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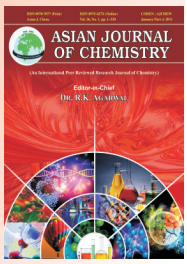

Some Physical and Chemical Characteristics, Molecular Typing of Isolated *E. coli* Strains of Fountain Waters, Turkey

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In this study, water samples collected from public fountains in Erzurum city centrum were analyzed and their drinking quality was evaluated. A total of 45 water samples were subjected to microbiological, chemical and physical analyses. Microbiological analysis results showed that total and fecal coliform bacteria were found in 10 samples (22.7 %) and *Clostridium perfringens* in only one sample. In addition, *Escherichia coli* were isolated from these 10 samples. RAPD-PCR technique was used for confirming phylogenetic diversity among *E. coli* species isolated from various fountains. Six of 45 typical *E. coli* isolates were determined to be different strains. Average colour, water temperature, turbidity, electrical conductivity, pH, dissolved oxygen, chloride, nitrite, nitrate, sulphate, phosphate, sodium, ammonium, potassium, magnesium, calcium, iron and manganese values of the samples were determined as 1.04 mg Pt L^{-1}, 21.43 ºC, 0.14 NTU, 456.80 µS cm^{-1}, 7.26, 5.36, 3029, 0.12, 2.09, 50.48, 22.71, 35.91, 0.32, 14.99, 31.20, 105.00, 0.16 and 2.64 mg L^{-1}, respectively.

Keywords: Fountain water, *E. coli*, Coliform bacteria, RAPD-PCR.

INTRODUCTION

Being an indispensable source for the existence and maintenance of life and a source which is in constant circulation in the nature, water can be contaminated with some physical, chemical and microbiological agents until it is delivered to the consumption point[1-3]. For this reason, provision of consumers with a quality and safe drinking water is very important for public health. The quality of water provided for the consumers, to a large extent, depends on the quality of water source, showing the necessary sensitivity to every stage from water's treatment to its distribution and the provision of appropriate water quality by taking drinking water standards into consideration[4].

Exposure of waters, which are to be used as drinking water, to contamination because of various reasons affects public health as much as it affects potability of the water[5,6]. As a matter of fact, various disease causes (*Salmonella typhimurium, E. coli, Aeromonas hydrophyla, C. perfringens* and *Shigella*) can be transmitted to humans through unhygienic waters [7]. According to World Health Organization 80 % of diseases in developing countries are reported to result from drinking water[8].

The best way to determine water's consumability quality in terms of health is to investigate the existence of microorganisms which indicate the existence of enteric bacteria in the water. The bacteria indicating that water is contaminated with feces are coliform bacteria such as *E. coli* which are members of *Enterobacteriaceae* family, which can reproduce at 44.5 ºC and are able to ferment lactose by forming acid and gas, indol (+) and tolerant of temperature and such as *Escherichia*, *Citrobacter*, *Enterobacter*, *Klebsiella* which are able to ferment lactose at 37 ºC[9]. Coliform bacteria are accepted as an appropriate microbial indicator in determining water quality for coliform bacteria can be easily detected and counted in water[10,11]. Drinking water with enteric bacteria also causes transmission of these bacteria among individuals[12-15].

Many analysis methods have been developed for microbiological control of waters. However, devices and methods utilized for this purpose are different for almost each microorganism and the existing analysis methods yield results in a long period of time for they are generally for cultivating microorganisms in the culture medium. It was discovered that drinking water can include many other microorganisms in addition to microorganisms which are called as indicator microorganisms and considered to reveal water's microbiological quality. All these facts revealed the need for safe and rapid analysis methods[16]. For this reason, polymerase chain reaction (PCR), which is a rapid method, is widely used today[17].

In this study, it was aimed to determine the usage and drinking quality of drinking water samples collected from some of the most frequently used public fountains in Erzurum city centrum through physical, chemical and microbiological analyses and to carry out the molecular typing of isolated *E. coli* strains by using RAPD-PCR method.

EXPERIMENTAL

Water samples: Two samples were taken from each one of the selected fountains for the study. These samples were put in sterile sample vessels, taken right away to the Microbiology Laboratory of the Department of Food Engineering in Atatürk University and kept at +4 ºC until the analysis.

Physical and chemical analyses: pH values of waters were detected at 25 ± 3 ºC using a laboratory-type pH meter (Hanna 211 pH-Meter)[18]. Turbidity measurement was realized by using a HF Mikro1000 (HF Scientific, USA) brand turbidimeter in accordance with the method expressed in Anonymous[19] and colour measurement was realized by using a DR 5000 spectrophotometer device (Hach, USA)[20]. A laboratory-type conductivity measurement device (Crison, Spain) was used to measure electrical conductivity[21]. Photoflex (WTW 82362n Weilheim, Serial Number 06440414, Germany) device was used in hardness analysis and an oxygen meter device (Thermo Scientific Orion Star, Singapore) was used to detect the amount of dissolved oxygen[22].

The amounts of free chlorine were detected by using Spectroquant (Merck) ready kits[22]; the amounts of nitrite, nitrate and ammonium were detected by using Dionex 3000 Ion Chromatography device[23]; the detection of other minerals, however, was realized by using a Thermo ICP-OES (Thermo Scientific, USA) device in accordance with the method given by Jenniss *et al.*[24] and Rubinson and Rubinson[25].

Microbiological analyses: Hydrophobic grid membrane filtration (HGMF) technique was used in the enumeration of total and fecal coliforms[26]. m-FC and m-ENDO media were used for isolation of fecal and total coliform. Filters were left to incubation on m-FC (Merck) at 44.5 ºC for 24 h and m-ENDO (Merck) media at 37 ºC for 24 h. At the end of incubation, enumerations were realized in these media[27]. *Clostridium perfringens* samples were directly added egg yolk after they were infiltrated and their cultivation was carried out on TSC (Tryptose Sulphite Cycloserine) (Merck) medium. After this medium became solid, a second layer (that does not contain egg yolk emulsion) of nearly 10 mL of the same medium was placed onto the cultivated petri plates and it was incubated under anaerobic conditions at 37 ºC for a period of 24 h. At the end of the incubation, black colonies with a diameter of 2-4 mm, around which is an opaque zone depending on lecithinase activity, were enumerated as *C. perfringens*[28,29]. For the detection of *E. coli* strains, colonies showing positive feature on m-FC and m-ENDO media were taken and they were cultivated on EMB (Merck) culture medium for confirmation of isolates. EMB agar plates were incubated at 37 ºC for a period of 48 h and metalic green colonies were evaluated as being suspicious *E. coli.* DNA isolation was carried out from the suspicious *E. coli* strains in accordance with the method expressed by Günal[30]. Amplification process was realized in a Thermo-Cycler (USA) device in accompany with $(GTG)_5$ primer with RAPD-PCR Method[22]. Amplified PCR samples were submerged in agarose gel electrophoresis at 70 V for a period of 1-1, 5 h and outcomes were obtained with a UV Transilluminator Screening device (Ultralum, USA)[22]. Genetic analysis was confirmed by using an *E. coli* GM 1402 strain. The strain obtained from Department of Food Engineering, Atatürk University, Erzurum, Turkey.

Statistical analysis: SPSS for Windows (Release 12.0) was used to statistically analyze the data obtained in the study, average values and their standard deviations were determined.

RESULTS AND DISCUSSION

Physical and chemical analyses: In the Table-1 are given the lowest, the highest and average colour, temperature, electrical conductivity and turbidity values of water samples which collected from some of the most frequently used public fountains from which Erzurum Metropolitan Municipality Water and Sewerage Administration collects samples in certain periods for control and analysis. As it is seen in the Table-1, the lowest, the highest and average colour, turbidity, electrical conductivity and temperature values of samples were determined as 0.12, 3.45, 1.04 mg Pt L^{-1}; 0.02, 0.45, 0.14 NTU; 141, 847 and 456.80 μS cm^{-1}; 17, 23 and 21.43 ºC, respectively.

TABLE-1
COLOUR, TURBIDITY, ELECTRICAL CONDUCTIVITY AND TEMPERATURE VALUES OF FOUNTAIN WATERS

Parameters	N	Lowest	Highest	Average
Colour (mg Pt L^{-1})	45	0.12	3.45	1.04±0.76
Turbidity (NTU)	45	0.02	0.45	0.14±0.10
Electrical conductivity (μS cm^{-1})	45	141.00	847.00	456.80±202.51
Temperature (ºC)	45	17.00	23.00	21.43±1.83

These values were found to be higher than the values given in Regulation concerning Water Intended for Human Consumption given in TS 266[31]. Agaoglu *et al.*[5] collected 30 water samples from 15 different sources in Van province and its vicinity and detected that only spring waters were completely colourless, odorless, clear and in normal taste. Günsen *et al.*[1] detected in his study where he examined water quality of 28 different spring water in Bursa Uludag that the samples taken from the outlet point comply with "Alimentary Products Law" in terms of colour, turbidity, odor and sediment. Abali *et al.*[32] collected water samples from 16 different points of Kula and Gökçeören's vicinity and detected that turbidity values of samples change between 0.20-0.37 NTU and electrical conductivity values of samples change between 396.00-699.00 μS cm^{-1}.

The pH, dissolved oxygen, chloride, nitrite, nitrate, sulphate, phosphate, sodium, ammonium, potassium, magnesium, calcium, iron and manganese values of water samples are summarized in Table-2. pH values of water samples were detected to change between 6.80-7.73 with an average of 7.26 mg L^{-1}; dissolved oxygen amounts to change between 4.80-6.30 with an average of 5.36 mg L^{-1}; chloride amounts to change between 16.22-58.17 with an average of 30.29 mg L^{-1}; nitrite amounts to change between 0.00-0.35 with an average of 0.12 mg L^{-1};

TABLE-2
pH, DISSOLVED OXYGEN AND SOME IMPORTANT MINERAL LEVELS OF FOUNTAIN WATERS

Parameters	N	Lowest	Highest	Average
pH	45	6.80	7.73	7.26 ± 0.175
Dissol. oxygen (mg L^{-1})	45	4.80	6.30	5.36 ± 0.313
Chloride (mg L^{-1})	45	16.22	58.17	30.29 ± 10.167
Nitrite (mg L^{-1})	45	0.00	0.35	0.12 ± 0.163
Nitrate (mg L^{-1})	45	0.43	5.91	2.09 ± 0.941
Sulphate (mg L^{-1})	45	24.66	72.20	50.48 ± 9.953
Phosphate (mg L^{-1})	45	13.75	27.38	22.71 ± 3.019
Sodium (mg L^{-1})	45	21.38	63.78	35.91 ± 9.450
Ammonium (mg L^{-1})	45	0.01	0.91	0.32 ± 0.186
Potassium (mg L^{-1})	45	3.76	40.86	14.99 ± 7.619
Magnesium (mg L^{-1})	45	7.22	48.91	31.12 ± 8.436
Calcium (mg L^{-1})	45	29.66	158.57	105.00 ± 27.874
Iron (mg L^{-1})	45	0.00	2.42	0.16 ± 0.479
Manganese (mg L^{-1})	45	1.10	6.80	2.64 ± 0.929

nitrate amounts to change between 0.43-5.91 with an average of 2.09 mg L^{-1}; sulphate amounts to change between 24,66-72,20 with an average of 50.48 mg L^{-1}; phosphate amounts to change between 13.75-27.38 with an average of 22.71 mg L^{-1}; sodium amounts to change between 21.38-63.78 with an average of 35.91 mg L^{-1}; ammonium amounts to change between 0.01-0.91 with an average of 0.32 mg L^{-1}; potassium amounts to change between 3.76-40.86 with an average of 14.99 mg L^{-1}; magnesium amounts to change between 7.22-48.91 with an average of 31.12 mg L^{-1}; calcium amounts to change between 29.66-158.57 with an average of 105.00 mg L^{-1}; iron amounts to change between 0.00-2.42 with an average of 0.16 mg L^{-1} and manganese amounts to change between 1.10-6.80 with an average of 2.64 mg L^{-1}.

It was detected that pH values and dissolved oxygen amounts of water samples comply with the values and amounts given in Regulation concerning Water Intended for Human Consumption. Dönderici *et al.*[32] found out that pH value of spring waters to be between 6.71 and 8.21. pH values of spring waters were detected to be 6.95-8.16 in Van province and its vicinity[5]; 6.8-7.4 in spring waters of Bursa Uludag[1]; 6.5-8.2 in Bursa Metropolitan Municipality spring waters[33] and 7.7-8.0 in Harbiye spring waters[34]. Alemdar *et al.*[33] collected 164 storage water and municipial water samples from Bitlis city centrum and its districts and detected pH values of these water samples to be at the level of 7.41 averagely. These results are parallel with our results. Abali *et al.*[31] reported pH values to change between 7.3 and 7.7 in a similar study.

As it is clearly seen in the Table-2, nitrite amounts of fountain waters were detected to be between 0.00-0.35 mg L^{-1}, nitrate amounts to be between 0.43-5.91 mg L^{-1}, chloride amounts to be between 16.22-58.17 mg L^{-1}, sulphate amounts to be between 24.66-72.20 mg $L^{-1}$and sodium amounts to be between 21.38-63.78 mg L^{-1}. These values were detected to comply with surface waters parametric values given by the Ministry of Health and Turkish Standards Institution (TS 266). Günsen *et al.*[1] detected nitrite in 7.03 % of municipial and drinking water samples in the study carried out in Bursa. Abali *et al.*[31] detected nitrite, nitrate, chloride and sulphate values of water samples that they examined in Kula and Gökçeören to be at the levels of 0.003-0.00, 3.05-9.32, 21.5-4.70 and 14.70-39.00 mg L^{-1}, respectively.

Phosphate, ammonium, potassium and magnesium values of water samples were detected to be between 13.75-27.38, 0.01-0.91, 3.76-40.86 and 7.22-48.91 mg L^{-1}, respectively. These figures were determined to be higher than the figures given in Regulation concerning Water Intended for Human Consumption.

Alemdar *et al.*[33] collected water samples from Bitlis province and its districts and detected magnesium values in these samples to be lower (3.81 mg L^{-1}) than drinking water samples of Güroymak district. Abali *et al.*[31], however, detected magnesium values in water samples to be between 10.1-92.94 mg L^{-1}.

Calcium, iron and manganese values of samples showed change between 29.66-158.57, 0.00-2.42 and 1.10-6.80 mg L^{-1}, respectively. These figures are parallel with the surface waters parametric values of the Ministry of Health and Turkish Standards Institution. Abali *et al.*[31], however, detected calcium, iron and manganese values of samples to be between 161.9-283.4, 0.00-0.01 and 10.1-92.94 mg L^{-1}, respectively. Dönderici *et al.*[32] detected iron values to be lower than the limiting value (1 mg L^{-1}) in 16 water samples and iron values to change between 2.40-86.64 mg L^{-1}in 14 water samples and reported these figures to be in compliance with the regulation. The research values that we obtained show similarity with the research results of Günsen *et al.*[1], Agaoglu *et al.*[5] and Abali *et al.*[31].

Microbiological analysis results: In Table-3 are given the lowest, the highest and average (per each/100 mL) numbers of total coliform, fecal coliform and *C. perfringens* belonging to analyzed 45 fountain waters.

TABLE-3
THE NUMBERS OF TOTAL COLIFORM, FECAL COLIFORM AND *C. PERFRINGENS* OF WATER SAMPLES

Parameters	N	Lowest	Highest	Average
Total coliform	45	0.00	98.00	6.87 ± 18.918
Fecal coliform	45	0.00	41.00	3.69 ± 8.878
C. perfringens	45	0.00	5.00	0.11 ± 0.745

As a result of microbiological analyses, the total numbers of total coliform, fecal coliform and *C. perfringens* in 45 samples were detected to be 10 (22.7 %), 10 (22.7 %) and 1 (2.3 %), respectively. Alemdar *et al.*[33] detected the positiveness rate of coliform bacteria in 164 samples collected from storage and municipal waters in Bitlis province and its districts to be 12 % (19/164). Günsen *et al.*[1] detected coliform bacteria in 7 samples out of 100 drinking and municipal water samples. Kirecci *et al.*[3] examined 1469 water samples taken from water used by military troops in Kars and Sarikamis with membrane filtration. Kirecci *et al.*[3] isolated *E. coli* from 439 (30 %) of all samples and could not detected *E. coli* in the rest of the samples. Avci *et al.*[34], detected temperature tolerant *E. coli* (fecal coliform) in 119 (34.7 %) and total coliform in 223 (65.3 %) of a total of 2495 drinking water samples as pathogen factors in the study carried out in Tokat province city centrum.

In our study, fecal contamination was detected in 15 of total 45 fountains and 45 *E. coli* strains were isolated from 15 contaminated fountains. *E. coli* isolates of 15 suspicious strains out of total 45 isolates was realized by cultivating them on

EMB culture medium from agar media whose fecal coliform enumeration were performed. Typical metalic bright green colonies developing on EMB agar were subjected to IMVIC test and 45 *E. coli* strains were isolated. RAPD-PCR technique was used for molecular *E. coli* typing. As a result of the analyses, the 45 isolates could be divided into six different fingerprint groups according to their different polymorphic bands. The band length of these separated 6 genotypes was detected to be between 750-1000 base pair. In order to reveal the validity of the genotyping method used, the same bands were obtained as a result of amplifications made with the same strains at different times (Fig. 1).

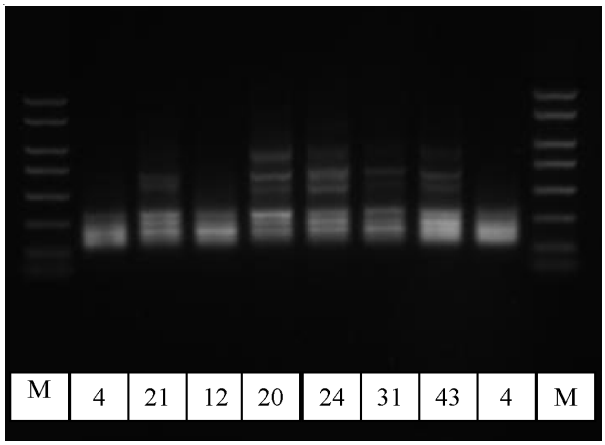

Fig. 1. DNA agarose gel electrophoresis image belonging to rapd-pcr results of fountain water samples

In Fig. 1 is given the agarose gel image of DNA bands obtained as a result of RAPD-PCR analysis of 6 different *E. coli* strains isolated from 45 fountain water samples. Figures on the image are fountain numbers, K is (control = No 4) and M is marker.

Balkay[35] detected *Salmonella, Shigella* and *E. coli* in Kars stream using Multiplex PCR method and detected *E. coli* especially in May and June, Balkay[35] detected *E. coli* only in samples collected from the outer borders of the city in July and August and could not detect this type of bacteria in September. Balkay[35] submerged the PCR amplifications of these bacteria into DNA agorose gel electrophoresis and determined band lengths of *E. coli, Salmonella* and *Shigella* strains as 147 base pair 526 base pair and 408 base pair, respectively. Hadise *et al*.[16] carried out the microbiological analysis of well water belonging to military troops in Ankara Garnison and detected *E. coli* in 14 water samples out of 28 water samples. Researchers using Quadro Multiplex PCR method detected band lengths of thermotolerant coliforms, *E. coli, Salmonella* and *Shigella* strains to be 326 base pair, 147 base pair, 526 base pair and 408 base pair, respectively. It is seen that band length values of *E. coli* strains isolated by these researchers are lower than the band length values of 6 *E. coli* strains that we examined. Using Taq Man PCR method, Frahm and Obst[36] detected *Enterococcus* in 96 % of 55 water samples while they detected *E. coli* in 98 % of these 55 water samples. Fode-Vaughan *et al.*[37] using PCR (DPCR) method and Tims and Lim[38] using Direct and Real-time PCR method detected *E. coli* O157:H7 in water and food samples from the market. Similarly, Sirilak *et al.*[39] and Maheux[40] detected the existence of *E. coli* in water samples using PCR method in their studies.

Conclusion

In terms of the parameters examined in this study, physical and chemical analysis results of water samples collected from the selected public fountains in Erzurum city centrum reveal that these waters are in compliance with the standards and do not show any negative quality. However, the detection of *E. coli* in some of these fountains shows that these waters were subjected to an external fecal contamination. 6 of all typical *E. coli* strains isolated from these fountains were detected to be different in molecular DNA structure. It is concluded that because of the potential health risk, it is necessary not to utilize waters of these fountains where fecal contamination was detected and it is necessary to warn consumers about this matter.

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