Sunsari Technical College Journal 2015; 2(1):54-57

ISSN: 2091-2102 (online)
: 2467-9224(print)

Original Research Article

BACTERIOLOGICAL EVALUATION OF BOTTLED WATER COMMERCIALLY AVAILABLE IN EASTERN NEPAL

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Received: 30.06.2015; Revised and Accepted- 23.08.2015

DOI: http://dx.doi.org/10.3126/stcj.v2i1.14801

Abstract

The demand for bottled water is high in these days and it has been a growing concern about the microbiological quality of such processed water. The objective of this study was to assess the heterotrophic contamination and total coliform. This cross-sectional study was carried out in randomly selected 24 different commercial brands of bottled water available in the commercial market of the eastern region of Nepal. Total coliform and heterotrophic bacteria were enumerated by the membrane filtration and spread plate technique, respectively. Among 24 water sample processed, 62.5% (n=15) samples showed the heterotrophic bacterial count above the acceptable range (<50 CFU/0.1ml) and 75% (n=18) samples showed the total coliform count crossed the WHO guidelines (0 CFU/ml) and not acceptable for drinking. *E. Coli* was detected in 54.2% of water samples and *Enterobacters aerogenes* (45.8%), *Enterococcus fecalis* (20.8%), *Pseudomonas* sps (20.8%), *Bacillus* species (16.6%) and *Staphylococcus aereus* (4.2%) were also detected from different samples. Only 25% of bottled water was acceptable for drinking purpose. The presence of heterotrophs and the coliform in the bottled water is a serious concern for public health. Concerned authority should prioritize this issue and continuously monitor the quality of available bottled water manufacturers in the eastern region to provide safe drinking water to the population.

key words:bottled drinking water, coliforms, heterotrophic count, membrane filter, water quality

INTRODUCTION

Water is life for all and it is commercialized in the market by bottling in plastic or glass bottles. Bottled water is any such form of consumable packaged water that is processed in food-grade bottles and brought down to markets or stores for sale.[1, 2] The sources of water for bottled water could be spring, dug wells, tube wells, underground water, rivers, tap water, glacial etc. The water collected from these sources are then processed as per the 'WHO guidelines for drinking water quality", i.e. no *Escherichia coli* in a 100ml water sample.[3]Bottled water is mainly recognized as either mineral water or spring water according to their sources of water.Bottled water is packaged in bottles made up of plastic or polyvinyl chloride (PVC), polyethylene terephthalate (PET) and with glass bottles. Mineral water may contain minerals both essential and toxic to humans, on the other hand, distilled water or de-mineralized water is usually tap water which is treated to eliminate toxic elements and that lowers the mineral elements in water.[4]Bottled water may be contaminated as ground water during manufacturing process due to lack of sanitation of production plant and use of contaminated water sources.[5] Microorganisms of genus such as *Pseudomonas, Aeromonas, Flavobacterium, Escherichia, Salmonella* and *Staphylococcus*etc may leads to contamination of water.[6] Although there is no direct relationship between heterotrophic plate count (HPC) values and human health effect in the absence of faecal contamination in the population at large but it indicates the wrong manufacture process.[7] Various water-borne diseases are caused due to drinking of water contaminated with human and animal faeces like cholera, dysentery, typhoid fever, hepatitis and other. *Salmonella* Typhiand *Vibrio cholerae* are most

common pathogenic bacteria transmitted by water route which causes typhoid fever and cholera respectively.[8]

Bottled water is one of the rapidly growing and consumed potable water due to its low cost, easy availability, and safety. Purity of drinking water cannot be detected by smell or taste. If drinking water contains a single coliform, it is unfit for drinking.[9]The main goal of this research is to evaluate total coliform and heterotrophs contamination in processed drinking water.

MATERIALS AND METHODS

Sampling of water samples: Altogether 24 processed drinking water bottles of commercially available different brands were collected from the local market, department store, and street vendors of Dharan sub-metropolitan city, Nepal in august 2015. Samples were transported in cool condition to the Microbiology Laboratory, Sunsari Technical College, Dharan, Nepal for microbial analysis. All samples were stored at 4^0C before analysis.

Microbial examination of water samples: Total coliform were detected by membrane filtration technique (0.45 µm, Pall Corporation, Washington). 100ml samples were filtered through membrane filters and filters were transferred to petri plates containing M-endo agar (Hi-media, India) with the grid side upward then incubated at 37^0C for 24 hrs. The colonies on membrane filter were counted. The heterotrophic bacteria count was done by using spread plate technique by spreading 0.1ml original water sample and 0.1ml of 10 times diluted water sample on plate count agar (Hi-media) separately and aseptically. Both plates were incubated at 37^0C for 24hrs all colonies were enumerated and mean was taken and all heterotroph were identified by various biochemical techniques.

RESULTS AND DISCUSSION

Out of total 24 samples, 62.5% (n=15) samples were found above satisfactory range in heterotrophic plate count (<50 CFU/0.1ml) and on the other hand 75% (n=18) samples were found above WHO guidelines (0cfu/ml) in coliform, 25% (n=6) samples were found within the WHO guideline (0cfu/ml) in coliform. Both heterotrophs as well as coliforms were found on 75% (n= 18) of the sample whereas no samples were free from both heterotrophs and coliform. Too much to count (TMTC) heterotrophs were found on 20.8% (n=5) samples and coliforms on 4.2% (n=5). Similarly, out of the total sample, only 37.5% and 85% of the sample bottled water were considered to be consumable on the basis of a number of heterotrophs and coliforms respectively according to national and WHO guidelines. Only 12.5% (n = 3) bottled water samples were microbiologically safe and bacteriological load is within the acceptable range for drinking purpose whereas; 87.5% (n = 21) sample brands of bottled water were not safe for drinking purpose as they crossed the heterotrophic and/or total coliform count standard range according to national and WHO guidelines.

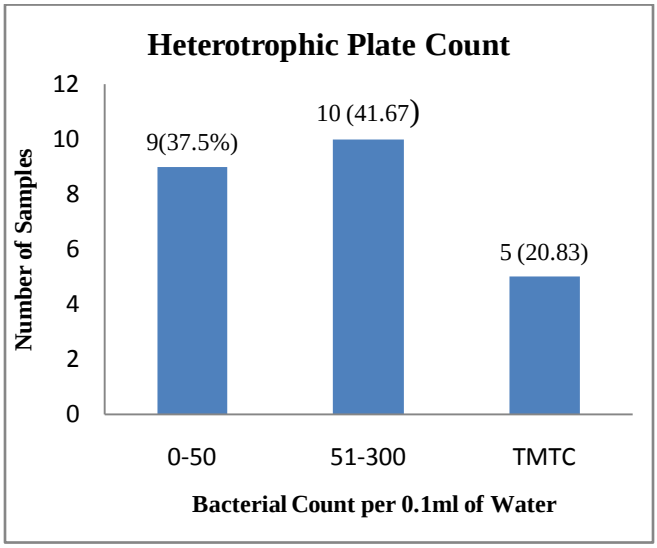

Figure 1. HPC of Bottled water

On a plate count agar, 0.1 ml of drinking water was spread and incubated overnight. The heterotrophs were counted and expressed as CFU/0.1 ml of drinking water.

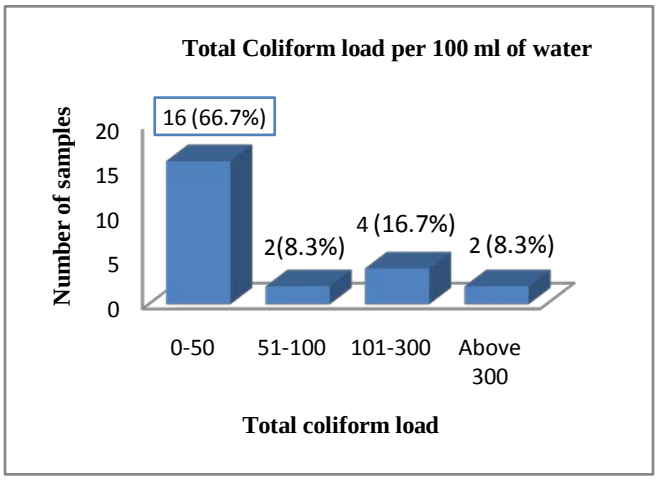

Figure 2. Total coliform Count by Membrane Filter Technique

Membane filter was done for 100 ml of bottled drinking water and filtered through the standard membrane filter of pore size 0.45 µm and membrane was inoculated in an M-Endo agar overnight for a total coliform count and it was expressed in CFU/100 ml of drinking water.

Bacterial analysis

On bacterial analysis, *E. coli* was found on 54.2% (n=13) of the water sample and *Enterobacter aerogenes* on 45.8% (n=11) samples whereas, *Pseudomonas* spp and *Streptococcus fecalis* were just above one fifth of samples.

Table 1: Recipe for the preparation of the mayonnaise

Bacterial species	Percentage
Escherichia coli	54.2 (n = 13)
Enterobacteraerogenes	45.8 (n =11)
*Pseudomonas*spps	20.8 (n = 5)
Enterococcus faecalis	20.8 (n =5)
*Bacillus*spps	16.6 (n = 4)
Staphylococcus aureus	4.1 (n = 1)
Escherichia coli	54.2 (n = 13)
Enterobacteraerogenes	45.8 (n =11)
*Pseudomonas*spps	20.8 (n = 5)
Enterococcus faecalis	20.8 (n =5)

In this study, randomly selected commercial bottled water samples were gathered from stores and market of Dharan Sub-metropolitan city and analyzed in the microbiology lab, Sunsari Technical College, Dharan, Nepal. Out of the 24 samples collected, though they were visibly clear, 62.5% (n=15) contained heterotrophic count to the unacceptable range and 75% (n=18) contained total coliform and were found in the unsatisfactory range of WHO guidelines (0cfu/ml) in coliform respectively. Nearly all bottled water selling in Dharan 87.5% (n = 21) brands of bottled water were unsafe for drinking purpose as they crossed the heterotrophic and/or total coliform count standard range according to national and WHO guidelines. The coliform study was perfomed by membrane filtration method for the total coliform count and plate count method for the heterotrophic count. This study revealed variation in the safety and quality of bottled waters sold in different shops and streets of eastern Nepal and raised a big question to the consumer's right to safe water.

This result had reported a low occurrence of total coliform then in a study by Rai et al on 2009 to 2011, which was 86.5%, in all types of the drinking water samples of all development region of Nepal.[10] In a similar study done by Timilshina et al; at 2012, total coliform had been reported slightly lower (56.6%) than this study.[11]*E.coli* was predominant (54.2%) bacteria among coliforms which notified a poor sanitary activity on commercially selling water bottle and 45.8% water sample had *Enterobacter* spps and one fifth of the water sample was found to be contaminated by *Pseudomonas* spp and *Streptococcus fecalis.* A study done in Kathmandu, Nepal had reported the lower presence of *E. coli* and *Enterobacter* spp on drinking jar water sample than this study.[12]Jayana et al on 2009 reported lower presence of *E. coli* (24.6%) and *Enterobacter* spps (29.5%) than this study.[13]

Heterotrophs, being natural microbial flora are freely available in the environment and can multiply even after bottling.[2] The presence of heterotrophs and coliforms in bottled water suggests fecal contamination in the source of water or incompetent manufacturing process.[14] Heterotrophs and coliforms can cause serious health hazard problems in children and immune compromised patients.[6] According to national and WHO guidelines, drinking water should not contain coliform and less than 50 CFU/ml for heterotrophs.[15]

The presence of total coliforms indicates a poor quality of drinking water and the occurrence of the coliform and unacceptable range of heterotrophic bacteria in commercially selling bottled water is a serious concern to the public and to who are immune compromised. The governmental and municipal authority should take an immediate attention to this type of issues which are directly impacting to public health as a hazard.

CONCLUSION

The results of the study on commercially available bottled drinking water showed an unsatisfactory condition for the consumption as they crossed an unsatisfactory range of coliform count and heterotrophic count. So it is concluded that the industry must follow the standard method of consumable water packaging and the concerned agencies must inspect regularly.

ACKNOWLEDGEMENT

The authors want to acknowledge to whole the management committee of Sunsari Technical College, Dharan, Nepal and all the members of the department of microbiology.

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Cite this article as: Rai R, Kumal B, Rai D, Keshari A, Bhandari R. Bacteriological evaluation of bottled water commercially available in Eastern Nepal. *STCJ 2015;2(1)*:54-57.