ORIGINAL ARTICLE

BACTERIOLOGICAL EXAMINATION ON BOTTLED WATER

Rajnarayan Mukhiya[1], Puja Thapa[1], Shrawasti Shakya[1], Soniya B.K[1], Rupa Nepal[1*], Ram Krishna Shrestha[1], Rajesh Kumar Thakur[2]

[1]*Department of Microbiology, Modern Technical College, Sanepa, Lalitpur*
[2]*Department of Laboratory Medicine, Manmohan Memorial Institute of Health Sciences, Kathmandu, Nepal*

ABSTRACT

Introduction: Bottled water are the packaged drinking water available commercially and manufactured by the company after treatment techniques like UV light, reverse osmosis or distillation, chlorination and carbon filtration etc. which are supposed to reduce the bacterial contamination to the safe level. Water-related diseases are of great concern in developing countries like Nepal. Every year, there are countless morbidity and mortality due to the consumption of unsafe drinking water. Recently, there have been increased uses of bottled drinking water in an assumption that the bottled water is safer than other sources of water and its use will help to protect from water related diseases. So, the main objective of this study was to analyze the bacteriological quality of bottled drinking water

Method: A community based cross-sectional study was conducted on bottled drinking water. A total of 60 bottled drinking water samples were collected for microbiological examination for the detection of total coliforms via membrane filtration technique followed by inoculation on M- endo agar and heterophilic count by pour plate method in Nutrient agar. The bacterial growth in these medium were counted as CFU/100ml and CFU/ml respectively. The bacterial isolates were identified by colony morphology, Gram staining and biochemical reaction.

Results: Among total 60 samples, 48(80%) were positive for bacterial growth and 12(20%) revealed no bacterial growth. 35(58.33%) samples were contaminated with coliforms. Similarly, among total 60 samples, 48(80%) samples were contaminated with heterophiles.

Conclusion: More than half of the bottled water showed bacterial growth in terms of both coliform and heterotrophs. Presence of coliforms indicate fecal contamination and lack of proper processing.

Key words: ***Bottled water, Membrane filtration, Coliform, Pour plate technique***

https://doi.org/10.3126/jmmihs.v10i1.77721

**Corresponding Author: Rupa Nepal, Deparment of Microbiology, Modern Technical College, Sanepa, Lalitpur*
Email: rupanepal@gmail.com
Received 8 April 2025 ; Received in Revised from 12 April 2025; Accepted 13 April 2025

INTRODUCTION

Water is one of the indispensable resources for the continued existence of all living things including human [1]. Drinking unsafe and unhygienic water can cause high prevalence of waterborne disease like diarrhea, typhoid and cholera[2,3].

Recently, there has been a considerable worldwide increase in the consumption of bottled water due to consumer's awareness regarding bottled water as a healthy alternative to tap water. Water treatment techniques like UV light treatment, reverse osmosis or distillation, chlorination and carbon filtration, etc. are used by bottled water manufacturing plant which reduces the bacterial contamination to the safe level. Gleick, Lalumandier and Anadu have stated that bottled water has been used instead of tap water for its convenience, better taste, and perceived purity[4-6].However, bottled water is not necessarily safer than tap water. Many studies have reported the presence of heterotrophic bacteria along with coliforms in bottled water in counts, exceeding national and international standards [7].

World Health Organization (WHO) has reported that about 30,000 people and children die every day from water-related diseases, more critically, in developing or least developing countries. According to the data published by public health department, Nepal government, every year, about 3500 children die due to water-related illnesses [7].

According to World Health Organization (WHO) recommendations, portable water should have < 20 CFU/ml heterotrophic bacterial count with complete absence of coliform bacteria, fecal coliforms, *E. coli, Enterococci and Pseudomonas aeruginosa.* Although, coliform organisms may not always be considered as indicator of fecal contamination their presence in drinking water suggests the potential presence of pathogenic enteric microorganisms such as *Salmonella spp. , Shigella spp. and Vibrio cholera* [8] .

Typically, there are three main parameters for drinking water quality standard such as physical, chemical and microbiological. Among them, microbial contamination is a major concern of water-related health burden [9]. The biggest health threat worldwide like waterborne diseases has been contributed between 70-80% of health problems in developing countries [10]. The presence of both non-coliform species like heterotrophic bacteria and total coliform bacteria may cause the special health risk for infants and young children and immune compromised adults [11].

According to the recommendation by World Health Organization (WHO), the microbial quality of drinking-water is measured using fecal indicator bacteria, preferably E.coli that indicate the presence of fecal contamination rather than identifying pathogens directly [9]. Edberg also reported that it is not necessary for analyzing drinking water for all pathogens but requiring an indicator of fecal pollution for public health protection. The presence of E.coli bacteria as indicator determines the risk of pathogenic contamination from fecal origin[12,13].

In this study we used total plate count technique for the isolation of the bacteria present in the bottled water samples and membrane filtration for the fecal coliform.

METHODS

A community based cross sectional study was conducted from February to April 2024 at Microbiology Laboratory of Modern Technical College and Central Department of Microbiology, TU Kirtipur. A total of 60 bottled water were collected from the local market. The condition of seal, storage site, manufactured and expiry date were checked. For the detection of total coliforms membrane filtration was done and the filter was placed on M- endo agar. After incubation for 24hrs at 37oC, the colonies were observed and counted. Differentiation of coliforms were done basis of colony morphology and biochemical reaction. For heterophilic count pour plate method was done after diluting the water (sample) upto 10^{-3} on nutrient agar. The nutrient agar plate was then

How to Cite

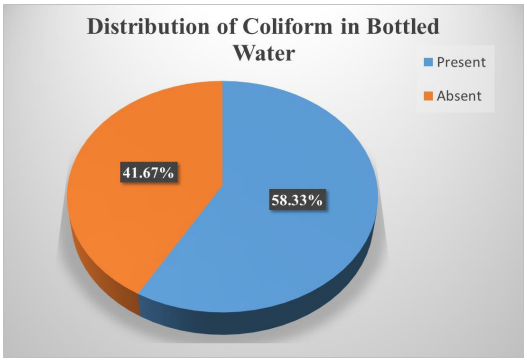
Mukhiya, R., Thapa, P., Shakya, S., B.K, S., Nepal, R., Shrestha, R. K., & Thakur, R. K. Bacteriological Examination on Bottled Water. Journal of Manmohan Memorial Institute of Health Sciences, 10(1), 36–38. https://doi.org/10.3126/jmmihs.v10i1.77721

incubated for 24hrs at 37°C. Colony count was done on following day. The bacterial colonies in M-endo agar medium was interpreted as CFU/100ml and that in nutrient agar as CFU/ml respectively. The bacteria were identified by colony morphology, Gram staining and reaction on biochemical medium.

RESULTS

Out of 60 bottled water examined 48(80%) showed bacterial growth and 12(20%) revealed no growth. Coliform was seen in 35 water sample; among which *Klebsiella pneumoniae*, *Enterobacter aerogenes* and *E. coli* were most predominantly isolated bacteria covering 47.36%, 36.84% and 15.78% of total positive outcomes respectively. Among the total sample processed 48(80%) samples were contaminated with heterotrophic bacteria and the remaining 12(20%) samples were free from it. The most prevalent heterophilic bacteria, 17(35.41%) were *Bacillus spp.*, followed by *CONS* 10(20.83%), *Staphylococcus aureus* 7(14.58%), *Clostridium spp.* 5(10.41%) *Pseudomonas spp.* 4(8.33%), *Proteus spp.* 4(8.33%) and 1(2.08%) was *Streptococci.*

Distribution of Coliform in bottled water



Distribution of heterophilic bacteria in bottled water

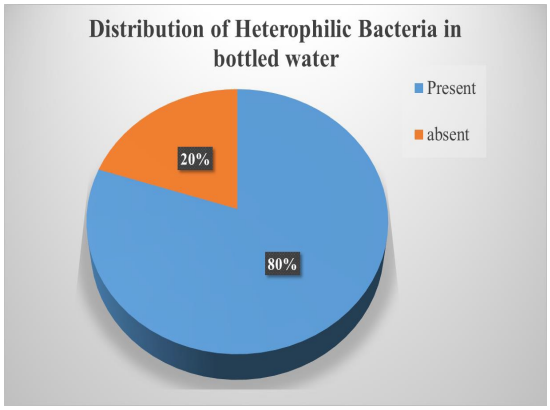


Table 1: Distribution of coliform

Coliform	Number	Percent
Klebsiella pneumoniae	18	47.4
Enterobacter aerogenes	14	36.8
E. coli	38	15.8

Table 2: Distribution of Heterophilic Isolates

Bacteria	Percent
Bacillus spp.	20.8
CONS	14.5
Staphylococcus aureus	10.4
Clostridium spp.	8.3
Pseudomonas spp.	8.3
Proteus spp.	2.08
Streptococci	2.08

DISCUSSIONS

This study showed that out of total 60 bottled water sample 48(80%) showed positive growth This finding is similar to the study conducted in Jaipur by Gangil R. et al 2013(45%) [17], in Northern Ethiopia by Kleb A. et al 2022(55.3%). Among the positive growth 35(58.33%) were positive for total coliform. A higher rate of Total coliform contamination (76.6%) was seen in the study of da silva. et al 2008[21]. Similarly, our study revealed the presence of heterotrophic bacteria as 48 (80%).This finding is similar to the study conducted in Jaipur by Gangil R. et al 2013(80%) [17].

The bacterial concentration in bottled water generally depends on the disinfection processes used by the factory [25]. Presence of bacteria may be indigenous from the natural source of water or may be introduced during processing or handling[26]. Although the microbial concentration in processed water is initially low, it can develop into high level during storage [27]. The reasons for this may be due to the high level of oxygen provided to the water during processing, larger surface area provided by the container, higher temperature, and the nutrients arising in the container[28].

Higher concentration of the bacteria may also occur through carriers like introduced flakes of human skin, particularly in non-ozonated and non-carbonated water[29]. In this study, *Klebsiella pneumonia* was the most prominent isolated coliform which covered 47.36% followed by *Enterobacter aerogenes*(36.84%) and *E. coli*(15.78%). This result is similar to a study conducted in Chennai by Venkatesan K.D. et al (2014) where 11.9% E. coli were found[15] and *Klebsiella pneumoniae* and *Enterobacter aerogenes* were 4.8% and 2.4% respectively, which is comparatively lower than this study[15]. The presence of *E. coli* in bottled water is strong indication of sewage or animal waste contamination of raw sources of water used. Since, *E. coli* can survive in drinking water at 15-18°C for 4-12 weeks and does not multiply significant in the external environment. Similarly, Heterotrophic growth revealed 45.82% Gram Positive rods followed by CONS (20.83%), *S.aureus*(14.58%), Pseudomonas spp (8.33%), *Proteus spp* (8.33%) and *Streptococcus spp* (2.08%) which is similar to the study conducted in Chennai by Venkatesan K.D. et al 2014, CONS (19%) and *Pseudomonas spp* (9.5%)[15], in Nisseria by Lamikanra et al 2014, *Staphylococcus aureus* (14%) [24].

CONCLUSIONS

More than half of the bottled water showed bacterial growth in terms of both coliform and heterotrophs. Presence of coliforms indicate fecal contamination and lack of proper processing. The types of heterotroph isolated are the potential pathogens. This marks the unacceptability of bottled water for drinking.

RECOMMENDATION

Recommendation- bottled water must be checked regularly for the presence of microbes. Regulatory bodies should monitor the companies which supply the bottled water.

REFERENCES

1. Cabral JP. Water microbiology. Bacterial pathogens and water. International journal of environmental research and public health. 2010 Oct;7(10):3657-703.
2. Oyedeji O, Olutiola PO, Moninuola MA. Microbiological quality of packaged drinking water brands marketed in Ibadan metropolis and Ile-Ife city in South Western Nigeria. African Journal of Microbiology Research. 2010 Jan 18;4(1):096-102.
3. Abd El-Salam MM, El-Ghitany EM, Kassem MM. Quality of bottled water brands in Egypt part II: biological water examination. J Egypt Public Health Assoc. 2008 Jan 1;83(5- 6):468-86.
4. Gleick PH, Wolff GH, Cooley H, Palaniappan M, Samulon A, Lee E, Morrison J, Katz D. The World's Water 2006-2007: The Biennial Report on Freshwater Resources. Island Press; 2013 Mar 5.
5. Lalumandier JA, Ayers LW. Fluoride and bacterial content of bottled water vs tap water. Archives of family medicine. 2000 Mar 1;9(3):246.
6. Anadu EC, Harding AK. Risk perception and bottled water use. Journal-American Water Works Association. 2000 Nov;92(11):82-

92.
7. Pant ND, Poudyal N, Bhattacharya SK. Bacteriological quality of bottled drinking water versus municipal tap water in Dharan municipality, Nepal. Journal of Health, Population and Nutrition. 2016 Dec;35:1-6.
8. Khatoon A, Pirzada ZA. Bacteriological quality of bottled water brands in Karachi, Pakistan. Biologia (Bratisl). 2010;56(1&2):137-43.
9. Guidelines for Drinking-Water Quality.(2008). 3rd.Vol.1. World Health Organization;Geneva, Switzerland: Recommendations.
10. ayana BL, Prasai T, Singh A, Yami KD. Assessment of drinking water quality of madhyapur-thimi and study of antibiotic sensitivity against bacterial isolates. Nepal Journal of Science and Technology. 2009;10:167-72.
11. Sharon, O., Buce, I. D., Wayne, W., & Sherry, W. (2013). Drinking water: Bacteria. Available from: http://extension.unl.edu/publication accessed.
12. Edberg SC, Rice EW, Karlin RJ, Allen MJ. Escherichia coli: the best biological drinking water indicator for public health protection. Journal of applied microbiology. 2000 Dec 1;88(S1):106S-16S.
13. Tharannum S, Sunitha S, Nithya J, Chandini M, Vanitha J, Manjula TS, Shyam Sundar C. Molecular confirmation of the presence of coliforms in drinking water using polymerase chain reaction. Kathmandu university journal of science, engineering and technology. 2009;5(2):130-6.
14. Joseph N, Bhat S, Mahapatra S, Singh A, Jain S, Unissa A, Janardhanan N. Bacteriological assessment of bottled drinking water available at major transit places in Mangalore city of South India. Journal of environmental and public health. 2018 Oct 25;2018.
15. Venkatesan KD, Balaji M, Victor K. Microbiological analysis of packaged drinking water sold in Chennai. International Journal of Medical Science and Public Health. 2014 Apr 1;3(4):472-6.
16. Abd El-Salam MM, El-Ghitany EM, Kassem MM. Quality of bottled water brands in Egypt part II: biological water examination. J Egypt Public Health Assoc. 2008 Jan 1;83(5- 6):468-86.
17. Gangil R, Tripathi R, Patyal A, Dutta P, Mathur KN. Bacteriological evaluation of packaged bottled water sold at Jaipur city and its public health significance. Veterinary World. 2013;6(1):27.
18. Gautam B, Gyanwali G, Ussery DW. Assessment of bacterial load in polyethylene terephthalate (PET) bottled water marketed in Kathmandu Valley, Nepal. International Journal of Polymer Science. 2021 Jan 12;2021:1-0.
19. G. Collee, A.G. Fraser, B.P. Marmion, A. Simmons (2023). Mackie and Mccartney Practical Medical Microbiology. Elsevier Health Science, India- 14th Edition
20. Keleb A, Ademas A, Sisay T, Lingerew M and Adane M (2022) Bacteriological Quality of Bottled Drinking Water and Municipal Tap Water in Northeastern Ethiopia. Front. Environ. Sci. 10:828335. doi: 10.3389/fenvs.2022.828335
21. M.E.Z. da Silva, R.G. Santana, M.Guilhermeti et al, 'Comparison of bacteriological quality of tap water and bottled mineral water' International journal of hygiene and environmental health., vol.211,no.5-6, pp.504-509, 2008.
22. Zeenat, A. Hatha, A.A., Viola L. and Vipra, K.(2009). Bacteriological quality and risk assessment of the imported and domestic bottled mineral water sold in Fiji. J water Health, 7(4):642-649.
23. Bharati G., Kumarjyoti G., Sona B., and Amrita K., Bacteriological analysis of bottled water- A multi- centric study in semi urban areas of West Bengal and Bihar. MedPulse International Journal of Microbiology; Print ISSN:2636-4646, Volume 12, Issue2, November 2019 pp32-35
24. Igbeneghu A. O., and Lamikanra A., The bacteriological quality of different brands of bottled water available to consumers in Ile-lfe, South-Western Nigeria BioMed central BMC Research Notes 2014, 7:859
25. Zamberlan da Silva ME, Santana RG, Guilhermetti M, Filho IC, Endo EH, Ueda- Nakamura T, et al. Comparison of the bacteriological quality of tap water and bottled mineral water. Int J Environ Health. 2008;211(5-6):504–9
26. Kassenga GR. The health-related microbiological quality of bottled drinking water sold in Dar es Salaam, Tanzania. J Water Health. 2007;5(1):179–85
27. Stickler DJ. The microbiology of bottled natural mineral waters. J R SocHealth. 1989;109(4):118–24.
28. Warburton DW, Bowen B, Konkle A. The survival and recovery of Pseudomonas aeruginosa and its effect upon salmonellae in water: methodology to test bottled water in Canada. Can J Microbiol. 1992;40(12):987–92.
29. Kassenga GR, Mbuligwe SE. Comparative assessment of physico-chemical quality of bottled and tap water in Dar es Salaam, Tanzania. Int J Biol ChemSci. 2009;3(2):209–17.

ACKNOWLEDGEMENT

The authors would like to thank Modern Technical College, Sanepa and Central Department of Microbiology Trivuwan University, Kirtipur for providing the working area to conduct our work. We are also grateful to Star Hospital Research Committee SHRC-IRC for providing ethical approval for the study

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

FUNDING

This research did not receive any specific grant from funding agencies