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**Original Article**

# Analysis of drinking water sources in Ankara city with PCR method

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drinking water, water analyses, PCR, multiplex PCR

**** This article was prepared from specialty thesis of Dr. Recai Ogur (Analysis of drinking water sources in Ankara city with PCR method. Ankara. 2000).***

**Abstract**

Aim: We aimed to analyze drinking and use waters of the Ankara Provincial Center by the polymerase chain reaction (PCR) technique which was widely used in many areas of health sciences.

Methods: Tap water, well water, and bottled water samples are included in this study. A total of 111 water samples were taken from different locations. Water samples were analyzed using a quadruplex PCR technique for the availability of thermotolerant coliform, *Escherichia coli*, *Shigella* and *Salmonella* bacteria. Also, *Giardia* parasite was investigated using standard PCR technique in water samples. The "freezing and thawing" method was used for the isolation of DNA in microorganisms. Mini-gel electrophoresis was used to show results.

Results: No cross-reactions have occurred between the primers. In 33.3% of water samples (tap water 25.9%, well water 50.0%, and bottled waters 32.0%), *E. coli* was identified. None of the water samples contained *Salmonella*, *Shigella* bacteria, and *Giardia* parasites. Although the standard bacteriological water analysis methods are completed in 24–48 hours and a single microorganism group is investigated, we completed analyses of four different bacteria in 4 hours by using quadruplex PCR technique.

Conclusion: Our results showed that quadruplex PCR could be used in the microbiological analysis of water samples in the epidemic and other emergencies when you need to examine waters in hours. Also, analyses have shown that not all water resources used as drinking water are safe. The relevant segments should be informed and more effective measures should be taken regarding the drinking water quality control that is closely related to community health.



## INTRODUCTION

The most important public health problems arising from drinking water are the occurrence of epidemic diseases in large populations resulting from disease-forming microorganisms in water [1-4]. Therefore, microbiological control of drinking water is one of the most important public health activities.

During the following years of the first definition and identification of Escherichia coli (E. coli) bacteria, the possible presence of it and similar microorganisms in the water has been one of the most important subjects of environmental microbiology and hygiene studies [5, 6]. In the 1910s, a microbiological analyses method was developed for the determination and counting of microorganisms in water and it was called "Most Probable Number" (MPN) method [5, 6]. MPN method has some limitations and may cause false negative results. About 40 years later, at the beginning of 1950s, another microbiological analyses method was developed for the same purpose and it was called as "Membrane Filtration Method". This method has been giving more reliable results, and also could be used in food microbiology [5, 6]. Besides these two analyses

methods, researchers developed a lot of methods but none of them had been used widely by public health laboratories. Almost all of the current analyses methods give a result at least in 24 hours. This means that you could be aware of a microorganism in water, after at least 24 hours of contamination and it would be late for some of the population. Another problem is the main bacteria which is searched during these analyses; almost all of the current methods try to find "indicator microorganisms" in water, so if you find an "indicator microorganism", you could consider that there may be a contamination and there could be disease-causing microorganisms in water. If you would like to learn the type of microorganisms, you should use another method and this takes more time [7]. All of these reveal the necessity of more specific and more reliable microbiological analyses methods should be developed.

Polymerase chain reaction (PCR) technique, which is one of the most commonly used in vitro nucleic acid amplification methods, has entered the agenda of water and environmental microbiology in the early 1990s, and it has been used particularly to isolate and define the microorganisms which are difficult to incubate in cultural environment [8-10]. But, standardized PCR methods are not available for rapid microbiologic water analyses. In this study; we aimed to develop a new multiplex PCR method for detecting multiple microorganisms that may be present in drinking water, in a short time. Although the multiplex PCR method was developed and widely used since 1988, the microorganisms examined in our study have not been examined in previous studies. There are numerous studies on the validity and reliability of the PCR technique in microbiological water analysis and this is beyond the interest of our study [8-19].

## MATERIAL AND METHODS

### Microorganisms

Samples of E. coli and total coliform were obtained from the Water Analysis Laboratory of Department of Public Health and the samples of Salmonella and Shigella were provided by the Department of Microbiology.

### Primers

List of primers, base sequences, and band sizes are shown in Table 1. All of the primers synthesized commercially (Operon Technologies Inc., USA).

### Collection and preparation of water samples

A total of 111 water samples were collected by using standard sampling techniques advised by World Health Organization (distribution of samples was 58, 28, and 25 for tap water, well water, and bottled water, respectively) [3, 4].

The samples were delivered to the laboratory within 2 hours and they were filtered by passing through 45 nm nylon microfilters in order to transfer microorganisms to the filter. These filters were transferred to 10 ml sterile tubes and added with 1 ml of distilled water. After the tubes were shaken vigorously for 30 seconds, the water in the tubes was transferred to 1.5 ml sterile microcentrifuge tubes. At this stage, the bacteria stocks to be used for positive control were diluted with distilled water and final volumes were completed up to 1 ml. The tubes were kept at +4°C during the analyses process.

### DNA Extraction

"Freezing and thawing" method was used to isolate DNA of bacteria [9, 15]. All microcentrifuges were kept in the freezer for 5 minutes (at −20°C) and then immersed into +60°C for 10 minutes. This cycle was repeated for three times and then tubes were stored at +4°C until the next step.

### Multiplex PCR

We used four primer sets to determine four bacteria together. For this purpose, we prepared PCR mix (10 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 200 μM dNTP, 0.5 μM primer and 2.5 units Taq DNA polymerase) and add DNA extractions of water samples to reach a final volume of 50 μl. Quadruplex PCR conditions are shown in Table 2.

### Mini Gel Electrophoresis

Mini gel electrophoresis was used to display PCR products. 2% agarose gel was prepared in 20 ml 0.5 x TBE solution by using 0.4 g agarose. This mixture was heated up to 100°C and added 5 ml of ethidium bromide and was poured into the gel chamber, then it was left for 15 minutes for cooling and solidification. In the horizontal mini gel electrophoresis (Sigma, USA) with 0.5 x TBE, the PCR products mixed with gel loading buffer were loaded into the wells in the agarose gel. Electrophoresis was performed for 30 minutes under constant voltage (120 V). Positive and negative controls were used for each analysis group. At the end of the electrophoresis process, the gel was extracted and viewed through the UV light (Alpha Innotech, TMW-20, USA).

**Table 1.** Primers

| Primer | Base | Base Pairs (BP) | Reference |
|---|---|---|---|
| ZL-1675<br>ZR-2025 | 5'-ATGAAAGCTGGCTACAGGAAGGCC-3'<br>5'-GGTTTATGCAGCAACGAGACGTCA-3' | 326 bp | 8,9,10 |
| ual 754<br>ual 900 | 5'-AAAACGGCAAGAAAAAGCAG-3'<br>5'-ACGCGTGGTTACAGTCTTGCG-3' | 147 bp | 8,9,10 |
| SalA 1144<br>SalB 1650 | 5'-ACGGTTGTTTAGCCTGATAC-3'<br>5'-CTGGATGAGATGGAAGAATG-3' | 526 bp | 17 |
| ShigA<br>ShigB | 5'-TTGACCGCCTTTCCGATAC-3'<br>5'-ACTCCCGACACGCCATAGA-3' | 408 bp | 11 |
| ABB97F<br>ABB220R | 5'-AGGGCTCCGGCATAACTTTCC-3'<br>5'-GTATCTGTGACCCGTCCGAG-3' | 163 bp | 20 |

**Table 2.** PCR conditions

| **Conditions** | Temperature (°C) | Time | Cycle |
|---|---|---|---|
| First Denaturation | 95 | 5 min | 1 |
| Denaturation | 95 | 25 sec | 30 |
| Annealing | 55 | 30 sec | |
| **Extending** | 72 | 55 sec | |
| Final **Extending** | 72 | 10 min | 1 |

After the last extension, the tubes were kept at +4°C.

**Table 3**. Results of water analysis

| **Water Samples** | **Bacteria (*E. coli*)** | | **Total** |
|---|---|---|---|
| | **Not detected** | **Detected** | |
| **Tap Water** | 43 | 15 | 58 |
| **Percent (%)** | (74.1) | (25.9) | (100.0) |
| **Well water** | 14 | 14 | 28 |
| **Percent (%)** | (50.0) | (50.0) | (50.0) |
| **Bottled water** | 17 | 8 | 25 |
| **Percent (%)** | (68.0) | (32.0) | (100.0) |
| **Total** | 74 | 37 | 111 |
| **Percent (%)** | (66.7) | (33.3) | (100.0) |

* $\chi^2 = 4.977$ ve $p = 0.083$

### Statistics

The data obtained was performed in the computer environment with SPSS for Windows statistical software. The chi-square test was used in the evaluation of the statistical significance and 0.05 was selected as a level of significance.

## RESULTS

In the preliminary studies, we performed before analysis, no cross-reactions were detected between the primers and the microorganisms examined (Fig. 1, 2).

In the bacteriological analysis using the quadruplex multiplex PCR method, specific DNA bands obtained (326 bp for thermotolerant coliform, 147 bp for *E. coli*, 526 bp for *Salmonella*, and 408 bp for *Shigella* were formed). 163 BP band were formed for *Giardia*.

Analysis of 111 water samples was detected in 37 (33.3%) and thermotolerant coliform bacteria. All of the thermotolerant coliform bacteria were *E. coli*.

As a result of the water analyses performed during our study, the *Salmonella* and *Shigella* bacteria and the *Giardia* parasite were not detected in any water sample.

Distribution of *E. coli* according to sample sources was showed in details in Table 3.

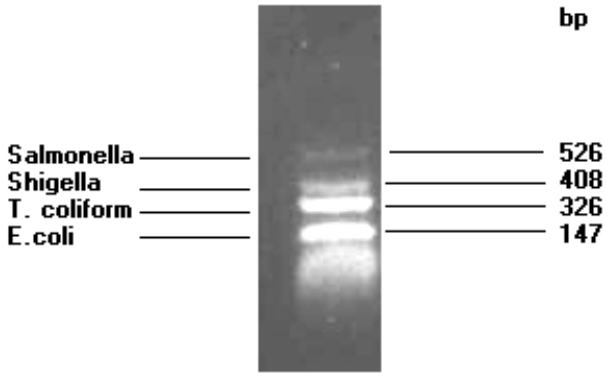


**Figure 1.** Quadruplex PCR

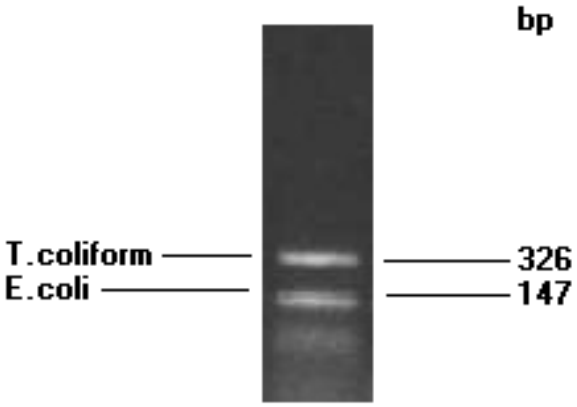


**Figure 2.** Thermotolerant coliform and *E.coli*

The bacteriological analysis of water samples performed using quadruplex PCR technique was completed within 4 hours (this period varies between 24–48 hours in standard methods).

## DISCUSSION

The results of our research show that all of the water sources that are preferred as drinking water does not have appropriate microbiological qualities. There are numerous studies on the validity and reliability of the PCR technique in microbiological water analysis, and this issue is beyond the interest of this study.

The primers we use in our study have been tested and used by other researchers. However, all of these primers were used by us for the first time in the same study as multiple (multiplex) and no cross-reactions were observed. The standard bacteriological strains that we used as positive control in our previous studies have created a unique band. The sizes of these bands are consistent with previous studies (8,9,10,11,17).

The primer that we use for thermotolerant coliform group bacteria has been tested and used for determining about 150 coliform bacteria (8,9,10). The primer that we used for *E. coli* bacteria could determine 4-methylumbelliferyl-beta-D-glucuronide negative and enteropathogenic, enterohemorrhagic, and enterotoxigenic disease-generating *E. coli* in addition to non-pathogenic *E. coli* (8,9,10). The primers we chose for *Salmonella* and *Shigella* bacteria were tested against 26 *Salmonella* and 3 *Shigella* strains (11,17). The primers selected for *Giardia* are specific to *Giardia lamblia* and G. *muris* parasites (20).

We found that about 25% of tap water samples contain coliform bacteria and this result is different from the results of other government laboratories. Tap water used in Ankara is treated water. It is presented to the public after sedimentation, filtration, and chlorination steps. As a result of sedimentation and filtration, large particles and therefore relatively large microorganisms are kept, and *Giardia* is one of the microorganisms that can be held during the filtration process. Subsequently, the gas chlorination process is aimed to destroy microorganisms by disintegration. However, because all microorganisms are not equally susceptible to chlorine, some may be given to the city. In addition, various factors in the grid system may cause microbiological contamination of the water after the treatment process. Microorganisms may temporarily lose their development and reproductive abilities as a result of various factors (such as cold, chlorine, etc.), and these called as "suppressed" microorganisms (stressed). Suppressed microorganisms do not immediately reproduce in normal incubating environments used in microbiological water analysis, they need 24–48 hours to regain their reproductive abilities. Therefore, the membrane filtration method which results in 24 hours and the multi-tube method that results in 24–48 hours can be inadequate for the

detection of such microorganisms. These microorganisms can then regain efficacy and cause disease (21,22,23,24). In the PCR method, it is possible to detect all active or suppressed microorganisms because this method analyzes the nucleic acid content of water but not the reproducibility of microorganisms. This also could explain the difference between our analyses results and results of other laboratories which use classical methods. Studies on the source of *E. coli* identified in the city network are much more comprehensive and may require a review of the entire network system that provides water to the entire city (2,25,26).

About one of three (32%) bottled waters that we analyzed contained *E. coli*. But, we could not find similar local studies that we can compare to the results of the analysis we obtained, but there are overseas studies which show that commercial waters are microbiologically worse than tap waters. A report by the U.S. National Resources Protection Agency, described in 1999; it was reported that approximately one-third of the sample of more than 1,000 bottled water samples did not conform to the microbiological and chemical drinking water standards [27].

Half of the water samples (50%) obtained from wells contained *E.coli* and this is very dangerous because most of the users of these waters believe that it is very healthy. Well waters are not analyzed periodically by government laboratories and these water sources might cause epidemics.

One of the main aims of our study is “use of the PCR technique in the microbiological analysis of water samples in the epidemic and other emergencies”, and our results showed that quadruplex PCR could be used for this purpose.

The data we obtain in our research shows that the lack of domestic data, which has the same feature to compare, causes a lack of water quality control which is directly concerning the health of society. Similar studies should be performed by independent researchers in addition to government laboratories.

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