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Quantitative variations in heterotrophic plate count and in the presence of indicator microorganisms in bottled mineral water

Maria Fernanda Falcone-Dias*, Adalberto Farache Filho

School of Pharmaceutical Sciences, UNESP- Univ Estadual Paulista, Araraquara SP, Brazil

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ABSTRACT

Quantitative variations in heterotrophic plate count (HPC) and in the presence of indicator microorganisms in 0.5, 1.5 and 20-L bottles of different brands of Brazilian mineral water were analyzed during their shelf life. No variations were identified in the presence of indicator microorganisms, but quantitative variations in HPC were observed in some brands, which suggests that changes may be occurring in the water quality during storage. The aim of this study was also to evaluate the quality of the bottled mineral waters and the presence of enterococci and *Pseudomonas aeruginosa* were verified in six and two bottles, respectively, which is in disagreement with the microbiological quality criteria established in the current legislation. Although no limit is set for HPC in mineral water, this study relies on the limit of 500 colony-forming units per mL of sample (CFU/mL). Seventy-two bottles presented levels above 500 CFU/mL and up to 560,000 CFU/mL. This study showed that the control of HPC (<500 CFU/mL) for non-returnable packaging seems to be adequate to ensure the quality of mineral water during storage. The high values of HPC and its variations detected during storage seem to fully justify the need for a reevaluation of the use of HPC in bottled mineral water quality management. More detailed studies on the potential health risk of HPC and its variations in mineral water are also needed.

1. Introduction

Natural mineral water means microbiologically wholesome water, originating in an underground water table or deposit and emerging from a spring tapped at one or more natural or bore exits. It may not be subjected to any treatment aside from the separation of unstable constituents and the elimination, introduction or reintroduction of carbon dioxide (Codex Alimentarius, 2001). Sales of bottled mineral water have been increasing all over the world. Dissatisfaction with the odor and taste of tap water due to chlorine, greater consumer concern about the safety of tap water and the use of bottled mineral water as a substitute for other beverages may have contributed to this increase (Varga, 2011). The successful promotion of bottled mineral water as clean, pure, safe and especially suitable for infants may also have increased its consumption (Bharath et al., 2003).

However, natural mineral waters are not sterile environments, but complex ecosystems with a high phenotypic and genetic diversity of autochthonous bacteria (Manaia, Munes, Morais, & Da Costa, 1990; Rosenberg, 2003). The autochthonous bacteria remain low in terms of population while the water is in its natural environment, but soon after bottling they begin to multiply rapidly (Leclerc & Moreau, 2002; Rosenberg, 2003) and can reach counts of 10^4–10^5 colony-forming units per mL of sample (CFU/mL) within a few days (Leclerc & Moreau, 2002). However, despite this multiplication being considered normal (Rosenberg, 2003), the long storage time of mineral water has a profound effect on the variation of the bacterial population, which may also indicate changes in water quality (Leclerc & Moreau, 2002; Morais & Da Costa, 1990). The reasons for this multiplication after bottling are still a matter of debate. The influence of materials, the relative contributions of attached (e.g., biofilms) to unattached microbes (Bischofberger, Cha, Schmitt, Konig, & Schmidt-Lorenz, 1990; Jayasekara, Heard, Cox, & Fleet, 1998; Schmidt–Lorenz, 1976; Zobell & Anderson, 1936), and the regrowth (from small initial populations) as opposed to resuscitation of existing microbes (Leclerc & Moreau, 2002) show the complexity of the issue. For these reasons, the study of variations of bacteria in bottled mineral water during storage can provide important data for excluding the possibility of microbial growth, thus protecting the product from deterioration and ensuring its quality to the consumer.

Because of the normal multiplication of autochthonous bacteria after bottling, the heterotrophic plate count (HPC) is not used as

* Corresponding author. Pharmaceutical Sciences, Univ Estadual Paulista-UNESP, Rod.Araraquara-Jau km 1 Campus Ville, 14801 – 902, Araraquara, São Paulo, Brazil. Tel.: +55 16 3301 6941; fax: +55 16 3301 6940.
E-mail address: mariafernandafdias@hotmail.com (M.F. Falcone-Dias).

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a quality parameter for bottled mineral water during marketing (ANVISA, 2005; Codex Alimentarius, 1985). Moreover, there is insufficient clinical and epidemiological evidence to conclude that high HPC in drinking water pose a risk to consumer health (Edberg & Allen, 2004; Otterholt & Charnock, 2011; Varga, 2011). However, members of some species that may be part of the mineral water microbiota can cause diseases, mainly in vulnerable individuals, i.e., the very young, the elderly, the immune suppressed population, and pregnant women (Otterholt & Charnock, 2011; Varga, 2011). In addition, multiple antibiotic-resistant bacteria were isolated from bottled mineral water using HPC procedures (Falcone-Dias, Vaz-Moreira, & Manaia, 2012; Massa, Petruccioli, Fanelli, & Gori, 1995; Mary, Defives, & Hornez, 2000; Messi, Guerrieri, & Bondi, 2005; Rosenberg & Hernandez-Duquino, 1989). It is also known that high levels of microbial growth can affect the taste and odor of drinking water and may well indicate the presence of nutrients and biofilms (Sartory, 2004). Therefore, HPC procedures are always recommended, although there is still much discussion about its importance to health (Rosenberg, 2003). It is believed that HPC should be used as an indicator in bottled water production, as it is in public water supply (Allen, Edberg, & Reasoner, 2004).

All those studies suggest that variations in bacteria counts in bottled mineral water may occur during storage, which may indicate changes in water quality, and that the use of HPC as a quality parameter for bottled mineral water still requires attention. Thus, the aim of the current study was to detect quantitative variations in HPC and in the presence of indicator microorganisms during the shelf life of bottled mineral waters originated from the State of São Paulo, Brazil. Their microbiological quality and the HPC limit were also evaluated and discussed.

2. Materials and methods

2.1. Sampling

Four brands for each size of packaging polyethylene (0.5, 1.5 and 20-liter) of non-carbonated natural mineral water were analyzed. These waters were distributed as: 0.5-liter (L) bottles – brands A, B, C and D; 1.5-L bottles – brands A, B, C and D and 20-L bottles – brands A, D, E and F (Fig. 1 and Table 1). Thirty-three 0.5 and 1.5-L bottles of the same batch were acquired for each brand and were analyzed during their shelf life (12 months); fifteen 20-L bottles of the same batch were also acquired for each brand and analyzed during their shelf life (2 months). Three bottles (same batch) of each brand were analyzed on each day of analysis, and all analyses were made in triplicate. A total of 324 bottles of mineral water was analyzed. Prior to the investigation, the bottles were stored at room temperature (22–25 °C) and kept away from the sun. All these mineral waters were purchased from retail outlet in Araraquara city (State of São Paulo, Brazil).

2.2. Microbiological analysis

2.2.1. Determination of total coliforms and Escherichia coli (E. coli)

To determine the presence of total coliforms and *E. coli*, the Enzyme Substrate Test was used as described in the standard methods for the examination of water and wastewater (APHA, 2005). The dehydrated Colilert® medium (IDEXX, Maine, USA) was added to 100 mL of each sample in a disposable sterile bottle, and incubated at 35 °C for 24 h. Following incubation, the samples were read for yellow color, which indicates coliform β-galactosidase activity from total coliforms and fluorescence as a result of the action of β -glucuronidase from *E. coli*. An ultraviolet (UV) lamp (at 365 nm) was used to identify the fluo rescence.

2.2.2. Determination of Pseudomonas aeruginosa (P. aeruginosa)

The presence of *P. aeruginosa* was determined by the multiple tube method as described in the standard methods for the examination of water and wastewater (APHA, 2005). For the presumptive test, 10-mL aliquots of sample were introduced into each of ten tubes with 10 mL double Asparagine broth (VETEC, Rio de Janeiro, Brazil) and incubated at 35 °C for 48 h. The production of pyoverdin in the asparagine broth, detected by fluorescence under a UV lamp (at 365 nm), meant that the presumptive test was positive. The confirmatory test was carried out by transferring 0.1-mL inocula from the positive asparagine broth tubes into Acetamide broth (VETEC, Rio de Janeiro, Brazil) and incubating the tubes at 35 °C for 24–36 h. Tubes that developed an alkaline reaction (purple coloration) were considered positive for the confirmatory test.

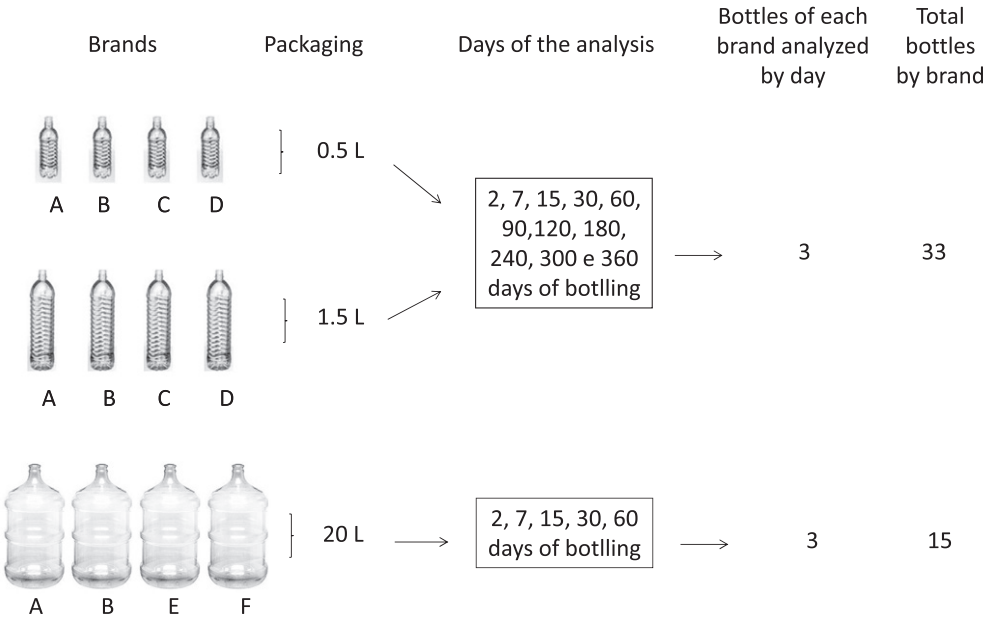

Fig. 1. Schematic representation of sampling.