

A Random Study of the Microbiological Quality of Bottled Drinking Water in Canada

Sonish Azam, Massimo Marino and Ali Khamessan
Microbiology Laboratory, C Crest Laboratories.

9390 Boul. Langelier
Montreal Quebec
H1P 3H8 Canada
Phone No. 514-324-1073
Fax No. 514-324-2543

Abstract

Microorganisms are ubiquitous in the environment. Drinking water along with food, air and soil is one of the numerous possible sources of microbes. This project focuses on the level of heterotrophic microorganisms in bottled drinking water which could be a health concern for the elderly, infants, pregnant women and immuno-compromised patients. Regulatory bodies such as Food and Drug Administration (FDA), Environmental Protection Agency (EPA), World Health Organization (WHO) and Health Canada do not specify a maximum allowed limit for the heterotrophic bacteria counts in bottled drinking water available in the market. However, according to the United States Pharmacopoeia (USP) not more than 500 CFU/mL of microbial contaminants should be present in the water used for drinking. In this study, different brands of packaged water (from 0.5L plastic bottles to 20L carboys) were analyzed for their microbiological quality, using different culture media. Heterotrophic microbiological count varies between less than 10 and 72,000 CFU/mL for ten different brands of bottled water. Whereas, the average heterotrophic microbial count for the tap water and USP water samples was 170 CFU/mL and less than 10 CFU/mL respectively. Morphological studies indicated the presence of five different kinds of colonies in the bottled water samples. There were no cases of fecal contamination or the presence of *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella* however, we could not rule out the possibilities of some random species of opportunistic pathogens which were found to sustain growth in the bottles. Bottled water is not expected to be free from microorganisms but the CFU observed in the samples was surprisingly high which indirectly reflects the poor sanitary practices during the packaging of the product. Since the significance of non-pathogenic heterotrophic microorganisms in relation to health and diseases is not entirely understood, there is an urgent need to establish a maximum limit for the heterotrophic count in the bottled water that should be tested and regulated periodically. Also, the unknown microbial isolates found in bottled water should be identified at species level and studied for their pathogenicity.

Overview

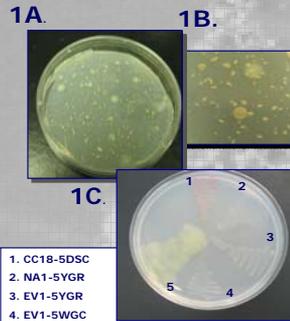
- Consumers assume that since bottled water carries a price tag, it is purer and safer than most tap water.
- Regulatory bodies do not specify a maximum limit for 'heterotrophic microbial count' in bottled water.^(1,2)
- Bottled water is regulated as food product and is not required to meet the guidelines for *Canadian Drinking Water Quality*.⁽²⁾
- Unknown heterotrophic microorganisms present in bottled water might act as opportunistic pathogens and cause serious health hazards in the vulnerable section of the population.⁽³⁾
- A number of cases of bottled water contamination and illness have been reported leading to product recall.^(4,5)
- Present study was initiated based on a complaint of sickness and foul taste after consuming bottled water.

Methods

- Bottled water samples were randomly selected for the study, irrespective of their packaging formats (0.5L to 20L).
- Water samples were analyzed within 24 hours of their purchase/ collection.
- Sample dilutions (1/10 or 1/100) were performed based on the number of CFU present in water.
- Water samples were diluted in phosphate buffer pH 7.0 and plated in different agar media (TSA, SMA and R2A) as per the 'USP pour-plate method'.⁽⁶⁾
- Agar media plates were incubated at 32°C for 7 days.

Results

Fig 1. (A & B) Heterotrophic bacterial count in bottled water. Randomly selected bottled water samples were analyzed for microbiological contamination. Water samples were diluted to 1/100 times in Phosphate buffer and were plated in R2A Agar. Majority of the samples were found to have more than 500 CFU/mL of microbial count which is over the United States Pharmacopoeia (USP) specification for drinking water (≤ 500 CFU/mL).



(C) Variety of microorganisms found in bottled water. A number of morphologically different colonies of unknown bacteria were found in different brands of bottled water. These bacteria have not been entirely elucidated for their safety with regard to human consumption.

1. CC18-5DSC
2. NA1-5YGR
3. EV1-5YGR
4. EV1-5WGC
5. FR18-5NGR

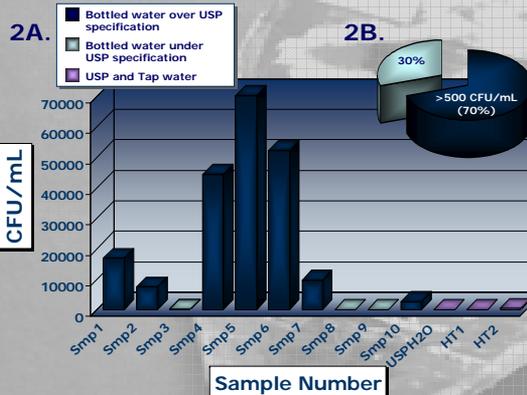


Fig 2. Number of CFU/mL of heterotrophic bacteria obtained in bottled water samples. Randomly selected bottled water, tap water and USP water samples were analyzed for the number of heterotrophic bacteria count. (A) The CFU/mL obtained in different brands of bottled water ranged between less than 10 and 7.2×10^4 . The highest CFU was found in Smp 6 which was more than 150 times the USP specified limit of 500 CFU/mL of microbes for drinking water. The number of microorganisms found in the tap water samples (HT1 & HT2) was lower than that of the bottled water. Moreover, USP water was found to have the lowest number of microorganisms (<10 CFU/mL) among all the water samples tested. (B) Approximately 70% of the randomly selected bottled water samples (Smp 1, 2, 5, 6, 7 & 8) did not meet the USP specification for drinking water. Experiments were done in duplicates and the data represents mean of the three independent analyses performed on three different lots of the same brand of bottled water.

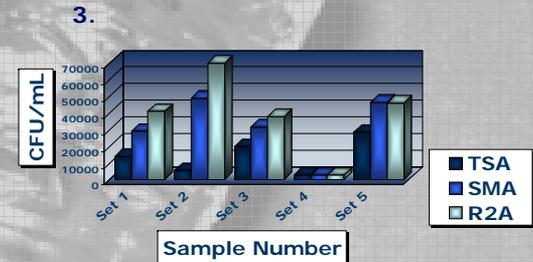


Fig 3. Effect of media on the growth of heterotrophic bacteria in bottled water. Three different agar media: Trypticase Soy Agar (TSA), Standard Media Agar (SMA) and Reasoner's 2A agar (R2A) were used to analyze five randomly selected brands of bottled water (Set 1-5). The results indicate that the heterotrophic bacteria count obtained in R2A agar for different brands of bottled water was higher than in TSA and SMA plates. In addition, some of the slow growing bacteria could only be recovered after 14 days of incubation (data not shown). Therefore, growth conditions such as the media, temperature and time of incubation are critical parameters for the recovery of different types of microorganisms present in bottled water. Experiments were done in duplicates and the data represents the mean of two independent analyses done on two different lots of the same brand of bottled water in three different media.

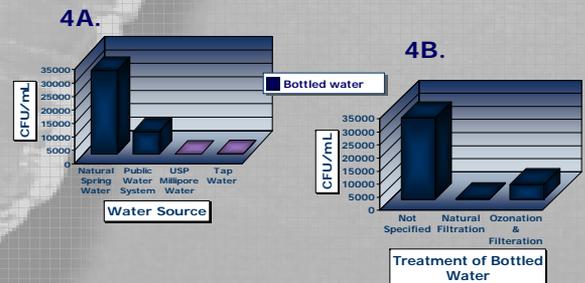


Fig 4. Effect of the source and treatment of bottled water on the CFU/mL of heterotrophic bacteria count. Randomly selected bottled water samples from different sources and with different purification treatments were analyzed in R2A agar by pour-plate method: (A) The average CFU/mL obtained in different brands of Bottled Natural Spring Water was found to be higher ($>30 \times 10^4$) than the Bottled Public Water. Millipore Water and Tap Water samples showed relatively lower microbial counts of <10 CFU/mL and 175 CFU/mL respectively. (B) Some bottled water labels do not clearly specify the purification treatments performed on its contents. Such samples with unknown or no treatment showed the highest number of CFU/mL of microbes. As expected, the average CFU/mL of microbes in treated samples such as Naturally Filtered and Ozonated bottled water were comparatively lower. Experiments were done in duplicates and the data shown here is the mean CFU/mL obtained for the three members of each group.

Conclusions

- Heterotrophic bacteria count in 70% of randomly selected bottled water brands in Canada exceeds the USP specified limit of 500 CFU/mL for drinking water.
- Growth conditions play a critical role in the recovery of heterotrophic bacteria in bottled drinking water. R2A agar, a low-nutrient media is preferred for the higher recovery of slow-growing bacteria which are otherwise suppressed by faster-growing species on a richer culture medium.
- This study puts a question mark on the safety of common public who consumes unknown microorganisms in bottled water.
- A maximum specific limit for the heterotrophic microbial count in the bottled water should be established. In addition to Coliforms, bottled water should be confirmed for the absence of other pathogens such as *Pseudomonas sp.* etc.
- Label on the bottled water should disclose the purification/treatment procedure and special instructions/precautions for weak or immuno-compromised individuals.

References

- Polaris Institute (2009). Murky Waters: The Urgent Need for Health and Environmental Regulations of the Bottled Water Industry. Ottawa, ON, Polaris Institute. Retrieved from <http://www.polarisinstitute.org/files/Murky%20Waters%20-%20The%20Industry%20News%20-%20Water%20-%20Environment%20-%20Regulations%20-%202009%20-%20Polaris%20-%20Water%20-%20Industry.pdf>
- Health Canada (2009). Frequently Asked Questions about Bottled Water. Retrieved December, 21st 2009 from: http://www.hc-sc.gc.ca/health/water/faq_bottled_water_eau_embouteille-eng.php
- J. Bartram, J. Cotruvo, M. Exner, C. Fricker, A. Glasmeier (2000). Heterotrophic Plate Counts and Drinking Water Safety. World Health Organization (WHO) Published by WHO Publishing, London, UK.
- Donato, Agnes E. Saigon Tribune. Crystal Waters Remains Closed. Nov. 14th, 2007.
- Sivola, Emily. 'Buddy Bottles Water' Post-22.com. December 4th, 2007.
- United States Pharmacopoeia (2010). USP32: NF 27.52.

Correspondence:

Dr. Ali _____