# Microbiological Investigation of Bottled Waters From Different Suppliers in Istanbul

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## Abstract

Some pathogenic bacteria found in drinking water may cause infections in many countries. The control of these infections is done by water treatment. It's very important to monitor faecal indicator bacteria such as faecal and total coliform in terms of the examination of the bacteriological quality of drinking water. In addition, the determination of total mesophilic aerobic bacteria is also used in measureing bacterial pollution. In our study 88 samples of bottled drinking water belonging to 22 different brands were examined. The water samples that were taken from plastic bottles of 19 L were examined for faecal coliform, total coliform, *Pseudomonas aeruginosa*, *Aeromonas* spp., total mesophilic aerobic bacteria, and presence of free-living amoeba. Faecal coliform, total coliform, *Pseudomonas aeruginosa*, *Aeromonas* spp., and the existence of free-living amoeba were determined by membrane filtration; and the total mesophilic aerobic bacteria numbers were studied using the pour plate method. It has been found that all of the samples examined in this study are not acceptable according to the standards of TSE and WHO. Although four different water samples were taken from the same brand, it has been shown that their were variations in the bacteriological quality of the water, from one water sample to another. Reusable methods of cleaning the bottles and the unhealthy conditions of filling facilities might cause these differences. In conclusion, the results of the present study have revealed that it is very important to ensure systematic microbiological monitoring and the control of bottled water which is used as drinking water. Also this control should be done by authorized foundations.

**Keywords:** Bacteriological quality, bottled water, *Pseudomonas aeruginosa*, *Aeromonas*, free-living amoeba
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## Introduction

The most common and widespread health risk associated with drinking water is microbial contamination from pathogenic organisms that are the source of waterborne diseases (Tallon et al. 2005; Jeena et al. 2006; WHO 2006). Disease-causing pathogens transmitted via drinking water are predominantly of faecal origin and therefore known as enteric pathogens (Ashbolt 2004). Also, the most frequently found pathogens in water sources are enteric pathogens (Rompre´ et al. 2002; Pianetti et al. 2004). In terms of public and environmental health, it is essential that water sources be free of these pathogenic bacteria and safe to drink (Rompre´ et al. 2002). The examination for the presence of all the possible pathogens in water is usually complex, expensive, time-consuming, and currently often not practically possible (Ministry of Health 2005). However, it is known that the presence of pathogens correlates well with the presence of faecal contamination (Tallon et al. 2005). For this reason, drinking water tests are based on faecal indicator bacteria which are detected in higher concentrations than pathogenic bacteria (Rompre´ et al. 2002; Tallon et al. 2005).

Indicator bacteria that are not themselves pathogenic are used as an index of both faecal contamination and the potential presence of entero-pathogens in water environments (Rompre´ et al. 2002; Standing Committee of Analysts 2002; Tallon et al. 2005). The drinking-water industry commonly uses indicator organisms such as total coliforms, faecal coliforms (thermotolerant coliforms), *Escherichia coli* (*E. coli*) (Ministry of Health 2005). Their presence in drinking water must at least be considered as a possible threat or indicative of microbiological water quality deterioration (Rompre´ et al. 2002).

In addition to the faecal indicator organisms, it is important to detect environmental microorganisms (Revantad 2008). Drinking-water entering the distribution system may contain free-living amoebas and environmental strains of various heterotrophic bacterial species (Parija and Jayakeerthee 1999). These bacteria have an epidemiological behaviour different from the traditional enteric bacteria and the capacity to behave as opportunistic pathogens (Pianetti et al. 2004; Revantad 2008).

Heterotrophic plate counts (HPCs) have been commonly used to assess the general bacterial content of the water and to monitor trends or rapid changes in water quality (Standing Committee of Analysts 2002; Pavlov et al. 2004). *Pseudomonas aeruginosa*, *Aeromonas* spp., and HPCs counts are used as process management indicators in bottled water production and not as health-risk indicators (Bartram et al. 2004).

The presence of free-living amoeba in source water and water distribution pipes is shown to be usually associated with thermally polluted waters (e.g. *Naegleria*) or inadequate disinfection of treated supplies (Harf 1993/94; Parija and Jayakeerthee 1999; WHO 2006).

The current study was designed to assess the bacterological quality of the bottled drinking water samples taken from the retail outles in Istanbul. According to Turkish legislation (TSE 2005) and World Health Organisation guidelines (WHO 2006), total and faecal coliform bacteria that are the indicators of faecal contamination were evaluated in water samples. In order to obtain a wider knowledge of the microbial characteristics of these waters, we also investigated bacteria counts belonging to *Pseudomonas aeruginosa*, *Aeromonas* spp., HPCs and the presence of free-living amoeba.

## Materials and Methods

### Collection of samples

Bottled drinking water samples were collected from the various retail outlets in Istanbul during the period of April 2007 to March 2008. Overall 88 samples belonging to 22 different brands were analysed.

The cap of each water bottle was carefully removed while avoiding touching the opening with bare hands. The bottle contents were then emptied into sterile bottles. The sample bottles were kept in an ice bath until the samples were transferred and then stored at 4°C in the laboratory (Kassenga 2007).

### Microbiological analysis

Four samples of each brand were analysed for levels of bacterial indicators (faecal and total coliforms), *Pseudomonas aeruginosa*, *Aeromonas* spp., total mesophilic aerobic bacteria, and the presence of free-living amoeba.

Total mesophilic aerobic bacteria were analysed by a pour plate method, using R2A agar. 0.1 ml of the undiluted water samples were aseptically plated on R2A agar and incubated at 25 °C for 96 h. All samples were plated in triplicate.

Total and faecal coliforms were detected by membrane filtration on selective media, Endo-NKS (Sartorious) for total coliforms and mFC-NKS (Sartorious) for faecal coliforms, according to the Standard Methods (APHA 1998). 100 ml water samples were filtered through a membrane filter (0.45 μm pore-size) (Sartorious), and were then placed on the selective media. The Endo-NKS and mFC plates were incubated at for 18–24 h 37°C and 44°C, respectively. The characteristic blue colonies were counted as FC. TCs were

detected by production of typical red colonies with a metallic surface sheen or atypical dark red colonies without sheen (Robles et al. 1999).

In order to detect levels of *Pseudomonas aeruginosa* and *Aeromonas* spp. bacteria, each water samples (100 ml) was filtered by a cellulose membrane filter (0.45 μm) and these filters were placed on Pseudomonas base agar (Oxoid) with added Pseudomonas CN selective supplement (Oxoid) and also, Aeromonas base agar (Oxoid) with added ampicillin. All plates were incubated at 27 °C for 48 hours (Donnell et al. 2005).

At the end of the incubation periods, colonies on all plates were counted by a Colony Counter Device (åCOLyte Super Colony Counter, Synbiosis) and expressed as colony-forming units per 1 ml (log cfu $ml^{-1}$) and per 100 ml (log cfu 100 $ml^{-1}$).

In the free-living amoeba analysis, the water samples (100 ml) were filtered by cellulose membrane filter (0.45 μm) and these filters were placed on non-nutrient agar (NNA) seeded with heat-killed *Escherichia coli*. All plates were incubated at 30°C in sealed polythene bags and examined microscopically daily from day 2 to day 10 in order to detect the growth (trophozoite and cyst) of amoeba (Jeong and Yu 2005; Ramirez et al. 2005). Each experiment was done in triplicate.

## Results

In the current study, overall 88 samples belonging to 22 different brands were analysed. At the end of this study, it was stated that 42%, 89%, 45%, 75%, 95%, 28% of the examined samples were faecal coliform, total coliform, *Pseudomonas aeruginosa*, *Aeromonas* spp., total mesophilic aerobic bacteria, and free-living amoeba, respectively.

In our investigation, we found total coliforms that are faecal contamination indicators in all of the brands. Of all the brands tested, 8 brands never showed the growth of faecal coliform bacteria. At the remaining brands, the levels of faecal contamination indicators fluctuated throughout the test period (Figs. 1 and 2).

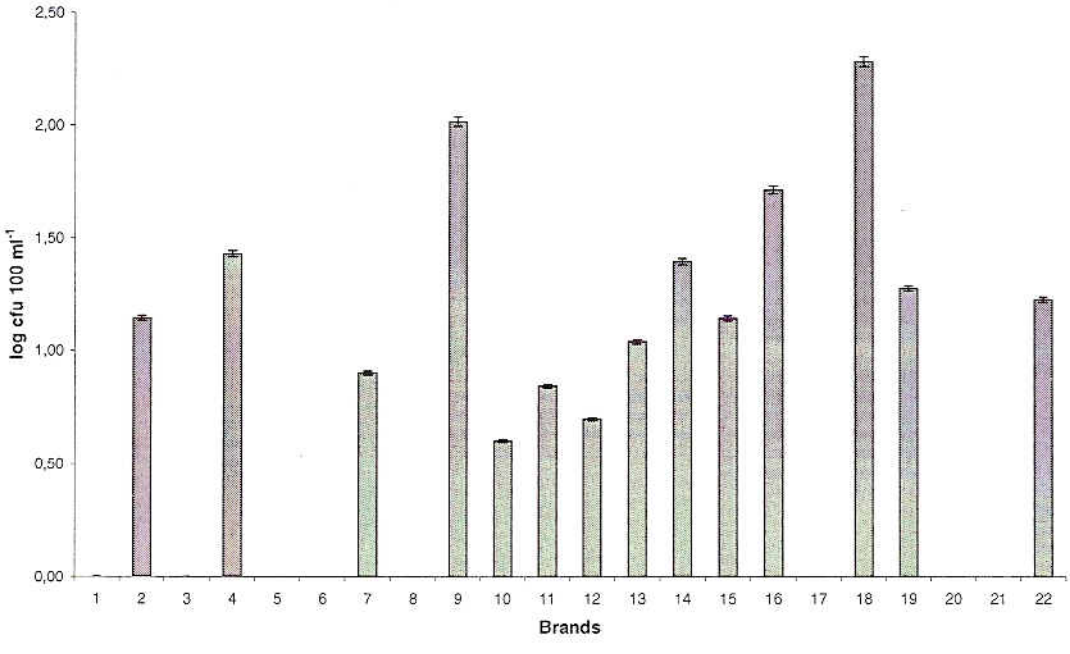


**Figure 1.** Faecal coliform counts in bottled drinking water samples

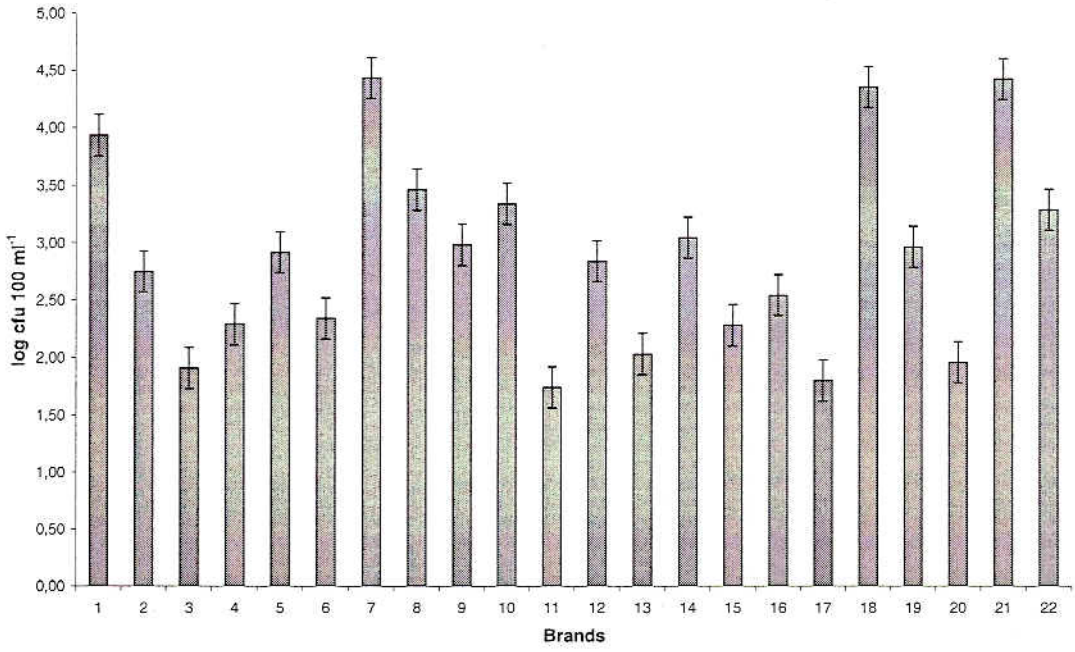


**Figure 2.** Total coliform counts in bottled drinking water samples

It was shown that in 10 of 22 brands tested there was no growth of *Pseudomonas aeruginosa* (Fig. 3). *Aeromonas* spp. bacteria were also identified in almost all the sampling brands but it was not present in some brands (3 and 18) (Fig. 4). Of the 22 brands, only 1 was detected as negative for total mesophilic aerobic bacteria (Fig. 5).

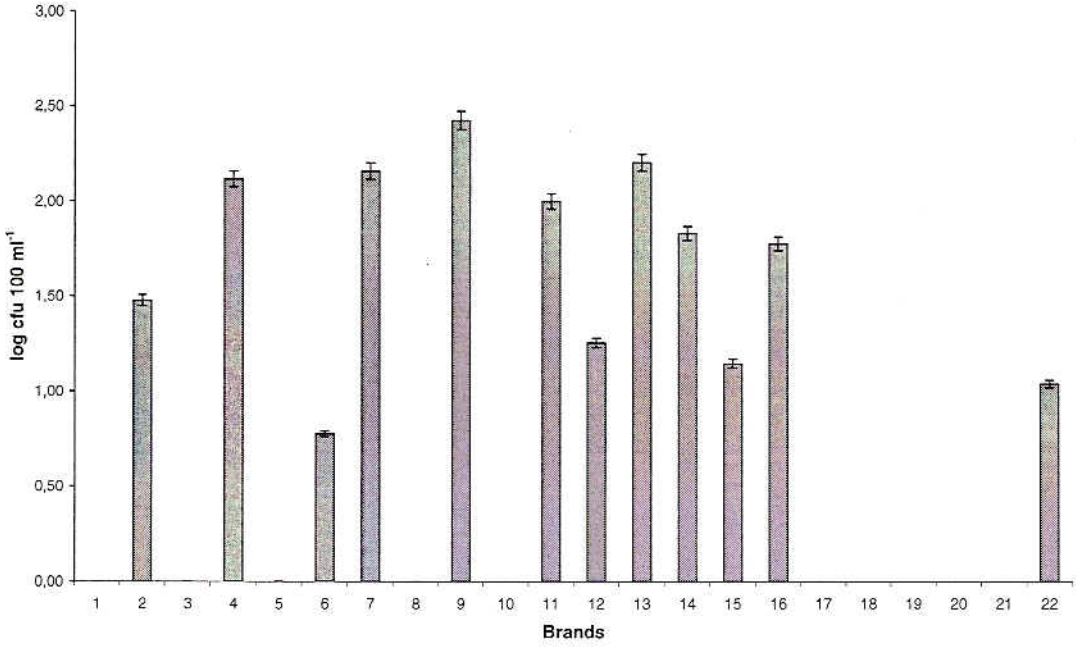


**Figure 3.** *Pseudomonas aeruginosa* counts in bottled drinking water samples

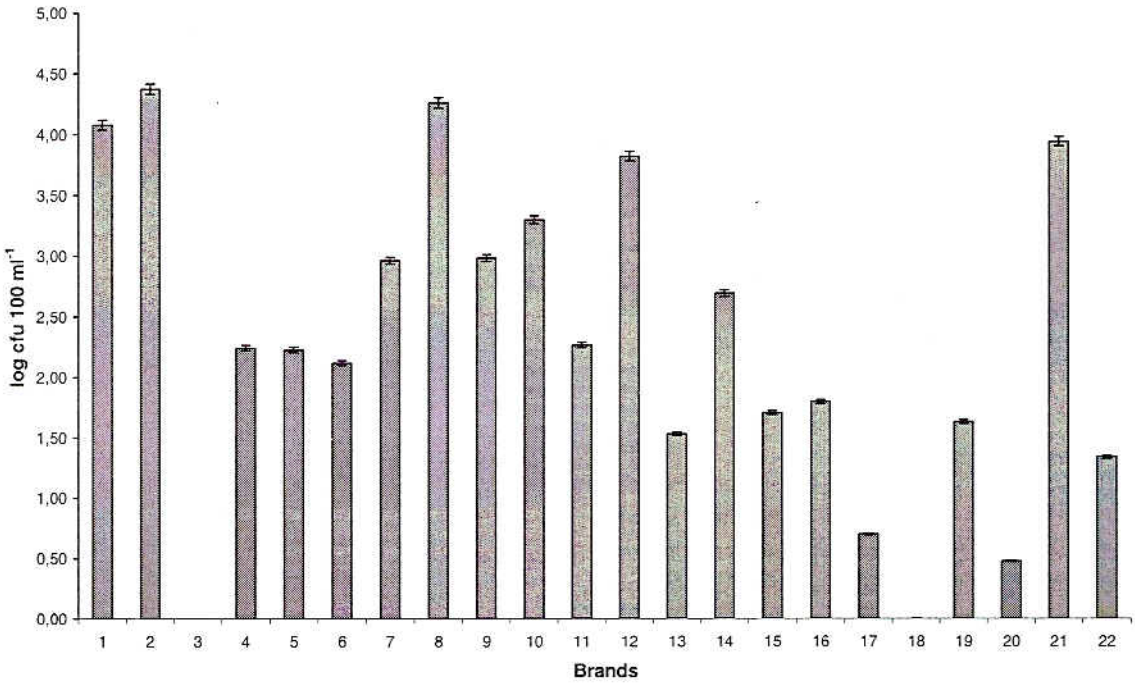


**Figure 4.** *Aeromonas* spp. counts in bottled drinking water samples

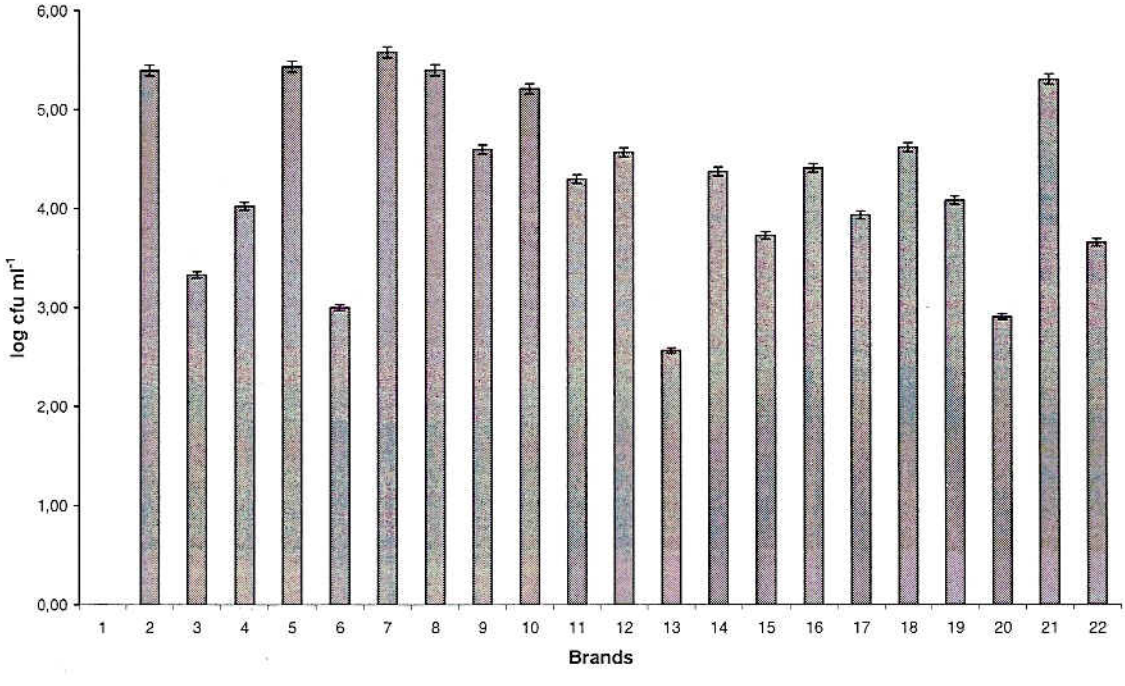


**Figure 5.** Total mesophilic aerobic bacteria counts in bottled drinking water samples

On the other hand, we found that in the bacteriological quality of four different water samples taken from the same brand their were variations from one sample and to another, despite their belonging to the same brand.

Finally, it has been found that none of the 88 samples is appropriate to the standards determined by TSE (2005) and WHO (2006).

## Discussion

Water for human consumption is required to be free from any bacteria that might pose a health risk (Zamberlan da Silva et al. 2008). Investigations pointed out that over 50,000 people died each day in the world from diseases relating to water (Schalenkamp 1990; Momba and Kaleni 2002). It is well known that the health of a community is significantly affected by drinking water quality. Studies have revealed that safe and sufficient water and sanitation would reduce child mortality by 50% and prevent 25% diarrhoea (Bailey and Archer 1997).

Water companies started to offer the 19 L polycarbonate bottled water since 1997. It has been known that about 60% of the houses in Istanbul consumed approximately 650.000 polycarbonate bottled drinking water (12.350.000L) per day.

Bottles containing drinking water can be used many times in our city. But, they must be disinfected and they must not be used for other purposes. Otherwise, they can easily be a cause of infections. Once the container is filled and sealed, bottled water may remain on the grocery shelf or stored in the home for weeks or sometimes months (Rosenberg 2003). It is well known that microorganisms attach on the surface walls of such containers during storage (Momba and Mnqumevu 2000). And, attached bacteria can detach from the surface walls and lead to continuous contamination of the water phase. In fact, we found high microorganism levels in these polycarbonate bottles.

It was determined that 42%, 89%, 45%, 75%, 95%, 28% of the examined samples were faecal coliform, total coliform, *Pseudomonas aeruginosa*, *Aeromonas* spp., total mesophilic aerobic bacteria, and free-living amoeba, respectively.

In a study done in Eskişehir, Kivanc et al. (1996) reported that coliform bacteria was found in 39.2% of 102 samples, and total bacteria was found in 87.25% of 102 samples.

Also, the results of a similar study done by Agaoglu et al. (1999) in Van, stated that 33.3% of drinking waters examined had coliform bacteria.

In 2006, a study related to the contamination level of drinking waters by Avci et al. (2006) in Tokat, it was shown that 65.3% was total coliform and, 87.3% of the water samples were not fit to drink.

To our knowledge, there are no reported studies relating to the determination of the levels of *Pseudomonas aeruginosa*, *Aeromonas* spp. and the presence of free-living amoeba in bottled drinking water in Turkey.

The presence of opportunistic pseudomonads in the water suggests the potential for problems in an immunocompromised population. *Pseudomonas aeruginosa* is usually an indicator of contamination during the bottling process (Tamagnini and Gonza´lez 2006). In the present study, it has been revealed that *Pseudomonas aeruginosa* counts in examined samples were higher than the standards. This indicates a contamination during the bottling or that the source has become polluted by organic material.

In the current study, we found that 28% of the water samples examined were positive for free-living ameoba. This result is in agreement with the results of Singh (2000) who reported that free-living amoebas were found in bottled drinking water samples in New Delhi. But, in another study in India, Reddy (2000) pointed out that no free-living amoeba was found in bottled drinking water samples.

Free-living amoebas are largely found in environmental waters and soils in association with other microorganisms in the same ecological niches. It has been known that a

with other microorganisms in the same ecological niches. It has been known that a number of bacteria such as *Vibrio cholerae* and *Listeria monocytogenes*, and nosocomial new pathogens, i.e. *Pseudomonas* spp., *Xanthomonas maltophilia*, *Flavobacterium* spp. have the ability to survive as intracellular parasite in mammalian cells. Some intracellular pathogens may even have acquired their virulence by first adapting to intracellular life in predatory free-living protozoa. This might be a survival mechanism for bacteria in an aquatic environment (Harf 1993/94). For this reason, it must also perform the presence and the cultures of free- living amoebas on non-nutrient agar in addition to the standard coliform test.

The heterotrophic bacteria counts from the bottled water samples showed that most of the bottled-water brands were excessively contaminated. The heterotrophic plate counts (HPCs) are used to assess the quality of treated drinking water supplies. Not much is actually known about the effects of HPC bacteria on human health, although some members of this group are considered opportunistic pathogens, such as *Aeromonas* and *Flavobacterium*. Burke at al. (1984) showed that many of the *Aeromonas* strains isolated from drinking water were positive for the same enterotoxins as clinically isolated strains. Additionally, a study of *Aeromonas* spp. within a Swedish distribution system concluded that some potentially pathogenic *Aeromonas* strains could persist for several months within drinking (Versteegh et al. 1989). The presence of *Aeromonas* in bottled drinking water was also reported by Jeena et al., (2006). In the present study, *Aeromonas* spp. bacteria were also identified in 75% of all the water samples.

The results of the present study have shown that most samples taken from the 19 L containers exceeded the maximum bacteriological limits according to TSE and WHO standards and the bacteriological quality of the 22 brands studied was extremely variable. These differences may originate from the water source, sanitation conditions of the process, unhygienic and high temperature conditions, defective packaging and lack of protective measures (Kassenga 2007; Revantad 2008). The handling and cleaning of the bottles is another important point. Water containing few organisms when bottled may show a logarithmic increase in the numbers of bacteria in a relatively short time (Rosenberg 2003; Tamagnini and Gonza´lez 2006; Revantad 2008). If the containers that are contaminated with bacteria are reused, it could lead to different bacteriological quality within the same brand. Therefore, periodic bacteriological monitoring of the water samples is required. Indeed, in the current study, although four different water samples were taken from the same brand, variations in the bacteriological quality of the water from one water sample to another have been shown. As mentioned above, this result is attributable to the inadequate washing and sanitizing of empty bottles before refilling.

With a view to getting rid of the bacteriologic contamination in the bottled drinking waters, we suggest that drinking waters are required to be sold in the single-use containers.

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