestern Mexico. *Microbiology Research*, 15(1), 385–403. DOI:https://doi.org/10.3390/microbiolres15010026

Basavaraju, M., & Gunashree, B. (2022). Escherichia coli: An Overview of Main Characteristics. *In IntechOpen eBooks*. DOI:https://doi.org/10.5772/intechopen.105508

Beata, S., Michał, T., Mateusz, O., Urszula, W., Choroszy, M., Andrzej, T., & Piotr, D. (2021). Norepinephrine affects the interaction of adherent-invasive Escherichia coli with intestinal epithelial cells. *Virulence*, 12(1), 630–637. DOI:https://doi.org/10.1080/21505594.2021.1882780

Beneduce, L., Spano, G., Nabi, A. Q., Lamacchia, F., Massa, S., Aouni, R., & Hamama, A. (2008). Occurrence and characterization of Escherichia coli O157 and other serotypes in raw meat products in Morocco. *Journal of food protection*, 71(10), 2082–2086. DOI:https://doi.org/10.4315/0362-028X-71.10.2082

Bisi-Johnson, M. A., Adedeji, A. A., Sulaiman, A. A., Adefisoye, M. A., & Okoh, A. I. (2023). Isolation and genotypic characterization of extended-spectrum beta-lactamase-producing Escherichia

coli O157: H7 and Aeromonas hydrophila from selected freshwater sources in Southwest Nigeria. *Scientific reports*, 13(1), 10746. DOI:https://doi.org/10.1038/s41598-023-38014-y

Chigor, V. N., Umoh, V. J., & Smith, S. I. (2010). Occurrence of Escherichia coli O157 in a river used for fresh produce irrigation in Nigeria. *African Journal of Biotechnology*, 9(2). DOI:https://doi.org/10.5897/AJB09.149

Dankovich, T. A., & Gray, D. G. (2011). Bactericidal paper impregnated with silver nanoparticles for point-of-use water treatment. *Environmental science & technology*, 45(5), 1992–1998. DOI:https://doi.org/10.1021/es103302t

El-Leithy, M., El-Shatoury, E. H., El-Senousy, W., Abou-Zeid, M., & El-Taweel, G. E. (2012). Detection of six *E. coli* O157 virulence genes in water samples using multiplex PCR. *Egypt. J. Microbiol*, 47, 171–188. DOI:https://doi.org/10.21608/ejm.2012.259

Erjavec, M. S. (Ed.). (2019). *The Universe of Escherichia coli*. BoD–Books on Demand.

Fiorati, A., Bellingeri, A., Punta, C., Corsi, I., & Venditti, I. (2020). Silver nanoparticles for water pollution monitoring and treatments: ecosafety challenge and cellulose-based hybrids solution. *Polymers*, 12(8), 1635. DOI:https://doi.org/10.3390/polym12081635

Fouda, A., Abdel-Maksoud, G., Abdel-Rahman, M. A., Salem, S. S., Hassan, S. E. D., & El-Sadany, M. A. H. (2019). Eco-friendly approach utilizing green synthesized nanoparticles for paper conservation against microbes involved in biodeterioration of archaeological manuscript. *International Biodeterioration & Biodegradation*, 142, 160–169. DOI:https://doi.org/10.1016/j.ibiod.2019.05.012

Gambushe, S. M., Zishiri, O. T., & Zowalaty, M. E. E. (2022). Review of Escherichia coli O157:H7 Prevalence, Pathogenicity, Heavy Metal and Antimicrobial Resistance, African Perspective. *Infection and Drug Resistance*, 15, 4645–4673. DOI:https://doi.org/10.2147/idr.s365269

Garrine, M., Matambisso, G., Nobela, N., Vubil, D., Massora, S., Acácio, S., ... & Mandomando, I. (2020). Low frequency of enterohemorrhagic, enteroinvasive and diffusely adherent Escherichia coli in children under 5 years in rural Mozambique: a case-control study. *BMC Infectious Diseases*, 20, 1–6. DOI:https://doi.org/10.1186/s12879-020-05380-1

Hamner, S., Broadaway, S. C., Mishra, V. B., Tripathi, A., Mishra, R. K., Pulcini, E., ... & Ford, T. E. (2007). Isolation of potentially pathogenic Escherichia coli O157: H7 from the Ganges River. *Applied and environmental microbiology*, 73(7), 2369–2372. DOI:https://doi.org/10.1128/AEM.00141-07

Hasan, H. K., Yassin, N. A., & Eassa, S. H. (2020). Bacteriological and Molecular Characterization of Diarrheagenic Escherichia Coli Pathotypes From Children in Duhok City, Iraq. *Science Journal of University of Zakho*, 8(2), 52–57. DOI:https://doi.org/10.25271/sjuoz.2020.8.1.702

Hessain, A. M., Al-Arfaj, A. A., Zakri, A. M., El-Jakee, J. K., Al-Zogibi, O. G., Hemeg, H. A., & Ibrahim, I. M. (2015). Molecular characterization of Escherichia coli O157: H7 recovered from meat and meat products relevant to human health in Riyadh, Saudi Arabia. *Saudi Journal of Biological Sciences*, 22(6), 725–729. DOI:https://doi.org/10.1016/j.sjbs.2015.06.009

Hua, Y., Bai, X., Zhang, J., Jernberg, C., Chromek, M., Hansson, S., ... & Matussek, A. (2020). Molecular characteristics of *eae*-positive clinical Shiga toxin-producing Escherichia coli in Sweden. *Emerging microbes & infections*, 9(1), 2562–2570. DOI:https://doi.org/10.6084/m9.figshare.13359562

Kadhim, F. A., & Kareem, I. Q. A. (2021). MOLECULAR IDENTIFICATION OF ESCHERICHIA COLI SEROTYPE O157: H7 FROM HUMANS AND CHICKENS IN IRAQ. *Plant Archives*, 21(1), 1183–1190. DOI: https://doi.org/10.51470/PLANTARCHIVES.2021.v21.S1.186

Korajkic, A., McMinn, B. R., & Harwood, V. J. (2018). Relationships between microbial indicators and pathogens in recreational water settings. *International Journal of Environmental Research and Public Health*, 15(12), 2842. DOI:https://doi.org/10.3390/ijerph15122842

Lane, D. (1991). 16S/23S rRNA sequencing. *Nucleic acid techniques in bacterial systematics*.

Loukiadis, E., Kérourédan, M., Beutin, L., Oswald, E., & Brugère, H. (2006). Characterization of Shiga Toxin Gene (stx)-Positive and Intimin Gene (*eae*)-Positive Escherichia coli Isolates from Wastewater of Slaughterhouses in France. *Applied and Environmental Microbiology*, 72(5), 3245–3251. DOI:https://doi.org/10.1128/aem.72.5.3245-3251.2006

Lu, J., Shen, C., Zhou, Z., Zhang, Y., Wang, J., Liang, Y., ... & Shao, J. (2024). A calibration-free, non-invasive, and accurate total viable count detection method based on tunable diode laser absorption spectroscopy combined with most probable number counting. *Optics and Lasers in Engineering*, 173, 107899. DOI:https://doi.org/10.1016/j.optlaseng.2023.107899

Lu, Y., Wang, R., Zhang, Y., Su, H., Wang, P., Jenkins, A., ... & Squire, G. (2015). Ecosystem health towards sustainability. *Ecosystem Health and Sustainability*, 1(1), 1–15. DOI:https://doi.org/10.1890/ehs14-0013.1

Menéndez-Serra, M., Triadó-Margarit, X., Castañeda, C., Herrero, J., & Casamayor, E. O. (2019). Microbial composition, potential functional roles and genetic novelty in gypsum-rich and hypersaline soils of Monegros and Gallocanta (Spain). *Science of the Total Environment*, 650, 343–353. DOI:https://doi.org/10.1016/j.scitotenv.2018.09.050

Olowe, B., & Arogbodo, J. (2022). Molecular identification of virulence and antibiotic-resistant gene in Escherichia coli O157 and non-O157 recovered from water samples. *Microbes and Infectious Diseases*, 3(2), 348–359. DOI:https://doi.org/10.21608/mid.2021.74664.1148

Oluwarinde, B. O., Ajose, D. J., Abolarinwa, T. O., Montso, P. K., DuPreez, I., Njom, H. A., & Ateba, C. N. (2023). Safety properties of Escherichia coli O157:H7 specific bacteriophages: recent advances for food safety. *Foods*, 12, p.3989. DOI:https://doi.org/10.3390/foods12213989

Oves, M., Aslam, M., Rauf, M. A., Qayyum, S., Qari, H. A., Khan, M. S., ... & Ismail, I. M. (2018). Antimicrobial and anticancer activities of silver nanoparticles synthesized from the root hair extract of Phoenix dactylifera. *Materials Science and Engineering: C*, 89, 429–443. DOI:https://doi.org/10.1016/j.msec.2018.03.035

Paletta, A. C., Castro, V. S., & Conte-Junior, C. A. (2020). Shiga toxin-producing and enteroaggregative Escherichia coli in animal, foods, and humans: pathogenicity mechanisms, detection methods, and epidemiology. *Current microbiology*, 77, 612–620. DOI:https://doi.org/10.1007/s00284-019-01842-1

Peterson, E., & Kaur, P. (2018). Antibiotic resistance mechanisms in bacteria: relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. *Frontiers in microbiology*, 9, 2928. DOI:https://doi.org/10.3389/fmicb.2018.02928

Razmi, N., Hasanzadeh, M., Willander, M., & Nur, O. (2022). Electrochemical genosensor based on gold nanostars for the detection of Escherichia coli O157: H7 DNA. *Analytical Methods*, 14(16), 1562–1570. DOI:https://doi.org/10.1039/d2ay00056c

Saeed, A. Y., & Ibrahim, K. S. (2013). Detection of Enterohemorrhagic Escherichia Coli O157 in Sheep and Goats Using Fluorogenic and Chromogenic Culture Media. *Science Journal of University of Zakho*, 1(1), 115–119. Retrieved from https://sjuoz.uoz.edu.krd/index.php/sjuoz/article/view/53

Samuel, M. S., Jose, S., Selvarajan, E., Mathimani, T., & Pugazhendhi, A. (2020). Biosynthesized silver nanoparticles using Bacillus amyloliquefaciens: Application for cytotoxicity effect on A549 cell line and photocatalytic degradation of p-nitrophenol. *Journal of Photochemistry and Photobiology B: Biology*, 202, 111642. DOI:https://doi.org/10.1016/j.jphotobiol.2019.111642

Sharef, A. J., & Dara, R. N. (2021). Greater zab river Basin planning (2050). *Iraqi Journal of Agricultural Sciences*, 52(5), 1150–1162. DOI:https://doi.org/10.36103/ijas.v52i5.1453

Sthapit, N., Malla, B., Tandukar, S., Ghaju Shrestha, R., Thakali, O., Sherchand, J. B., ... & Kazama, F. (2022). Occurrence and reduction of Shiga toxin-producing Escherichia coli in Wastewaters in the Kathmandu Valley, Nepal. *Water*, 14(14), 2224. DOI:https://doi.org/10.3390/w14142224

Subramanian, S., & Kearns, D. B. (2019). Functional regulators of bacterial flagella. *Annual Review of Microbiology*, 73(1), 225–246. DOI:https://doi.org/10.1146/annurev-micro-020518-115725

Urnukhsaikhan, E., Bold, B. E., Gunbileg, A., Sukhbaatar, N., & Mishig-Ochir, T. (2021). Antibacterial activity and characteristics of silver nanoparticles biosynthesized from Carduus crispus. *Scientific reports*, 11(1), 21047. DOI:https://doi.org/10.1038/s41598-021-00612-w

Wang, X., Lee, S. Y., Akter, S., & Huq, M. A. (2022). Probiotic-mediated biosynthesis of silver nanoparticles and their antibacterial applications against pathogenic strains of Escherichia coli O157: H7. *Polymers*, 14(9), 1834. DOI:https://doi.org/10.3390/polym1409183

Wolfson, E. B., Elvidge, J., Tahoun, A., Gillespie, T., Mantell, J., McAteer, S. P., Rossez, Y., Paxton, E., Lane, F., Shaw, D. J., Gill, A. C., Stevens, J., Verkade, P., Blocker, A., Mahajan, A., & Gally, D. L. (2020). The interaction of Escherichia coli O157:H7 and Salmonella Typhimurium flagella with host cell membranes and cytoskeletal components. *Microbiology*, 166(10), 947–965. DOI:https://doi.org/10.1099/mic.0.000959

Yang, F., Yang, L., Chang, Z., Chang, L., & Yang, B. (2018). Regulation of virulence and motility by acetate in enteropathogenic Escherichia coli. *International Journal of Medical Microbiology*, 308(7), 840–847. DOI:https://doi.org/10.1016/j.ijmm.2018.07.010

Yang, X., Sun, H., Fan, R., Fu, S., Zhang, J., Matussek, A., & Bai, X. (2020). Genetic diversity of the intimin gene (*eae*) in non-O157 Shiga toxin-producing Escherichia coli strains in China. *Scientific Reports*, 10, 3275. DOI:https://doi.org/10.1038/s41598-020-60225-w

Zhang, Y., Liao, Y., Salvador, A., Sun, X., & Wu, V. C. H. (2020). Investigating the Whole-Genome Sequence of a New Locus of Enterocyte Effacement-Positive Shiga Toxin-Producing Escherichia coli O157:H7 Strain Isolated from River Water. *Microbiology Resource Announcements*, 9(12). DOI:https://doi.org/10.1128/mra.00112-20