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A Study on the Microbiological Quality of Drinking Water and Changes During Storage

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Abstract: To assess possible risks from the consumption of drinking water from various sources, a survey of the microbiological quality of tap water, commercial bottled drinking water which is exploited from natural mineral water, and natural spring water was conducted. A total of 4 different brands of commercial bottled drinking water, and 4 types of spring water from different sources, and tap water from 4 private houses were tested for four index microorganisms, and the microbial quality changes of the water during the storage at room temperature or refrigerated temperature for 7 days. Aerobic plate counts of all of the initial water samples were still within 100 CFU/m*l* (drinking water standard of Korea). Total coliforms, fecal coliforms, and *E. coli* were not detected in all of the water samples at initial. However, aerobic plate counts of three types of spring water and three types of bottled drinking water stored at room temperature showed higher levels than the standards in 5 days. Total coliforms were detected in three types of spring water after one day's storage at room temperature, and in one type of bottled drinking water after 5 days' storage. These results indicate that some of the spring water surveyed are not safe to drink, and the spring water and bottled drinking water after opening the lid should not be stored at room temperature, if they are used for drinking.

Keywords: tap water, bottled drinking water, spring water, microbiological quality changes, storage

Introduction

During the past decade, there has been a considerable increase in the consumption of bottled drinking water which is originated from natural mineral water in Korea (Im, 2004), and this trend is expected to continue. Moreover, many people living in urban environments prefer natural spring water, so called medicinal water because it is associated with naturalness (Kim *et al.*, 1997; Kim and Lee, 1997; Choi, 1989; Jeong and Zong, 1989; Saad *et al.*, 1998), objection to unpleasant tastes and odors from municipal water supplies (Tamagnini and González, 1997), and is often regarded as safer and healthier than tap water (Armas and Sutherland, 1999, Chae *et al.*, 1989).

Spring water is not pasteurized or otherwise treated to remove or destroy microorganisms. In case of natural mineral water, the number of bacteria recovered at the source is generally very low, around 10 CFU/m*l* (Armas and Sutherland, 1999). However, there are several reports that viable counts increase, notably in uncarbonated water, to 10^4-10^5 CFU/m*l* after 1-3 weeks of storage (González *et al.*, 1987; Bischofberger *et al.*, 1990; Mavridou, 1992; Mavridou *et al.*, 1994; Tsai and Yu, 1997).

The public health significance of the high numbers of index bacteria such as aerobic plate counts, coliforms, and *E. coli* which develop is clear. Ecological data, especially the diversity and microbiological properties are essential together with epidemiological studies in order to perform safety evaluation for drinking water. The purpose of this study were to evaluate the microbiological status of tap water, spring water, and commercial bottled drinking water and to determine any microbiological quality changes occurring during storage.

Materials and Methods

Water Samples

Natural spring water was sampled from 4 different public springs at a southern area of Korea (Daegu area). Tap water was sampled at four private houses at this area. The water samples were collected midstream in a sterilized glass bottle by the

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recommendations of the World Health Organization (WHO, 1984) from July to August. A total of 4 different brands of commercial bottled drinking water produced in Korea were purchased from a local supermarket.

Sample Preparation for Microbiological Examination

Each type of water sample was tested immediately after carrying in an ice box and was sampled after storage for 7 days in shaded daylight at ambient temperature in the laboratory or in a refrigerator. Microbial numbers of the water samples were determined every two days by decimal dilution in sterile phosphate buffered saline.

Determination of Aerobic Plate Count (APC)

The pour plate method was employed to determine the presence of aerobic bacteria using plate count agar (PCA) (Difco Lab., MI, U.S.A.). Serial dilutions of the water samples were prepared in sterile saline (0.85% sodium chloride). Duplicate plates of the PCA were then inoculated with 1.0 m*l* of the diluted samples and incubated at 37°C for 24 h. Enumeration was done using a Quebec colony counter (Korea Manhattan Co., KMC-1301) and was expressed as CFU/m*l* of sample.

Determination of Total and Fecal Coliforms

To determine the presence of total and fecal coliforms in water samples, the multi-tube fermentation technique for coliform analysis was used, followed by plating on Endo agar (Difco Lab.) and incubated at 37°C and eosin methylene blue (EMB) agar (Difco Lab.) incubated at 44.5°C, respectively.

Detection and Enumeration of *E. coli*

To detect *E. coli*, EMB agar (Difco Lab.) plates were inoculated with 1.0 m*l* of water samples and then incubated at 37°C overnight. Counts at the highest dilutions using sterile saline were then expressed as CFU/m*l* of sample. Gram stain and IMVic test were necessary in some cases for final identification.

Statistical Analysis

The numbers of aerobic bacteria and total coliforms in tap water, bottled drinking water, and spring water were compared. Also, the microbial prevalence in the three kinds of drinking water at room temperature and refrigerated temperature was compared. The data from samples were compared by analysis of variance. Significant differences among means were determined by Duncan's multiple range test.

Results and Discussion

Table 1 shows aerobic plate counts, total coliforms, fecal coliforms, and *E. coli* in the tap water, commercial bottled drinking water, and natural spring water sampled. The prevalence of aerobic plate counts (APCs) of all of the initial water samples were still within 100 CFU/m*l*, the drinking water standards of Korea (Ministry of Environment, Republic of Korea, 2003). The overall mean APCs per m*l* of bottled water in the present study is considerably lower than was found in bottled water reported by Bharath (2003) ($3.6\times10^3\pm 1.8\times10^4$ CFU/m*l*). Similarly, Armas and Sutherland (1999) reported that heterotrophic plate counts in eight brands of uncarbonated bottled water were high and variable. In this study total coliforms, fecal coliforms, and *E. coli* were not detected in all of the initial water samples.

Figs. 1 and 2 shows the changes of aerobic bacteria and their mean counts in the tap water,

Table 1. Microbiological quality of drinking water samples

Drinking water	Aerobic plate counts (CFU/m*l*)	Total coliforms /100 m*l*	Fecal coliforms /100 m*l*	*E. coli* /100 m*l*
Tap water	7.8 ± 3.6	ND	ND	ND
Bottled drinking water	26.8 ± 7.4	ND	ND	ND
Natural spring water	13.0 ± 3.4	ND	ND	ND

Each value represents the mean ± S.E. of four types. ND: Not detected

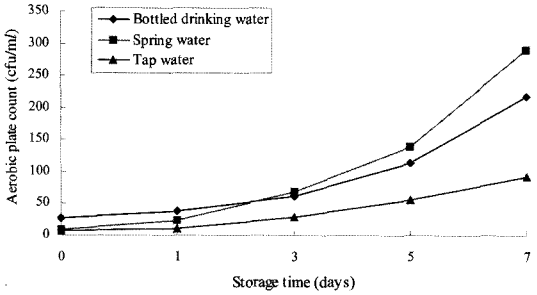

Fig. 1. Changes in aerobic plate counts of drinking water during storage at room temperature for 7 days.

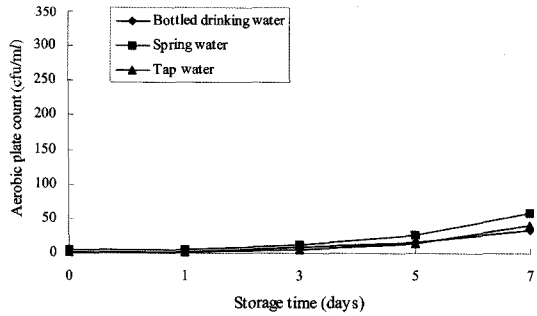

Fig. 2. Changes in aerobic plate counts of drinking water during refrigerated storage for 7 days.

bottled drinking water, and spring water sampled on the day of sampling and after 7 days' storage, with different storage temperatures. Initial APCs of all the water samples were below 100 CFU/m*l*. However, during the 7 days' storage at room temperature, there appeared to have been an overall increase in aerobic bacterial numbers. Also, the APCs of three types of spring water and three types of bottled drinking water showed higher levels than the standards in 5 days. A count over 3.0×10^2 CFU/m*l* on PCA plates was obtained from three types of spring water, and a count over 2.0×10^2 CFU/m*l* from two types of bottled water (Fig. 1). The differences of APCs among the three kinds of drinking water stored at room temperature were statistically significant ($p<0.05$). In case of refrigerated water samples, APCs were generally <100 CFU/m*l*, with the exception of a spring water (1.03×10^2 CFU/m*l*), although the microbial numbers increased over time (Fig. 2).

The prevalence of total coliforms in bottled drinking water and spring water during storage at room temperature is shown in Fig. 3. Three of

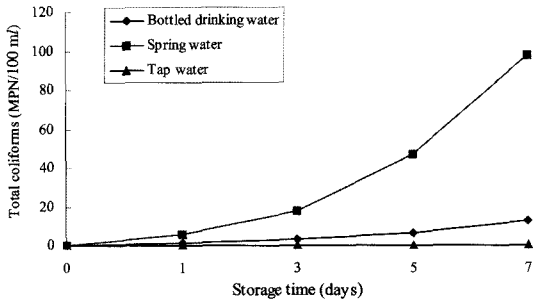

Fig. 3. Changes in total coliforms of drinking water during storage at room temperature for 7 days.

four types of spring water (75%) were positive for coliforms after one day's storage at room temperature, and one of four types of bottled water (25%) was positive after 5 days' storage compared with 0 (0.0%) of tap water. Failure to detect fecal coliforms and *E. coli* in the positive samples tested in this study partially agrees with previously published reports (Ogan, 1992; Warburton *et al.*, 1992). In case of refrigerated water samples, coliforms were not detected during the storage period.

The occurrence of coliforms in the water samples during storage at room temperature suggests the potential presence of enteric pathogens. It also has health implications, as these microorganisms are considered an indicator of fecal pollution (Eaton *et al.*, 1995; Bharath, 2003). Pathogenic microorganisms such as *Pseudomonas* spp., *Salmonella* spp., and *Vibrio cholera* should be assayed for the positive samples, but not in the present study. Although bottled water is generally considered safe if it meets the coliform standards (Dutka, 1973; Bharath, 2003), waterborne epidemics have been reported from water that met coliform standards, since the presence of indigenous opportunists or other pathogens is not always adequately correlated with coliform counts (WHO, 1984; WHO 2004).

It was interesting to find that although all of the drinking water were negative for total coliforms at initial, but 3 of 4 types of spring water and 1 of 4 types of bottled drinking water were positive during the storage period. This indicates that there might be other opportunists of contamination in the storage condition as well as intrinsic factors.

In this study the APCs were similar for initial water samples, however, the overall mean APCs

of tap water stored at room temperature was considerably lower than was found in bottled drinking water or in spring water. It has been documented that after storage, microorganisms in bottled water may multiply and exceed 10^5 CFU/ml (Warburton *et al.*, 1993; Hunter 1993). The storage temperature of bottled water has also been demonstrated to affect the rate of multiplication and survival of microorganisms (Gonzalez *et al.*, 1987; Bischofberger *et al.*, 1990; Warburton *et al.*, 1992; Armas and Sutherland, 1999; Bharath 2003).

It was not possible to determine in this study whether the microflora in the drinking water samples was autochthonous or not. Further characterization of the microflora detected in the spring water and bottled drinking water should be necessary in order to assess its origin and potential risks.

Conclusions

The findings of this investigation have several implications. Based on the recommended zero tolerance for coliforms in potable water (Bordner *et al.*, 1978), some of natural spring water in this survey could not be considered fit for human consumption. If the spring water and bottled drinking water after opening the lid are stored at room temperature, they may pose a health risk to consumers. The length of storage of drinking water at the various temperatures can also affect the microbial load. Therefore, there is a need for public awareness and education about the quality and potential health risks associated with the consumption of spring water and bottled drinking water.

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