original article

Detection of *E. coli* O157: H7 by immunological and real-time PCR methods in the water treatment plant

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ABSTRACT

Aims: There is limited data on the occurrence of *E. Coli* O157: H7 in water. Therefore, this study aims to detect *E. Coli* O157: H7 in the Water Treatment Plant (WTP).

Materials and Methods: This cross-sectional study was conducted in Isfahan WTP, central of Iran. Immunological methods were implemented with anti-serum kits and the molecular method of reverse transcription-polymerase chain reaction (RT-PCR) was used to detect *E. Coli* O157: H7 in eight locations of the WTP; the sludge of the sedimentation basin and filter backwash water were also monitored. The survival of *E. Coli* O157: H7 in the sludge samples of the sedimentation basin was indicated by the formation of agglutination particles using the immunological method, and through indicator probes using the RT-PCR method.

Results: *E. Coli* O157: H7 was not detected in the water samples from the WTP units. The removal percent of *total coliforms* (TC), *fecal coliforms* (FC), and *Heterotrophic Plate Count* (HPC), respectively, were as follows: 59.5, 49, and 54.8% in the sedimentation basin; 66, 45.8, and 57% in the ozonation system; 98.8, 98, and 78.8% in the filtration system; and 96, 100, 91% in the disinfection system.

Conclusions: This study revealed the existence of the pathogenic coliform of *E. Coli* O157: H7 in the sludge of the sedimentation basin. The absence of *E. Coli* O157: H7 in the finished water indicated that the WTP units were able to eliminate these pathogenic bacteria before reaching the final units of the plant, including the filtration and disinfection systems.

Key words: *E. Coli* O157: H7, HPC, real-time polymerase chain reaction, total and fecal coliform, water treatment plant

INTRODUCTION

From a microbiological perspective, the primary objectives of drinking water treatment are to ensure the absence of any pathogenic bacteria in the finished product and limit any uncontrolled growth during the distribution of water. Historically, there has been a relationship between the incidence of disease and water quality. According to the reports of the World Health Organization (WHO), one-third of the world's population suffering from disease is afflicted by contaminated drinking water. Every year, about13 million people die as a result of waterborne infections, approximately two million of whom are children.[1]

In 1996, Grabow introduced heterotrophic bacteria as an index of the quality of drinking water. The Heterotrophic Plate Count (HPC) in drinking water has been determined to be between 100 and 500 Colony Forming Units per ml (CFU/ml).[2]

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Escherichia coli (*E. Coli*) is abundant in water and is one of the most resistant pathogens that can be transmitted through water. Therefore, *E. Coli* is used as an indicator to determine the level of pollution by wastewater in the drinking water. The detection of *E. Coli* in the water samples indicates inadequate filtration, disinfection, and contamination after treatment.[3]

At present, the upgrading of water treatment plants is considered the main objective for the optimal control of plant unit performance. It prevents the spread of waterborne diseases. In some cases of such diseases, contamination of public water systems by *E. Coli* O157: H7 has been reported.[4] *E coli* O157: H7 has been the most important factor worldwide in food- and waterborne diseases, in the last 20 years.[5] Its first outbreak through drinking water, in the USA, was reported in 1989. Contamination of drinking and recreational water by *E. Coli* O157 has emerged as an important cause of human disease.[6]

The concern of the WHO, in 1997, was the prevalence of *E. Coli* O157: H7, as shigellosis was one of the important causes of mortality among children in developing countries such as South Africa.[7] The incidence of *E. Coli* O157: H7 in drinking water sources in Ontario, Canada, resulted in the infection of 2300 people and seven deaths.[8] Therefore, it is necessary to control water resources, to detect microbial pathogens, for public health protection. Water supply facilities, especially water treatment plants, should consider appropriate treatment processes according to the water type, raw water quality, and preliminary water treatment units, as important parameters.

Gastrointestinal pathogens such as *E coli* O157: H7 are generally present in very low concentrations in water resources, and their presence in drinking water must be considered at least as a potential threat of microbiological water quality deterioration.[9] It is alarming that the ingestion of only 10 to 100 organisms of this type may be sufficient to cause an infection.[10]

Most human infections related to *E. Coli* are caused by the consumption of contaminated water and food. Water is considered as an important source of contamination of enterotoxigenic *E. Coli* (ETEC) and enterohemorrhagic *E. Coli* (EHEC). *E. Coli* can also produce Shiga-like toxins. These toxins (stx_2, stx_1) can demolish the epithelial cells of the intestinal lining, damage red blood cells, induce hemorrhagic colitis, destroy the kidneys, and cause blood clots in the brain, which may lead to paralysis.[11]

Although detection of *E. Coli* O157H7 from drinking water has been reported worldwide, there is very little data on the prevalence of this microorganism in water, in Iran. Therefore, the objective of the current study is to detect *E. Coli* O157: H7 by using microbiological, immunological, and real-time polymerase chain reaction (PCR) methods in the intake and various units of the Isfahan Water Treatment Plant. In addition we analyzed the trend in eliminating TC, FC, and HPC.

MATERIALS AND METHODS

This cross-sectional study was conducted over a time period of nine months in the intake, processing, and operation units of the water treatment plant in Isfahan, Iran, with a water flow rate of 12.5 m^3/s, through two phases (6 m^3/s and 6.5 m^3/s) and four streams that produced potable water for approximately four million people. The sampling locations are specified in eight points in the schematic diagram shown in Figure 1. A total of eight samples was collected.

Microbiological method

Total coliforms

FC and HPC were counted according to the standard methods.[12] In the microbiological method, Lactose Broth and Brilliant green cultures were used for Total coliforms (TC) in all presumptive and confirmed tests, EC-broth for FC and R_2 Agar for HPC. TC and FC were reported as the most probable numbers (MPNs).

Immunological method

In addition to the eight sampling locations mentioned above, raw river water, waste sludge from the sedimentation tank, and filter backwash water were also analyzed for *E. Coli* O157: H7 detection. MPN tubes showing growth were inoculated onto MacConkey and sorbitol-MacConkey(SMAC) agar (Oxoid) for confirmation, because of its non-sorbitol fermenting properties. *E. coli* O157: H7 produced colorless colonies on the SMAC agar and EMB agar plates.

For the detection of Antrobacteriacea, differential tests have been performed on three media: Triple sugar iron (TSI) agar, citrate, and SIM (sulfide-indole-motility). *E. Coli* O157: H7 is one of the hundreds of species of *E. Coli* bacteria that can ferment lactose. It is indistinguishable from other *E. Coli* species in cultures containing lactose. *E. coli* isolates have

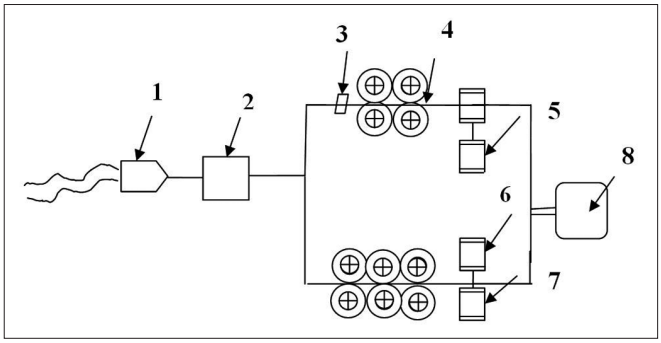

Figure 1: Schematic design of the water treatment plant and sampling locations in this study, including: 1- Intake, 2- Raw water influent, 3- Ozonation, 4- Clarifier, 5- Filter II, 6 -Filter III, 7- Filter IV, 8 -Treated water

been subjected to serotyping by the slide agglutination test.

Unlike the other (approximately) 80% of the *E. Coli* species, most strains of *E. Coli* O157: H7 did not ferment sorbitol. To detect this organism, the suspected colonies in the sorbitol MacConkey agar medium were cultivated for 18 to 24 hours, at a temperature of 35°C±0.5°C. These environmental conditions facilitated the production of colorless colonies, because the bacteria were slow fermenters of sorbitol. In this study, *E. Coli* O157: H7 NCTC 12900 was used as a positive control sample. Using anti-serum kits for these bacteria, the colorless colonies grown were examined with respect to agglutination formation.

Real-time polymerase chain reaction method

Colonies in non-fermentative sorbitol were used for the ultimate detection of *E. Coli* O157: H7. The primers used to amplify the fragments of antigenic and virulent genes are shown in Table 1.

The TaqMan probes used in this method were oligonucleotides that contained fluorescence material at the 5′ end and a quencher marker at the 3′ end.[10]

DNA extraction

DNA extract buffer, proteinase K enzyme, and SDS 10% were added to 200 µl of the centrifuged sample and were then placed in a 60°C water bath for two hours. Next, the phenol-chloroform extraction method was performed as follows.

For the PCR, $MgCl_2$, specific primer probes, dNTPs, Taq polymerase enzyme, and buffer were added to the tubes and they were placed in a teal-time PCR machine (Corbett Research Model 6600) after mixing. To determine the PCR spectra of the samples, the samples were placed in a cycle of 95°C for four minutes, 40 cycles of 94°C for 15 seconds, 60°C for one minute.

Turbidity and total organic carbon

Six samples were tested to determine turbidity and total organic carbon (TOC). In this study, the HACH-2100N model (England) turbidity meter and the SHIMADZU TOC-VCSH model (Japan) TOC-measuring device were used.

Table 1: The sequences of the oligonucleotide, primers, and probes used in this study

Primer	Oligomer	Primer sequence 5′-3′
EO157 eaeA	F	CAATTTTTCAGGGAATAACATTGC
EO157 eaeA	R	AAAGTTCAGATCTTGATGACATTG
Probe		FAM-TCAAGAGTTGCCCATCCTGCAGCAA-BHQ1

RESULTS

Microbiological method

The results for TC and FC in terms of MPN/100 ml and HPC in terms CFU/ml at the eight sampling points are represented in [Figure 2a-c].

The performance profiles of different units of the WTP in terms of the elimination of the total and fecal coliforms, HPC, turbidity, and TOC are displayed in Table 2.

Turbidity and total organic carbon

Findings related to the trend of the turbidities and TOC in the WTP units are displayed in Figure 3.

Immunological method

The positive and negative controls and positive sample of the sludge from the sedimentation basin are shown in Figure 4.

Real-time polymerase chain reaction method

A graph of the real-time PCR results is presented in Figure 4.

In this figure, the baseline or comparative threshold (CT) represents the difference between the positive and negative samples. The number of cycles and diffused fluorescence are indicated along the horizontal and vertical axes, respectively. The diagram of the baseline (CT) shows that the samples related to the sludge from the sedimentation basin in the water treatment plant produce a positive response to the cycle numbers below 40.

DISCUSSION

Figure 2a-c, show that with seasonal conditions and the treatment unit used, the efficiency of the WTP for the removal of TC, FC, and HPC varies. As shown in Figure 2b the HPC count of one to three colonies per milliliter, during spring, represents the worst effluent condition of filter Nos. 2, 3, and 4 and was zero for the finished water of the WTP. The average removal of microbes 1 log by coagulation and clarification, 2 logs by filtration, and 3 logs by disinfection resulted in an average removal of 6 logs for the entire treatment process.[13] Winter [Figure 2a] represented the best seasonal conditions, during which the HPC count was

Table 2: Performance profile of the units of the water treatment plant to remove

Sampling point	TC	FC	HPC	TOC	Tur.
Clarifier	59.5 ± 23	49 ± 22.6	54.8 ± 29	1.5 ± 0.3	2.2 ± 0.7
Ozonation	66 ± 31.4	45.8 ± 27	57 ± 26.5	1.3 ± 0.3	2.2 ± 0.5
Filtration	98.8 ± 1.2	98 ± 1.7	78.8 ± 18	0.9 ± 0.3	0.6 ± 0.6
Disinfection	96 ± 14.6	100	91 ± 21.3	0.9 ± 0.3	0.6 ± 0.5

TC, FC, HPC, turbidity, and TOC (in percent)

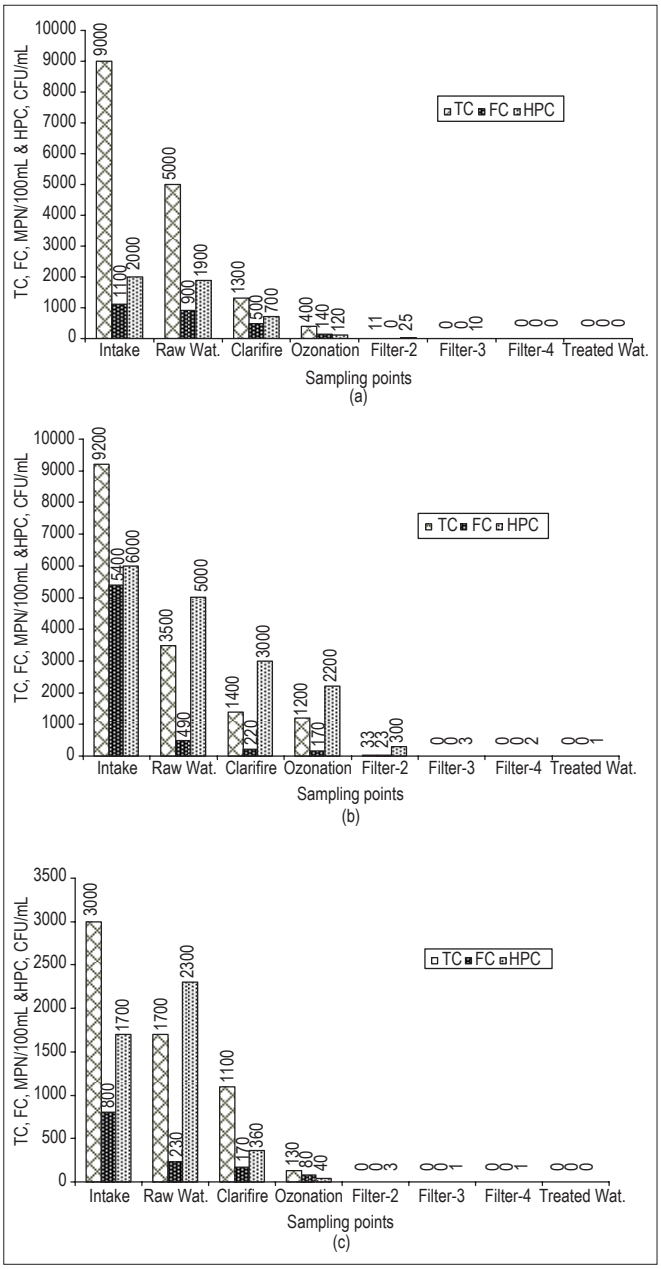

Figure 2: Total and fecal coliforms and heterotrophic counts in the units of the water treatment plant in the three seasons: (a) winter, (b) spring, and (c) summer

zero in the finished water of the WTP. In Switzerland, the legal limit of HPC bacteria was set at 20 to 300 CFU/ml after treatment and within the distribution system, respectively. This range met the worldwide accepted guidelines, such as those set by the WHO.[14]

According to Table 2, the efficiency of the sedimentation unit (including coagulation and flocculation) in eliminating TC, FC, and HPC was 59.5% (0.77 log), 49% (0.69 log), and 54.8% (0.74 log), respectively. These log removals are close to the proposed 1 log microbial reduction by coagulation and clarification.[13]

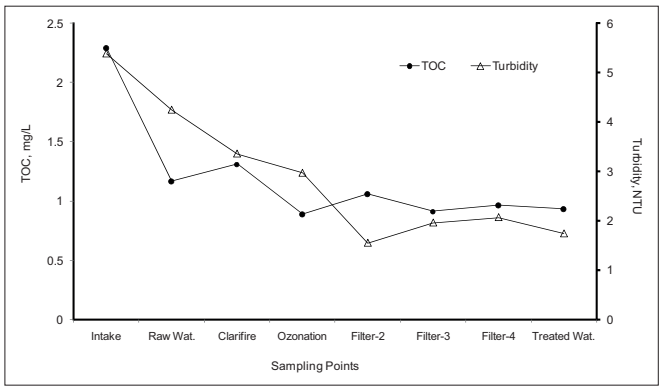

Figure 3: The profile of turbidity and TOC changes in the units of the water treatment plant

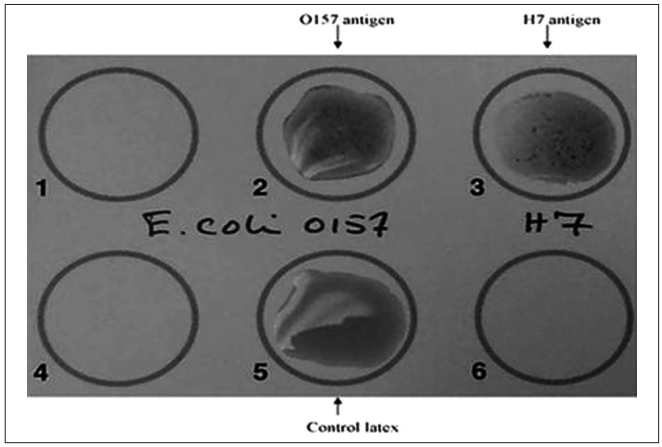

Figure 4: Seroagglutination test samples of sludge from sedimentation basin in the water treatment plant

In one study, the average microbial elimination by the coagulation and flocculation units was reported to have been32 to 87% for bacteria and 27 to 74% for viruses.[12] These data agree with the findings of the current study.

Given the fact that ozone is used in two stages of the first phase of the WTP, it is expected to affect the removal of TC, FC, and HPC.

Table 2 reveals a reduction of 2 logs for TC and FC and 1 log for HPC through the filtration units of the WTP. This can be compared with the 2-log microbial reduction reported in literature.[13]

The profiles of the turbidities and TOC changes in the units of the WTP [Figure 3] show that the amount of dissolved organic carbon reaches values ranging from 2.5 mg/l raw water to less than 1 mg/l in the finished water. These values approach the criterion of 2 mg/l of TOC for treated waters.[15]

Moreover, in our study, the turbidity of raw water was determined to be more than 5 NTU. Under undesirable conditions, the turbidity of treated water and the effluent

of filter number 2 reached less than 2 NTU. The turbidity of the filtered water had to be less than 0.3 NTU in 95% of the measurements performed during one month and should not exceed 1 NTU.[15]

Immunological method

Negative sorbitol colonies are colorless in the SMAC medium. In the samples cultivated in this study, only in the sludge of the sedimentation basin, colorless colonies have been observed.

The formation of agglutination particles with the antiserum of *E. Coli* O157: H7 was only observed in the sludge samples of the sedimentation basin.

Muller (2001) studied 204 samples from15 locations in South Africa to detect suspected *E. coli* culture colonies in the SMAC medium and examine the samples in terms of coagulation with the antiserum of the bacteria. The results obtained in that study indicated that there was an infrequent incidence of *E. Coli* O157: H7 in the water examined, suggesting that the likelihood of acquiring disease through the ingestion of these waters was low.[7]

Real-time polymerase chain reaction method

The DNA probes are useful molecular tools for identifying specific microorganisms in the water and surrounding environment.[16] In this research, experiments carried out by RT-PCR confirmed the existence of positive samples in the agglutination method. Figure 5 shows a graph of the RT-PCR results.

The CT baseline, in lines 3 and 4 of Figure 5, indicates that the sludge samples of the sedimentation basin of the WTP are below40 cycles and show a positive response. The CT values equal to 40 or above do not indicate any increase, and this value is not added to the calculations.[16]

Jin (2005) has detected *E. Coli* bacteria using a rapid and sensitive molecular method and by using microarrays.[17] Specific oligonucleotide probes of the infection genes of *E. Coli* O157: H7 have been identified by Jordan in 45 samples from water sources using the PCR method.[4] Hammes was able to determine the concentration of bacteria in water samples from treatment plant units using nucleotides labeled with Cyber Green I.[18]

This study determined the presence of *E. Coli* O157: H7 infectious strains in the sludge sedimentation of the WTP. The lack of this strain in the treated water of the WTP indicated that the various processes performed by treatment plant units were able to remove *E. Coli* O157: H7 before the water reached the final treatment processes, such as filtration and disinfection. The use of an immunological method confirmed this contamination.

The TC, FC, and HPC counts showed a declining trend from water intake to the finished water output, which showed the

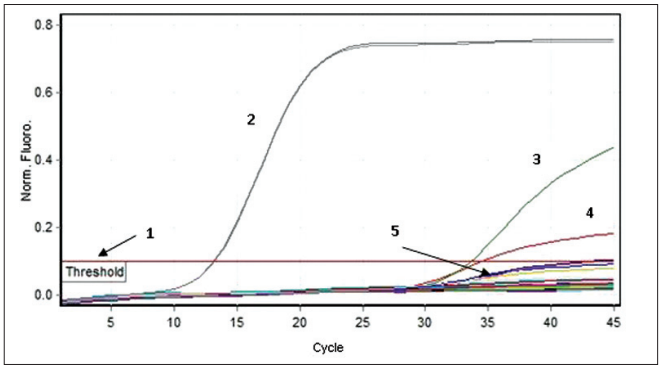

Figure 5: The finding of Real time PCR in units of the water treatment plant, including: 1 - line threshold comparison or base line (Comparative Threshold: CT); 2 - PCR graphs of the positive control; 3 - positive sample sedimentation sludge number 1;4 - positive sludge sample sedimentation sludge number 2; 5 - negative samples from 15 points, including raw water of the river, eight points of sampling [Figure 1] the sludge of another sedimentation basin, waste water from washing reverse filters, and sludge from water disinfection

effective performance of different treatment plant units in decreasing the microbial load of raw water.

The undesirable efficiency of some units of the treatment plant, such as the sedimentation basin and some filters, in removing coliforms, could be attributed to the operating conditions of the units, weather conditions, and river water quality.

Watershed protection is suggested as the optimum method for preventing the entrance of *E. Coli* O157: H7 into the WTP.

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