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Detection and characterization of *Escherichia coli* O157:H7 in drinking water from Baghdad city using phenotypic, immunological, and molecular methods

Sudad Jasim Mohammed

*Corresponding author. Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq. E-mail: sudad@mracpc.uobaghdad.edu.iq

Adil Turki Al-Musawi

Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq. E-mail: adilalmusawi@mracpc.uobaghdad.edu.iq

Raafat A. Abu-Almaaly

Market Research and Consumer Protection Center, University of Baghdad, Baghdad, Iraq. E-mail: raafat@mracpc.uobaghdad.edu.iq

Abstract

Objective

The aim of this study was to isolate and identify *Escherichia coli* O157:H7 from drinking water intended for human consumption in Baghdad City. The isolates were characterized using conventional phenotypic and biochemical methods. Then, their ability to produce Shiga toxin (Stx) was determined using ELISA. In addition, molecular identification and genotyping were performed. For detecting the presence of *stx1* and *stx2* genes, multiplex polymerase chain reaction (MPCR) was used. Another goal was to compare these genes as diagnostic and epidemiological indicators with traditional diagnostic methods.

Material and methods

Thirty drinking water samples were collected from both sides of Baghdad City (Karkh and Rusafa) between July and August 2023. Samples were collected in sterile 250-mL glass bottles containing 3% sodium thiosulfate. Cefixime-tellurite sorbitol MacConkey agar (CT-SMAC) was used to isolate *E. coli* O157:H7. Biochemical identification was performed using the API 20E system. Moreover, ELISA was used to determine the production of Shiga toxin. Molecular detection of *stx1* and *stx2* genes was conducted using the PCR technique.

Results

Out of the 30 drinking water samples analyzed, 25 *E. coli* isolates were obtained. Biochemical tests identified 10 isolates as *E. coli*. While, the API 20E system confirmed 6 isolates. Serological examination using the rapid latex agglutination test confirmed that four isolates belonged to the *E. coli* O157:H7 serotype. According to the ELISA report it was found that all four studied isolates can produce Shiga toxin. Based on the results of MPCR analysis, it was determined that one of the isolates carried the stx1 gene with a length of 180 bp. On the other hand, the stx2 gene with a length of 255 bp was found in all four isolates.

Conclusion

Verotoxin is another name for Shiga toxin (Stx). This toxin is an AB5 toxin. Certain strains of E. coli, particularly E. coli O157:H7, can produce this toxin. Furthermore, it is closely related to the toxin produced by *Shigella dysenteriae*. The presence of *stx1* and *stx2* genes in drinking water isolates can highlight the potential public health risk. It also can support the use of molecular methods as reliable tools for the detection and surveillance of pathogenic *E. coli* in drinking water.

Keywords: Baghdad city, drinking water, ELISA, *E. coli* O157:H7, *Stx1* and *Stx2* genes

Paper Type: Research Paper.

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Introduction

Access to safe and potable drinking water is one of the most critical global challenges. It is more critical particularly in developing and Third World countries. Water is essential for life. It is also recognized as a fundamental human right. Consequently, drinking water pollution has become one of the primary issues that confirmed by scientists and environmental health specialists worldwide (Chang et al., 2011; Sharon et al., 2021). In 2002, the United Nations issued General

Comment No. 15 formally recognized the human right to water. It emphasized that all individuals must have access to clean, safe, and affordable water for personal and domestic use. All of this shows that we need to take water security and public health protection seriously (Mohammed et al., 2022; Maphanga et al., 2024). The water access situation in Iraq is getting worse every day. Because the water in the Tigris and Euphrates rivers is getting less and less every day. This is because many dams and reservoirs have been built in this country. On the other hand, the water quality is getting worse day by day due to the discharge of industrial wastewater and petrochemical activities into the rivers and the production of hydroelectric power. Unfortunately, the water treatment processes are also not carried out properly and are incomplete (Marabini et al., 2007; Al-Musawi et al., 2025a). Therefore, one of the fundamental and complex challenges is to ensure that clean and safe drinking water is provided to the people. Access to safe drinking water is one of the key indicators of health and social well-being presented by the World Health Organization (WHO) and has been introduced as a prerequisite for sustainable development. Contaminated water is a major and serious threat to human health. Unfortunately, about 1.1 billion people in the world are forced to consume unsafe drinking water (Shen et al., 2020; Al-Maliki et al., 2025). One of the most serious water pollutions in the world is microbial pollution. These pollutions endanger human health. Pathogenic bacteria are from this group of microbes. Therefore, water must be evaluated before drinking to reach the standard for consumption. It should be noted that not all water bacteria are harmful. Only pathogenic microorganisms are dangerous to human health and should be considered. Pathogenic bacteria in water cause diseases in the digestive tract. These infections, especially dysentery, cause hospitalization and death of many people, especially children (Khairy et al., 2020; Al-Musawi et al., 2025b). Many diseases are caused by waterborne pathogens. Some of them are gastrointestinal infections, dysentery, hepatitis, typhoid fever, and cholera. Among these pathogens, *Escherichia coli* holds particular importance from both health and environmental perspectives. It is widely used as an indicator organism for fecal contamination of water, originating from either human or animal sources. International water quality standards rely on the detection of coliform bacteria, including members of the family *Enterobacteriaceae*, as indicators of water suitability for human consumption (Martinson and Walk, 2020). *Escherichia coli* was first described in 1885 by the German pediatrician Theodor Escherich. He isolated the bacterium from the feces of an infant. It is a natural component of the intestinal microflora of humans and animals. However, certain strains are pathogenic and are responsible for numerous water- and foodborne disease outbreaks worldwide (Rohatgi and Gupta, 2021). Most pathogenic strains, particularly *E. coli* O157:H7, pose a serious threat to public health. It is more serious especially in developing countries, due to their transmission through contaminated water and food. *Escherichia coli* O157:H7 is the most

prominent serotype of enterohemorrhagic *E. coli* (EHEC). It is considered a major food- and waterborne pathogen (Bjørklund et al., 2020). This strain is responsible for severe clinical conditions. Some of these conditions are hemorrhagic colitis and hemolytic uremic syndrome (HUS), particularly among infants and young children. In severe cases, these infections may lead to kidney damage, renal failure, and death. The pathogenicity of *E. coli* O157:H7 is largely attributed to its ability to produce Shiga toxin (Stx). It represents one of its most important virulence factors (Ramires et al., 2020). Unfortunately, biochemical tests cannot identify many bacteria, viruses, and parasites with 100% accuracy and certainty (Ahsani et al. 2010; Khabiri et al., 2025). One of the newest and most practical techniques and methods for rapid and accurate diagnosis of infectious diseases is PCR (Mohammadabadi et al. 2004; Khabiri et al. 2023). Various results and reports from around the world and in different disciplines have shown that PCR is a very fast, accurate and reliable method. That is, the test can be completed and the result can be seen within a few hours (Mohammadabadi et al. 2011; Mohammadabadi et al., 2025). PCR can detect and report infections directly and rapidly in clinical samples (Shahdadnejad et al. 2016; Mohammadabadi et al. 2024). Since E. coli O157:H7 contamination poses serious risks to human health, drinking water should be examined and tested for the presence of this pathogen. Therefore, our aim in conducting this study was to isolate and identify E. coli O157:H7 from drinking water samples collected in Baghdad city using phenotypic, biochemical, immunological and molecular detection methods.

Materials and methods

Sample collection: For this study, thirty drinking water samples were collected from different locations in Baghdad city (both sides of Karkh and Rusafa). The study was conducted between July and August 2023. To neutralize the residual chlorine in the water samples, 3% sodium thiosulfate solution ($Na_2S_2O_3 \cdot 5H_2O$) was added to each bottle. This was done during collection and transportation (Sauvala et al., 2023).

Isolation of *Escherichia coli* O157:H7 from water samples: The method of Bjørklund et al. (2020) was used to isolate E. coli O157:H7 from the collected samples. To homogenize the water samples, each water sample was shaken vigorously 25 times. Then, filtration was performed through a membrane filtration unit equipped with a membrane with a 0.20 µm pore size. Sterile forceps were used to sterilely transfer the membrane filter into the modified Trypticase Soy Broth culture medium. Then, incubation was performed for 4 to 6 hours as an enrichment step to enhance bacterial growth at 41.5°C. After this step, a loop of the enriched culture was plated on Cefixime Tellurite-Sorbitol MacConkey Agar (CT-SMAC) plates. These plates were incubated for 18 to 24 hours at 37°C to allow selective growth of E. coli O157:H7.

Phenotypic and serological identification of *E. coli* O157:H7: Evaluation of colony morphology, microscopic appearance, and biochemical characteristics were used to identify E. coli O157:H7 (Li et al., 2017). The API 20E detection system (bioMérieux, France) was used according to the manufacturer's instructions to perform biochemical identification. Serological confirmation of the isolates was carried out using a rapid latex agglutination test (Oxoid DR620). It was performed to detect the O157 somatic antigen and the H7 flagellar antigen. In addition, enzyme-linked immunosorbent assay (ELISA) was used to determine the ability of serologically confirmed isolates to produce Shiga toxin. The ELISA test was performed using the Premier EHEC ELISA kit (Meridian Bioscience Europe, Italy) according to the manufacturer's protocol.

Detection of *stx1* and *stx2* genes by Polymerase Chain Reaction (PCR): Multiplex polymerase chain reaction (MPCR) was used for molecular detection of stx1 and stx2 genes. This was done for isolates that were confirmed by serology. Two pairs of primers specific for *stx1* and *stx2* (Inat and Siriken, 2010), were used. Primer sequences and expected amplicon sizes are shown in Table 1. The PCR reaction mixture was prepared in a final volume of 25 µL using the Promega PCR Core System II (USA). The mixture consisted of 5× PCR buffer (100 mmol/L Tris-HCl, 35 mmol/L $MgCl_2$, 750 mmol/L KCl, pH 8.8), 1 µL of 10 mmol/L dNTPs, 1 µL (10 pmol/L) of each primer (*stx1F*, *stx1R*, *stx2F*, and *stx2R*), 0.2 µL of Taq DNA polymerase (0.1 U), 12.3 µL of sterile nuclease-free distilled water, and 2 µL (100 ng) of template DNA. Negative controls contained sterile distilled water instead of template DNA. While, positive controls included template DNA from confirmed *E. coli* O157:H7 strains. A thermal cycler was used to carry out PCR amplification. The following conditions was applied for PCR: an initial denaturation step at 94 °C for 5 minutes for 1 cycle, then 35 cycles including denaturation at 94 °C for 2 minutes, annealing at 32 °C for 1 minute, and extension at 72 °C for 2 minutes. A final extension step was performed at 72 °C for 10 minutes in 1 cycle. The amplification protocol was performed according to Faúndez et al. (2024). PCR products were analyzed by electrophoresis on a 1% agarose gel. The gel was stained with an appropriate DNA dye and run at 90 V for 40 minutes. The sizes of the amplified fragments were estimated by comparison with a 100 bp DNA ladder (molecular size marker).

Results and discussion

Isolation on Cefixime Tellurite-Sorbitol MacConkey (CT-SMAC) medium: A total of twenty-five *Escherichia coli* isolates were obtained from the thirty drinking water samples analyzed. These samples represented 83% of the total samples examined. When cultured on CT-SMAC agar, these isolates exhibited differential sorbitol fermentation patterns. Of the total isolates, 83% were identified as non-sorbitol fermenters. This characteristic is commonly

associated with *E. coli* O157:H7. Moreover, twelve isolates (40% of the total) produced pink colonies on MacConkey agar. It indicates their ability to ferment lactose. These lactose-fermenting isolates were subsequently purified. Then they subjected to further identification procedures (Table 2).

Table 1. Primers used for amplification of *stx1* and *stx2* genes in the current study

Gene	Primer	Primer sequence (5′-3′)	Expected product size (bp)
stx2	*stx2*F	GGCACTGTCTGAAACTGCTCC	255
	*stx2*R	TCGCCAGTTATCTGACATTCTG	
stx1	*stx1*F	ATAAATCGCCATTCGTTGACTAC	180
	*stx1*R	AGAACGCCCACTGAGATCATC	

Table 2. Number and percentage of *E. coli* O157:H7 isolates identified using different diagnostic methods

Sample type	Total number	Percentage
Drinking water samples	30	100%
Non-sorbitol-fermenting isolates on CT-SMAC	25	83%
Lactose-fermenting isolates on MacConkey agar	12	40%
Isolates identified by biochemical tests	10	33%
Isolates identified by API 20E	6	20%
Isolates confirmed by latex agglutination test	4	13%
Isolates positive by ELISA	4	13%
Isolates positive for *stx1* gene by MPCR	1	-
Isolates positive for *stx2* gene by MPCR	4	-

The findings of this study are consistent with the results of Yousefipour et al. (2023). They documented significant contamination of irrigation water in central Iraq. *E. coli* counts ranged from 5.5 to 12.1 CFU/mL. Their values exceed the permissible limits established by the U.S. Food and Drug Administration (FDA). This is particularly concerning given that many drinking water treatment and bottling facilities in central Iraq rely on water from the Tigris and Euphrates rivers. Similarly, Al-Hmani et al. (2024) reported seasonal variations in *E. coli* contamination in drinking water sources in Mosul City. Their isolation rates reached up to 28% during summer months.

Biochemical identification of *Escherichia coli* isolates: Biochemical characterization of the twelve presumptive isolates revealed that ten isolates (33%) were confirmed as *E. coli*. These isolates did not ferment cellobiose sugar and showed tolerance to potassium cyanide (KCN). All confirmed isolates demonstrated hemolytic activity when cultured on sheep blood agar after 24 hours of incubation. Furthermore, all isolates yielded negative results for the 4-

methylumbelliferyl-β-D-glucuronide (MUG) test. It is a distinguishing characteristic of *E. coli* O157:H7 strains (Table 3).

Table 3. Biochemical characteristics of *Escherichia coli* O157:H7 isolates

Bacterial isolate	Number	MUG test	Cellobiose fermentation	Growth in KCN	Hemolysis
E. coli O157:H7	10	Negative	Negative	Positive	Positive

These results agree with those reported by Malvano et al. (2018) and Mohan and Lyons (2022). They demonstrated that biochemical screening methods provide an effective preliminary approach for identifying Shiga toxin-producing *E. coli* (STEC). In addition, Clermont et al. (2013) reported high isolation rates of pathogenic *E. coli* from fecal samples of hospitalized children in Baghdad. They highlighted that contaminated water and food are major transmission routes. Suzuki et al. (2023) further emphasized the association between elevated ambient temperatures and increased isolation rates of *E. coli* O157:H7 from surface water sources.

API 20E and ELISA identification of *Escherichia coli* isolates: When the API 20E identification system was used, six of the twelve isolates (20%) were identified as E. coli (Figure 1). Subsequent serological confirmation using the latex agglutination test revealed that four isolates belonged to the E. coli serotype O157:H7, accounting for 13% of the total isolates. All four isolates were found to produce Shiga toxin by ELISA analysis. It supports their pathogenic potential (Table 2). These findings partially differ from those reported by Clermont et al. (2013). They detected *E. coli* O157:H7 in surface water during summer but not winter. It suggests a strong seasonal influence. However, the present results align with studies by Abong'o and Momba (2009) and Lu et al. (2015). They demonstrated that latex agglutination testing is a highly sensitive and specific method for detecting the O157 somatic antigen and H7 flagellar antigen. The O157 antigen, composed of lipopolysaccharide (LPS), is heat-stable and highly immunogenic. While, the H7 antigen is heat-labile and composed of flagellin proteins responsible for bacterial motility.

Molecular detection of *stx1* and *stx2* genes by multiplex PCR: Multiplex polymerase chain reaction (MPCR) analysis demonstrated high efficiency in detecting the *stx1* and *stx2* genes. These genes encode Shiga toxins in *E. coli* O157:H7 isolates. Among the four confirmed isolates, one isolate carried the *stx1* gene. This gene produces an amplicon of 180 bp. Whereas, all four isolates carried the *stx2* gene, with an amplicon size of 255 bp (Figure 2).

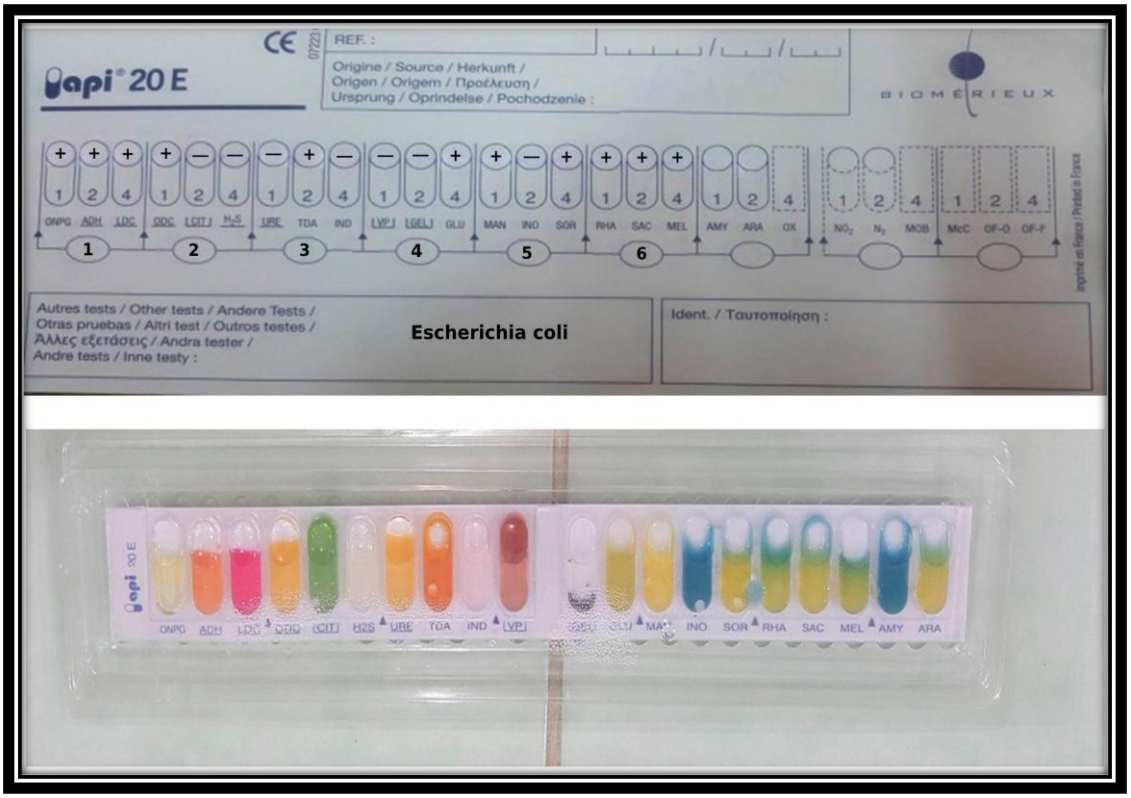

Figure 1. API 20E identification profile of *Escherichia coli* isolates in the current study

Molecular detection of *stx1* and *stx2* genes by multiplex PCR: Multiplex polymerase chain reaction (MPCR) analysis demonstrated high efficiency in detecting the *stx1* and *stx2* genes. These genes encode Shiga toxins in *E. coli* O157:H7 isolates. Among the four confirmed isolates, one isolate carried the *stx1* gene. This gene produces an amplicon of 180 bp. Whereas, all four isolates carried the *stx2* gene, with an amplicon size of 255 bp (Figure 2).

These findings are consistent with those reported by Tsen and Jian (1998) and Paton and Paton (2005). They noted that some *E. coli* O157 strains may lose *stx* genes during isolation or subculture. This leads to reduced toxin detection. Results from various studies have shown that loss of stx genes can complicate laboratory diagnosis (Schmidt et al., 1999; Ferens and Hovde, 2011). These changes occur even when the strains remain clinically and epidemiologically related. The amplicon sizes observed in our study confirm the results reported by Manzanas et al. (2023) and Rodesari et al. (2025). They showed that the lengths of the stx1 and stx2 genes are approximately 180 and 255 bp, respectively. Detection of the stx gene is very important in differentiating enterohemorrhagic E. coli (EHEC) strains from non-EHEC strains and should be emphasized (Amerotto et al., 2024). In another study, McDonald et al. (2024) showed that E. coli O157:H7 carries both stx1 and stx2 genes by studying drinking water and river water samples, which is consistent with the results of our study. Additionally, their findings showed long-term

survival of E. coli O157:H7 in both treated and untreated water. These results demonstrate how dangerous it can be to consume contaminated drinking water.

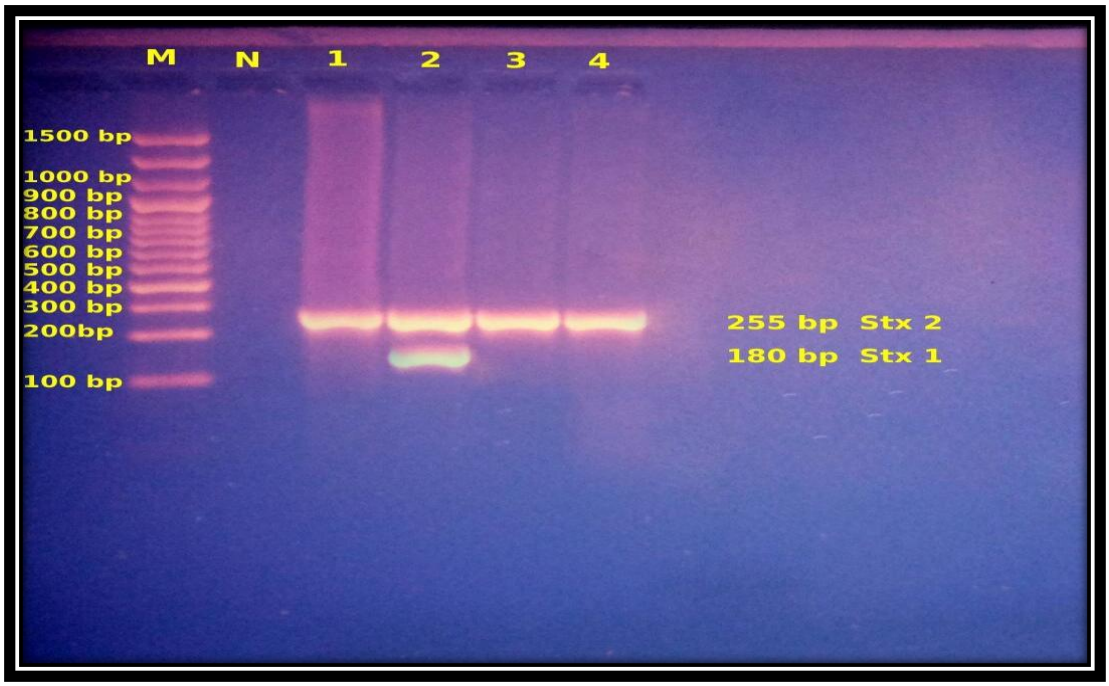

Figure 2. Electrophoresis of MPCR products on 2% agarose gel using 70 V for 60 min. Lane M is 100 bp DNA ladder, Lane N is negative control, Lanes 1-4 are *E. coli* O157:H7 isolates positive for *stx2* (255 bp), and Lane 2 is isolate positive for *stx1* (180 bp)

Conclusion: In order to have a healthy society and a sustainable environment, our drinking water must be safe and hygienic. Therefore, one of the vital priorities of humanity is to identify pathogenic bacteria in water sources. The results of this study showed that Escherichia coli O157:H7 is present in drinking water samples from Baghdad and is a serious and dangerous risk to public health. It was found that the combined use of culture-based, biochemical, serological and molecular techniques is effective for the accurate identification of this pathogen. In addition, the detection of stx1 and stx2 genes in confirmed isolates highlights the pathogenic potential of the identified strains. It also emphasizes the need to use molecular detection methods that are more reliable. On the other hand, it was found that conventional water treatment and monitoring methods cannot be sufficient to eliminate highly pathogenic E. coli strains. Consequently, continuous microbiological monitoring of drinking water sources should be carried out and advanced diagnostic methods should be used to ensure water safety in order to protect public health in urban environments.

Authors' Contributions

A.T.M.: Methodology and Experimental Design, S.J.M.: Data Collection and Statistical Analysis, and R.A.: Writing the Original Manuscript. All authors reviewed, discussed, and approved the final version of the manuscript.

Data Availability Statement

The data and samples for this study are available at the Scientific and Health Research Center at Koya University. Moreover, they are available from the authors upon reasonable request.

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Ethical Approval

The present study was conducted at the Market Research and Consumer Protection Center, University of Baghdad, from July to August 2023, following approval from the institutional ethical committee (Approval No. MRCPC-68).

Conflict of Interest

The authors declare that they have no conflicts of interest.

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شناسایی و ویژگی‌یابی *Escherichia coli* O157:H7 در آب آشامیدنی شهر بغداد با استفاده از روش‌های فنوتیپی، ایمنی‌شناسی و مولکولی

سداد جاسم محمد

*نویسنده مسئول. مرکز تحقیقات بازار و حمایت از مصرف‌کننده، دانشگاه بغداد، بغداد، عراق. ایمیل: sudad@mracpc.uobaghdad.edu.iq

عادل ترکی الموسوی

مرکز تحقیقات بازار و حمایت از مصرف‌کننده، دانشگاه بغداد، بغداد، عراق. ایمیل: adilalmusawi@mracpc.uobaghdad.edu.iq

رافت ع. ابوالمعالی

مرکز تحقیقات بازار و حمایت از مصرف‌کننده، دانشگاه بغداد، بغداد، عراق. ایمیل: raafat@mracpc.uobaghdad.edu.iq

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چکیده

هدف: هدف از این مطالعه جداسازی و شناسایی *Escherichia coli* O157:H7 از آب آشامیدنی مورد مصرف انسان در شهر بغداد بود. ایزوله‌ها با استفاده از روش‌های متداول فنوتیپی و بیوشیمیایی مشخصه‌یابی شدند. سپس توانایی آن‌ها در تولید سم شیگا (Stx)با استفاده از آزمون الایزا (ELISA) تعیین گردید. علاوه بر این، شناسایی مولکولی و تعیین ژنوتیپ انجام شد. برای شناسایی ژن‌های *stx1* و *stx2* از واکنش زنجیره‌ای پلیمراز چندگانه (MPCR) استفاده شد. هدف دیگر، مقایسه این ژن‌ها به‌عنوان شاخص‌های تشخیصی و اپیدمیولوژیک با روش‌های تشخیصی سنتی بود.

مواد و روش‌ها: در مجموع ۳۰ نمونه آب آشامیدنی از دو سوی شهر بغداد (کرخ و رصافه) در فاصله زمانی ژوئیه تا اوت ۲۰۲۳ جمع‌آوری شد. نمونه‌ها در بطری‌های شیشه‌ای استریل ۲۵۰ میلی‌لیتری حاوی ۳ درصد تیوسولفات سدیم برداشت شدند. برای جداسازی *E. coli* O157:H7از محیط کشت مک‌کانکی سوربیتول حاوی سفیکسیم-تلوریت (CT-SMAC) استفاده شد. شناسایی بیوشیمیایی با سیستم API 20E انجام گرفت. همچنین، تولید سم شیگا با روش ELISA بررسی شد. شناسایی مولکولی ژن‌های *stx1* و *stx2* با استفاده از تکنیک PCR صورت پذیرفت.

نتایج: از میان ۳۰ نمونه آب آشامیدنی بررسی‌شده، ۲۵ ایزوله *E. coli* به‌دست آمد. آزمون‌های بیوشیمیایی ۱۰ ایزوله را به‌عنوان *E. coli*شناسایی کردند، در حالی که سیستم API 20E شش ایزوله را تأیید نمود. بررسی سرولوژیک با آزمون آگلوتیناسیون سریع لاتکس نشان داد که چهار ایزوله به سروتیپ *E. coli* O157:H7 تعلق دارند. نتایج ELISA نشان داد که هر چهار ایزوله قادر به تولید سم شیگا هستند. تحلیل MPCR نشان داد که یک ایزوله حامل ژن *stx1* (۱۸۰ جفت‌باز) بوده و هر چهار ایزوله دارای ژن *stx2* (۲۵۵ جفت‌باز) بودند.

نتیجه‌گیری: سم شیگا (Stx) که با نام وروتوکسین نیز شناخته می‌شود، یک سم AB_5 است که توسط برخی سویه‌های *E. coli*، به‌ویژه *E. coli* O157:H7، تولید می‌شود و ارتباط نزدیکی با سم تولیدشده توسط *Shigella dysenteriae* دارد. وجود ژن‌های *stx1* و *stx2* در ایزوله‌های آب آشامیدنی می‌تواند خطر بالقوه‌ای برای سلامت عمومی را نشان دهد و همچنین بر کاربرد روش‌های مولکولی به‌عنوان ابزارهایی قابل اعتماد برای شناسایی و پایش *E. coli* بیماری‌زا در آب آشامیدنی تأکید می‌کند.

کلمات کلیدی: آب آشامیدنی، الایزا، ژن‌های *Stx1* و *Stx2*، شهر بغداد، *E. coli* O157:H7

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