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# Comparison of the bacteriological quality of tap water and bottled mineral water

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## Abstract

The bacteriological quality of tap water from municipal water supplies, 20-L bottles of mineral water from water dispensers and samples collected from new 20-L bottles of mineral water were comparatively studied. Total coliforms, termotolerant coliforms, *Escherichia coli*, fecal streptococci, *Pseudomonas aeruginosa, Staphylococcus* spp. and heterotrophic plate count were enumerated. The results showed that 36.4% of the tap water samples from municipal water systems and 76.6% of the 20-L bottles of mineral water from water dispensers were contaminated by at least one coliform or indicator bacterium and/or at least one pathogenic bacterium. The bacteriological quality of municipal tap water is superior when compared with the 20-L bottles of mineral water collected from water dispensers and samples collected from new 20-L bottles of mineral water before installation in the dispensers. This highlights the need for an improved surveillance system for the bottled water industry. For the municipal water systems, it is recommended to perform the *Pseudomonas* enumeration periodically, in addition to the routine data collected by most systems.


***Keywords:*** Bottled mineral water; Tap water; Coliform; Streptococci; *Staphylococcus* spp.; *Pseudomonas aeruginosa*

## Introduction

The transmission of waterborne diseases is still a matter of major concern, despite worldwide efforts and modern technology being utilized for the production of safe drinking water (Venter, 2000). This problem is not confined to the developing world where water treatment may not exist or is inadequate. There may also be contamination during storage, a lack of regulations and limited understanding and awareness among the population (AAM, 1996). It may also assume serious proportions in industrial countries (Kramer et al., 1996). Mechanical failure, human error or deterioration in the quality of the source water can lead to failure even in the best treatment systems and disinfection processes (Mac Kenzie et al., 1994; Roefer et al., 1996).

Water quality is often related to the degree of bacterial contamination. Drinking water distribution

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systems are colonized by saprophytic heterotrophic microorganisms that grow on biodegradable organic matter (Servais et al., 1992). Potentially pathogenic microorganisms (e.g., *Pseudomonas aeruginosa*) and microorganisms of fecal origin (e.g., *Escherichia coli*) may also find favorable conditions and proliferate in these systems. The quantity of bacteria in commercial mineral water is generally dependent on the disinfecting process of natural spring-water use at the factory. It is well known that natural mineral water is characterized by its bacterial flora, chemical and physical composition. The quantity of microbial flora of spring water is usually high. Spring water contains a natural microbiota composed mainly of species of the genera *Achromobacter*, *Flavobacterium*, *Alcaligenes*, *Acinetobacter*, *Cytophaga*, *Moraxella* and *Pseudomonas*. If these microorganisms are not adequately removed during processing and bottling, bacterial multiplication may occur for 1–3 weeks after bottling, and the bacterial count can reach $10^3$–$10^4$ bacteria $mL^{-1}$ at 37 °C (Tamagnini and Gonzalez, 1997). In addition to natural contamination, the product can also deteriorate before it reaches the consumer.

The purpose of the present study was to compare the bacteriological quality of tap water from municipal water supplies, 20-L bottles of mineral water from water dispensers and samples collected from new 20-L bottles of mineral water before installation of the bottles in the dispensers.

## Materials and methods

### Water samples

Twenty residences and 20 workplaces were randomly selected from the list of the Companhia de Saneamento do Paraná of Maringá city, Paraná State, Brazil. To be included in the study, owners of water dispensers had to have 20-L bottles of mineral water supplied by a recognized company among nine bottling companies sampled in this study. This analysis was performed over a period of 10 months, between June 2001 and April 2002. For bacterial analysis, samples were taken from the dispensers (>15 days to 3 months after installation) and from the most-often-used faucet. To make sure that the samples were representative of the water consumed, we did not flush before sampling, and there was no attempt to sterilize the outer surfaces of the faucets. In conjunction, water samples were collected for bacterial analysis from 20-L bottles of water (representing the nine most important bottling companies in the region) before installation of the bottles on the water dispenser. The samples for microbiological analyses were collected in 1.0 L sterilized plastic bottles containing sodium thiosulfate (10% w/v) and transported to the laboratory in ice. Analyses were carried out within 4 h of sampling.

### Culture media

The microbiological parameters determined were total coliforms (TC) and fecal coliforms (FC), fecal streptococci (FS), *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus* spp. and aerobic and facultative anaerobic heterotrophic bacteria (HPC). TC, FC, FS, *E. coli*, *P. aeruginosa*, *Clostridium* spp. and *Staphylococcus* spp. were quantified by membrane filtration. Media for bacterial analyses were obtained from Difco Laboratories, Detroit, MI, or from Becton Dickinson and Company, Cockeysville, MD. A volume of 100 mL of the samples was filtered through membrane filters with 0.45 μm pores (Millipore, MA, USA). The membranes were placed on solid media employed for each bacteria. The media, the bacteria enumerated and conditions for bacterial enumeration were as follows: m-Endo-Les, TC at 35 °C for 24 h; m-FC, FC in water by membrane filtration at 44.5 °C for 24 h; m-TEC, *E. coli* first at 35 °C for 2 h followed by incubation at 44.5 °C for 22 h; m-E, FS at 41 °C for 48 h; m-PAC, *P. aeruginosa* at 41.5 °C for 24 h; m-CP, *Clostridium* at 45 °C for 24–36 h in an anaerobic atmosphere; and Baird Parker, *Straphylococcus* spp. at 35 °C for 48 h and Plate Count Agar, HPC at 35°C for 72 h. Confirmation consisted of EIA at 41 °C for 20 min and Milk Agar at 35 °C for 24/48 h, cytochrome oxidase test, Gram stain and BBL crystal identification systems (Becton Dickinson). The heterotrophic plate count (HPC) was determined by the pour plate technique as described by the standard methods (APHA, 2005).

### Statistical analysis

Results were analyzed by linear regression and *t*-test, at $p<0.05$ of confidence level.

## Results

The physico-chemical characteristics of the tap water and bottled water were compared with the US EPA (2001) drinking water standards. The US EPA standards for drinking water were used since some guideline levels for physico-chemical characteristics were lacking in the Brazilian standards. All water quality constituents in bottled mineral water and tap water analyzed were within acceptable limits when compared with the US EPA directives (data not shown). Table 1 shows the standards for microbial quality of water for human consumption in Brazil.

The results of the microbiological analysis performed on samples of tap water from municipal water supplies, 20-L bottles of mineral water from water dispensers and samples collected from new 20-L bottles of mineral water before installation of the bottles in the dispensers appear in Table 2. TC bacteria were detected in 3 out of

96 (3.1%), 31 out of 77 (40.2%) and 5 out of 22 (22.7%) of the tap water from municipal supplies, 20-L bottles and new 20-L bottles of mineral water, respectively. Three (3.1%) of the tap water, 8 (10.3%) of the 20-L bottles and 1 (4.5%) of new 20-L bottle samples were positive for FC. Five (6.4%) samples from 20-L bottles were contaminated with *Escherichia coli*. The finding that 31/77 or more than 1/3 of the bottled water contains coliform organisms suggests the need for an improved surveillance system for the bottled water industry. Similar results were observed in new 20-L bottles; 5/22 or almost 1/4 bottles of water were positive for coliforms. FS were detected on the EIA plates in 7 out 77 (9.0%) and 1 out of 22 (4.5%) of the 20-L bottles and new 20-L bottles of mineral water, respectively. According to WHO (2003), the term ''fecal streptococci'' refers to those streptococci generally present in the feces of humans and animals. Their primary value in water quality examination is therefore as additional indicators of treatment efficiency. *Pseudomonas aeruginosa* contamination was evident in 43% of the samples; over 2/3 (58.4%) of the 20-L bottles were contaminated, which was higher than the new 20-L bottles (50%) and tap water samples from municipal supplies (29.1%).

The number of samples with HPC over the maximum level legally permitted in Brazil (500 colony forming units $mL^{-1}$) were 4 (4.17%), 67 (87.01%) and 10 (45.46%) for tap water, 20-L bottles and new 20-L bottles of mineral water, respectively. HPC ranged from 14 to 300,000 CFU $mL^{-1}$ among 77 samples of bottled water examined, including 67 samples with levels above 500 CFU $mL^{-1}$. Moreover, bacterial count in samples from new 20-L bottles ranged from 2 to 226,000 CFU $mL^{-1}$, counting 10 (45.46%) samples with levels above 500 CFU $mL^{-1}$. Of the 96 tap water samples from municipal supplies, only 4 had bacterial count above 500 CFU $mL^{-1}$. Considering that HPC is an indicator of hygienic condition and that disinfection does not completely eliminate these bacteria, different ranges of total bacterial densities were established.

On the basis of results obtained with a sampling of initial streams of water, the bacteriological quality of municipal tap water is superior to the quality of mineral water. Of the 195 samples examined, 109 (55.8%) were contaminated by at least one coliform or indicator bacterium and/or at least one pathogenic bacterium, including 35 (36.4%) of the 96 tap water samples from municipal supplies, 59 (76.6%) of the 77 20-L bottles and 15 (68.1%) of the 22 new 20-L bottles of mineral water. No *Clostridium* was found in any of the 195 samples. Table 3 shows the comparison among the absence of one indicator with the absence of all the other indicators.

**Table 1.** Brazilian microbiological limits[a] for mineral water and tap water from municipal supplies

| Characteristic | Tap water | Mineral water |
|---|---|---|
| Heterotrophic plate count[b] | 500 | 500 |
| Total coliforms | 0 | 0 |
| Fecal coliforms | 0 | 0 |
| *Escherichia coli* | 0 | 0 |
| *Staphylococcus* spp. | – | – |
| *Pseudomonas aeruginosa* | – | 0 |
| Fecal streptococci | – | 0 |
| Sulfite-reducing clostridia | – | 0 |

– Not determined.
[a]Colony-forming units per 100 mL.
[b]Colony-forming units per mL.

**Table 2.** Microbiological quality of tap water and bottled mineral water

| Indicator bacteria or pathogen | Number (%) of samples positive | | |
|---|---|---|---|
| | Tap water[a] ($n = 96$) | 20-L bottles[b] ($n = 77$) | New 20-L bottles[c] ($n = 22$) |
| Total coliforms | 3 (3.1) | 31 (40.2) | 5 (22.7) |
| Fecal coliforms | 3 (3.1) | 8 (10.3) | 1 (4.5) |
| *Escherichia coli* | 0 (0.0) | 5 (6.4) | 0 (0.0) |
| Fecal streptococci | 0 (0.0) | 7 (9.0) | 1 (4.5) |
| *Pseudomonas aeruginosa* | 28 (29.1) | 45 (58.4) | 11 (50.0) |
| *Staphylococcus* spp. | 6 (6.2) | 25 (32.4) | 2 (9.0) |
| HPC (CFU $mL^{-1}$) | | | |
| $< 1$ | 47 (49.0) | 0 (0.0) | 1 (4.5) |
| 1–500 | 45 (46.9) | 10 (13.0) | 10 (50.0) |
| $> 500$ | 4 (4.2) | 67 (87.0) | 11 (45.5) |
| Range | 0–2350 | 14–300,000 | 2–226,000 |
| Median | 95 | 22,658 | 12,665 |

[a]The most often-used faucet.
[b]20-L bottles.
[c]Bottled mineral water before installation of the bottles on water dispensers.

**Table 3.** Comparison among the absence of one indicator with the absence of all the other indicator

| Indicators bacteria | % of negative samples for the other indicators | |
|---|---|---|
| | Tap water[a] ($n = 96$) | Mineral water[b] ($n = 99$) |
| Total coliforms | 65.6 | 39.1 |
| Fecal coliforms | 65.5 | 26.1 |
| *Escherichia coli* | 63.5 | 25.0 |
| Fecal streptococci | 63.5 | 25.7 |
| *Pseudomonas aeruginosa* | 89.7 | 56.2 |
| *Staphylococcus* spp. | 67.7 | 34.6 |

[a]The most often-used faucet.
[b]20-L bottles.

## Discussion

The Brazilian directives (ANVISA, 2004) regulate water from municipal water supplies on the basis of coliform content and HPC, whereas more stringent bottled mineral water regulations prohibit the presence of a group of potentially pathogenic bacteria (*Pseudomonas*, FS and Clostridia).

Coliform organisms have long been recognized as a suitable microbial indicator of drinking-water quality, largely because they are easy to detect and enumerate in water (WHO, 2003). In drinking water from municipal supplies, the coliform test can be used as an indicator of treatment efficiency and of the integrity of the distribution system. Although coliform organisms may not always be directly related to the presence of fecal contamination, the presence of coliforms in drinking water suggests the potential presence of pathogenic enteric microorganisms such as *Salmonella* spp., *Shigella* spp., and *Vibrio cholerae*. Coliform bacteria are the only microbiological contamination to be regulated by law in both tap and bottled water. For tap water, the Brazilian directives state that for public water supplies that test at least 40 samples per month, contamination must not be present in 95% of the samples taken throughout any 12-month period. In the case of minimum frequencies, one sample every week for waterworks with a surface water source and one sample every 2 weeks for waterworks with a groundwater source must not be detectable in any 100 mL sample.

The presence of *E. coli* in water is nearly always associated with recent fecal pollution and it is the preferred indicator organism for this purpose (APHA, 2005). In the present study, the occurrence of coliform bacteria was significantly higher than the isolation of *E. coli*. However, the isolation of *E. coli* from 6.4% of the mineral water samples, with all the positive samples being mineral water from water dispensers, also has health implications, as these microorganisms are considered to be an indicator of fecal pollution. A Brazilian directive recognizes *E.coli* as the best indicator of fecal contamination, since several culture media, which enumerate FC may also enumerate *Klebsiella* spp., which are often present in industrial wastes.

The fact that neither *E.coli* nor FS was found in any of the 96 tap water samples in this study suggests the absence of fecal contamination of these samples. However, the finding that 3.1% of the tap water sampled in the current study failed to meet the Brazilian standard for both FC and TC in drinking water should, therefore, be of concern.

*P. aeruginosa* is an opportunistic pathogen that is known to cause urinary tract infections, respiratory system infections, dermatitis, soft tissue infections, bacteremia and a variety of systemic infections, particularly in patients who are severely immunocompromised, those with catheters, open wounds or cystic fibrosis. Outbreaks caused by this organism have been reported in various settings. The strain responsible for the outbreak may be spread via the hands of healthcare workers or by environmental sources of transmission such as contaminated water (Kolmos et al., 1993; Richard et al., 1994).

A particular feature of *Pseudomonas aeruginosa* is its ability to grow in low-nutrient water. Besides being a primary cause of disease, *P. aeruginosa* is often monitored as an indicator of other bacterial contamination of fecal origin (Warburton, 1992). The public health significance of the high number of *Pseudomonas* spp. which develop is unclear. Many *Pseudomonas* spp. recovered from water are resistant to antimicrobial agents (Hernandez Duquino and Rosenberg, 1987).

Water for human consumption is required to be free from any bacteria that might pose a health risk. The presence of biofilms in the drinking water distribution system may play a role in the presence of potential pathogens in the drinking water supply. During an outbreak of gastroenteritis in 28 children living in a small neighborhood of Cuernavaca city (Mexico), a survey was performed to evaluate the confidence in coliform bacteria as sole indicators of potability of drinking waters (Victoria and Galvan, 2001). A primary infection by *E. coli* and a secondary infection by *Pseudomonas aeruginosa* were diagnosed in 5 of the children and the drinking water provided by a well was suspected to be a transmission source. According to these authors a probability of correspondence between the presence of this bacterium and the secondary gastrointestinal infection diagnosed was found pointing towards a need for the inclusion of other microorganisms, one of which may be *P aeruginosa*, as indicators of health risk associated with drinking waters.

According to Payment and Franco (1993), *C. perfringens* is a better choice as an indicator of virus and cyst inactivation as well as an overall indicator of treatment efficiency. Testing for the spores of this bacterium can probably provide an added margin of safety in the evaluation of treatment. Also, it has been reported that *C. perfringens* could be a suitable indicator of the presence of pathogens of fecal origin in surface waters (Sorensen et al., 1989).

The European Directive on drinking water quality, unlike the previous one, has included *C. perfringens* as one of the microbiological parameters to be determined in order to control the quality of water for human consumption (EU, 1998).

In the present work, contamination with *Clostridium* was not observed in the tap water and bottled water. Unfortunately, we were unable to discern the dominant factor responsible for the absence of this indicator. Fewtrell et al. (1997) analyzed 1082 samples of bottled water and found that only one sample contained sulfite-reducing anaerobes (0.1%). In the study by Kohnen et al. (2005) 35% of the inner surfaces of the bottles were colonized with coliforms identified as *E. cloacae* and, in two cases, as *K. pmeumoniae*. Enterococci were found in 6% and *P. aeruginosa* in 12% of the bottles. Contamination with *E. coli* or spore-forming sulphite-reducing anaerobes was not observed in the tap water, in the carbonated water, on the inner surface of the bottle or on the gas tubing.

When the bacteria associated with fecal contamination and those derived from environmental sources were considered, the results shows that the absence of *P. aeruginosa* produced the highest percentage of negative samples for the other indicators in tap water (89.7%) and bottled mineral water (56.2%).

A correlation matrix was also established to compare the degree of association among microbiological indicators used in this study (data not shown). A positive correlation was observed among *P. aeruginosa*, FC and *Staphylococcus* spp., as well as between TC and *E. coli* for samples of mineral water. Contrarily, no correlation between the bacteria associated with fecal contamination and those derived from environmental sources was observed for samples of tap water from municipal water supplies.

According to Brazilian regulations, disinfection or sterilization of commercially available mineral water is not permitted. Therefore, generally high HPC are found a few days after bottling that results only from an increase in bacteria present in the source water. The number of bacteria recovered at the source is generally very low, around $10\,CFU\,mL^{-1}$, but there are many reports that viable counts increase, notably in uncarbonated water, to $10^4$–$10^5\,CFU\,mL^{-1}$ after 1–2 weeks of storage (Tamagnini and Gonzalez, 1997; Bischofberger et al., 1990; Mavridou, 1992; Mavridou et al., 1994; Tsai and Yu, 1997).

## Conclusion

The bacteriological quality of municipal tap water is superior when compared with the 20-L bottles of mineral water collected from water dispensers and samples collected from new 20-L bottles of mineral water before installation in the dispensers. This highlights the need for an improved surveillance system for the bottled water industry. Obviously, better efforts are necessary to eliminate planktonic bacteria and the biofilm, both sources of contamination that can concentrate opportunistic pathogens like *P. aeruginosa.* Moreover frequent cleaning of water dispensers would help eliminate various contaminants from the water and therefore lower the possibility of waterborne illness.

For the municipal water systems, the enumeration for *Pseudomonas* should be performed periodically (e.g. once a week or once a month depending on the system size and analytical capabilities), plus the routine data collected by most systems (coliforms, total bacteria, chlorine, pH, etc.).

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