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Evaluation of tap water quality and molecular identification of *Escherichia coli O157* in Al-Kut City, Iraq

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ABSTRACT

Escherichia coli is a pathogen which can result in gastrointestinal infection. It can be transferred in a many methods, like water and food. This study is aimed to evaluate the potable water quality in Al- Kut City, Iraq, in addition to diagnosis of *E. coli O157:H7* by PCR protocol. The method includes examination for total dissolved solids (TDSs), pH and electrical conductivity (EC), as well as the bacterial DNA extraction by DNA extraction kit, then detection via PCR. Primer "Rfb" was used for detection of O157 gene. The bands of PCR product was 292 bp, which has been visualized using gel electrophoresis. The results gained revealed that the advanced procedure by molecular detection may be used as a routine examination for drinking water pollution. Moreover, it is a precise procedure providing results at a less time compared to the classical culture methods.

Keywords: PCR, Rfb, *E.coli*, TDS, pH, EC.
Article type: Research Article.

INTRODUCTION

Water is a valuable source vital for supporting life. Thus, the human life style was based on the water accessibility (Omidi *et al.* 2021; Fatih Ali *et al.* 2021; Farabi *et al.* 2022; Heidari *et al.* 2022). High-quality drinkable water could be used with no side effects (Gray 1998; AL Dulaimi & Younes 2017), as it is free from risky ratios of microorganisms, minerals and other contaminations. Besides, it is free of flavour, colour, turbid, and scent. The main water parameters are TDS, pH, electrical conductivity (EC). (Sasikaran *et al.* 2012; AL Dulaimi & Younes 2017). In fact, pH is a measurement of acidic/alkaline level of water. The pH is a gauge of free H^+ and OH^- ions in water. The range of acidity is pH < 7, while a pH > 7 is alkaline. Acidic water contains more free H^+ ions, while alkaline more free OH^- ions (USGS 2016; Islam *et al.* 2017). In fact, pH of water controls the solubility in addition to life accessibility (quantity which could be consumed via water being) of chemical components for example P, N_2, C. For instance, how much phosphorus is plentiful in water, pH controls whether water life could use it. In addition to heavy metals (cadmium, copper), the level of soluble heavy metals controls the toxicity. Metals are having toxin effect in lesser pH since they are highly soluble. Contamination can alter the pH of water, that hurt organisms living in water (USGS 2016; Islam *et al.* 2017). In fact, TDS refer to the inorganic salts in addition to minor quantities of organic substance existing in aquatic area. The main components are commonly magnesium, calcium, potassium and sodium cations in addition to hydrogencarbonate, carbonate, sulfate, chloride and nitrate anions (WHO 1996). Water comprising not < 250 ppm of TDS is from underground water, might be "mineral water" which is defined via its comparative ratios of minerals as well as trace elements from the source (Islam *et al.* 2017; USFDA 2017). In fact, TDS associates with the EC as well as influencing pH. High TDS reveal high EC and low pH. The existence of TDS in water can influence its sense of taste (Bruvold & Ongerth 1969). The tastiness of drinking water are measured by the ratio of taste to TDS in this manner: < 300 mg L^{-1} (excellent);

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300-600 mg L^{-1} (good); 600-900 mg L^{-1} fair; 900-1200 mg L^{-1} (poor); > 1200 mg L^{-1} (intolerable; Bruvold & Ongerth 1969; WHO 2004). Water containing very low TDS level can be improper due to the presence of flat, insipid flavour (Bruvold & Ongerth 1969; Islam *et al.* 2017). In fact, it is essential to observe the TDS ratio in addition to pH of drinking water. Water containing an elevated TDS ratio or low pH, reveals the presence of additional dangerous pollutants in water. In fact, TDS and pH are measured easily and in the case of presence of e.g., contamination. TDS and pH ratios will change revealing a warning indicator in the water (Islam *et al.* 2017; SDWF 2017). Hence this study was intended to evaluate pH, conductivity as well as TDS values of drinking water in Iraq. Microbiological examination of drinking water is significant to discover waterborne infections, for example gastroenteritis, typhoid fever, and hepatitis A. Bacteriological examination of coliforms is performed to detect the microbiological value of water. Furthermore, stool coliforms can occur in water due to the home sewage of human and animal waste (Ahmad & Bajahlan 2009; Hadi Dehghani *et al.* 2011; AL Dulaimi & Younes 2017).

E. Coli as a significant biological indicator

Identification approaches of the waterborne microorganisms are commonly expensive, technically challenging and time-consuming. *E. coli* is a kind of the normal microbiota in mammals and is considered as an indicator of drinking water. (Edberg *et al.* 2000) *E. coli* is the main agent of bloody diarrhoea. Most *E. coli* contagions are food-borne. (Griffin 1995; Meng *et al.* 1997). The RfbE gene encoding an enzyme plays an important role in the biosynthesis of O:157 antigen. It is different from rfb locus encode O antigen. In fact, Rfb gene is not able to distinguish O157:H7 from the other O157 isolates. For more accurate identification of H7 antigen, Flich primer are used and offers a full O157:H7 antigen profile (Bonetta *et al.* 2011; Imtiaz *et al.* 2013). The aim of the current study was to diagnose *E. coli O157:H7* gene in drinking water by PCR.

MATERIALS AND METHODS

The current study has been conducted at the Microbiology Laboratory in Wasit University, Iraq.

Water sampling

In the present study, 15 samples of tap water were collected according to Ahmad and Bajahlan (Ahmad & Bajahlan 2009). Samples were obtained from 15 areas, in the period of May- June 2021. All samples were preserved in glass container (250 mL) and transported to the laboratory. Afterward, the physiochemical analyses of some parameters (total dissolved solids (TDS), pH, conductivity) was carried out according to Greenberg and Clesceri. (Greenberg & Clesceri 1992; AL Dulaimi & Younes 2017). In addition, microbiological and molecular detection of *E.coli* was performed. *E. coli* has a specific power as a biological sign because of its availability, low-cost, easy approaches, so it can be directly detected from water (Edberg *et al.* 2000).

The physiochemical analysis

pH assessment

The pH value of drinking water was measured by electrochemical technique via pH meter. (Islam *et al.* 2017)

TDS and conductivity Assessment

The TDS value of drinking water was measured via TDS meter. TDS meter assesses conductivity and TDS. TDS is assessed via water electrical conductivity. The water electrical conductivity is associated with the dissolved ionized solid concentrations within water. The solid dissolved ion in H_2O produces water capability of electrical conductivity. Electrical conductivity could be measured by TDS meter or conventional conductivity meter. In correlation to TDS assessment, conductivity support an estimated value for TDS concentration, generally within accuracy of 10%. (Islam *et al.* 2017).

Bacteriological examination

The bacteriological examination for drinking water is essential for the determination of the microbiological quality of water samples, as well as detection and the control of waterborne pathogens, for example *Vibrio cholera* and enterobacteriacea. (AL Dulaimi & Younes 2017). In the current study, water samples were obtained in sterile containers, preserved in the presence of ice, and examined within 6 h. All isolates of *E. coli* were isolated via the method of membrane filter method. Concisely, a volume of water (100 mL) has been filtered by sterilized filter

(0.45 µm) pore size, then, filter was positioned on Eosin Methylene Blue agar, followed by incubation at 37 °C for 24 h. Next day, colonies having green metallic sheen were recognized as *E.coli* isolates. The growth results was documented for each *E.coli* isolate. All *E.coli* isolates were diagnosed biochemically by API 20E test. (AL Dulaimi & Younes 2017).

Molecular identification

All *E. coli* samples were identified using PCR technique. Genomic bacterial DNA was extracted according to the instruction of Geneaid Extraction kit (Geneaid Biotech Ltd., Taiwan). All samples were subjected to amplification of 30 cycles. The program of PCR was as follows: denaturation (95 °C for one min), annealing (55.5 °C for two min), extension (72 °C for one min). Then, the PCR product was subjected to gel electrophoresis on 1.5% agarose gels. Afterward, the PCR product was subjected to safe view stain (ABM) and UV-trans-illuminator visualization. (Gunzer *et al.* 1992) The DNA ladder (Promega) 100-1500 bp was used to define the PCR product size.
The incidence of *E. coli* O157:H7 gene was detected using these primers which are:
Rfb-F (GTGTCCATTTATACGGACATCCATG), and
Rfb-R (CCTATAACGTCATGCCAATATTGCC). (Imtiaz *et al.* 2013).

RESULTS AND DISCUSSION

In fact, The excellence of drinking water depends on water origin, handling and storage route and circulation grid (Batarseh 2017). The levels of the physiochemical factors are shown in Table 1. The pH of water is a significant quality indicator of water. In the current study, the pH values ranging from 6.5-8.5, was in agreement with the Environmental Protection Agency (EPA) criteria (Hendrickson 2017). Moreover, no significant differences were found between the sampling locations. Consequently, it may be established that water processing and refining procedures has a minor influence on the concentration of H^{+1} ion. The pH is an excellent sign of water hardness or softness. The pH value of pure water is 7 (Hendrickson 2017). Furthermore, the TDS concentration and electrical conductivity (EC) of drinking water from various locations did not display substantial differences, ranging from 490 - 780 mg L^{-1} and 1000-1309 µS cm^{-1} respectively. The TDS concentration significantly diverse in different areas as a result of the variances in mineral solubility as well as indigenous circumstances, e.g., water origin in addition to handling route. Even though the concentrations of TDS in Al-Kut City were in satisfactory values. Generally, water with a TDS value < 300 is healthy, while with a TDS > 1000 is undesirable (Faysal & Juliana 2017). On the other hand, the variances in TDS and EC values in various areas in Al-Kut City might be in association with differences in the drinking water origin and handling procedures.

Table 1. Physiochemical parameters and presence of *E.coli.*

Water source	EC, µS cm^{-1}	TDS, mg L^{-1}	pH	Presence of *E.coli*
District-1	1119	580	6.7	+
District-2	1282	490	6.6	+
District-3	1283	590	6.6	+
District-4	1287	550	6.7	+
District-5	1067	780	6.8	+
District-6	1309	760	8.5	+
District-7	1250	740	6.5	-
District-8	1257	780	8.5	+
District-9	1287	670	6.8	-
District-10	1282	780	6.7	-
District-11	1254	670	6.8	+
District-12	1120	740	6.9	-
District-13	1058	500	6.8	-
District-14	1000	490	6.8	-
District-15	1254	590	6.8	+

Bacteriological examination

The existence of coliform bacteria in water reveals the probability of water pollution by microorganisms found in faeces. The results of this study display the *E.coli* existence in all 15 districts. The *E. coli* existence in water is a sign of faeces pollution. The incidence of faeces pollution is an indicator of a probable health hazard to people using that contaminated water (Bain *et al.* 2014).

Molecular detection of O157 gene by Rfb primers

In this study, O157 gene was amplified and appeared in 10 out of 15 samples. The bands appeared in the band size 292 bp at 55.5 ºC (Fig. 1).

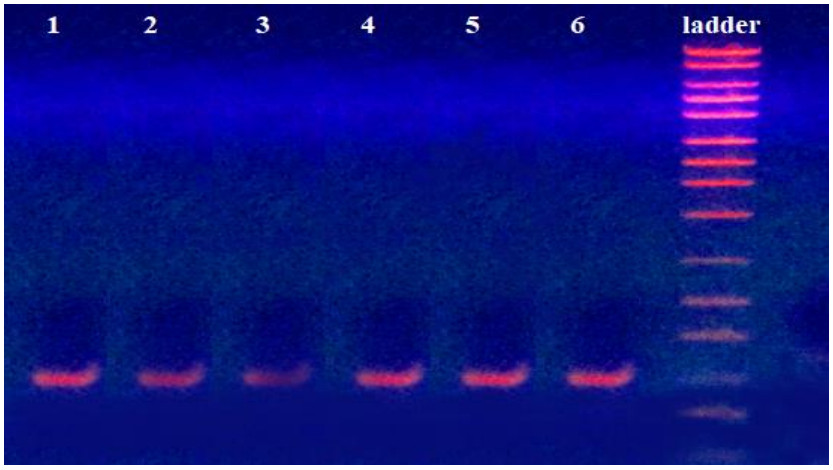

Fig. 1. Agarose gel electrophoresis result for PCR bands of *E. coli* O157:H7, Lane-7 displaying ladder, lane (1, 2, 3, 4, 5 and 6) and O157 gene (292bp) obtained by Rfb primers.

This study improved a procedure for microbial investigation of drinking water as well, to study the incidence of O157 gene in the water. The Rfb primer was amplified at 55.5 ºC, via setting the parameters such as the concentrations of template, Mg^{+2} ion, and primer along with annealing temperature, and a good bands of PCR product gained. Accordingly, all the aforementioned parameters are effective on Taq DNA polymerases activity. (Innis *et al.* 1990). The checking of *E. coli* existence in the drinking water is essential to confirm the community health. *E. coli* O157:H7 generally is accompanying with occurrences of human infections. Its clinical signs are intestinal problems, for example abdominal pain and diarrhoea. (Tsen & Jian 1998) The PCR for O157 gene recognition in *E. coli* gives a potent enhancement to the routine approaches for a precise risk evaluation and checking pathogens in drinking water. (Parma *et al*. 1996). In this study, was recognized the RfbE gene encoding the enzyme which plays a role in O157 antigen biosynthesis. This gene was deviated from rfb locus encodes O antigen. The Rfb could not distinguish the O157:H7 from other O157. So, Flich primers are useful to distinguish H7 antigen in future studies, as well as offering a whole description of O157:H7 antigen. (Bonetta *et al.* 2011).

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