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Evaluation of bottled drinking water and occurrence of multidrug-resistance and biofilm producing bacteria in Nepal ☆

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Highlights

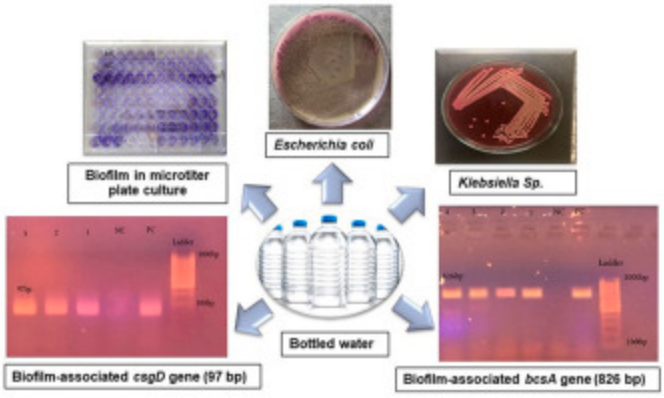
- Bottled drinking water available in the market isn't as safe as it is perceived for drinking.
- The antibiotic test found that 28.57% of the bacterial isolates were multidrug-resistant.
- *E. coli* produced the most biofilms than Klebsiella spp.

Abstract

Health consequences arising from unsafe drinking water and water insecurity lead to increased reliance on usage of bottled water. Biofilm-producing antibiotic-resistant bacteria

in bottled water may pose a risk to public health. This study aims to assess the quality of bottled drinking water with a focus on biofilm-producing and drug-resistant coliform. We analyzed 60 bottled water samples of 30 different brands purchased from Kathmandu for physicochemical and microbial analysis. The parameters pH, iron, total coliform count, Escherichia coli count, and fecal coliform count exceeded National Drinking Water Quality Standards, 2022 in 30.00%, 16.67%, 66.67%, 23.33%, and 16.67% of samples, respectively. Water quality index measurement showed 36.67% and 6.67% of bottled water samples were categorized as grade A and grade B indicating excellent and good water quality, respectively. However, 56.67% of bottled water samples fall under grade E meaning unsuitable for drinking. Among 14 coliform isolates, 85.71% and 14.29% were identified as *E. coli* and Klebsiella spp, respectively. The antibiotic susceptibility testing revealed that 28.57% of the isolates were multidrug-resistant and Gentamicin resistant isolates comprised 71.43%. However, none of the isolates were carbapenem (meropenem) resistant. In this study, 42.87% of the isolates were found biofilm producers with 14.29% each of strong, moderate, and weak biofilm producers. The genetic potential of biofilm-producing capacity of the isolates was assessed by Polymerase Chain Reaction amplification of bcsA and *csgD* genes. Our results showed that 66.67% and 50.00% of the isolates harbored bcsA and *csgD* genes, respectively. This study highlights potential public health hazards associated with the consumption of bottled water containing biofilm-producing and drug-resistant bacteria in Nepal.

Graphical abstract



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Introduction

The existence of high-altitude mountains, lakes, and rivers in Nepal has traditionally been the primary sources of water. Most people depend upon groundwater sources and surface water for their daily consumption. Groundwater quality can vary and faces problems of coliform, iron, turbidity, ammonia, etc. (Shrestha et al., 2023). The availability of safe drinking water has, however, gotten worse due to factors like rising population, urbanization, and water resource degradation (Pandey, 2021). The direct disposal of contaminated sewage, leakage of septic tanks, and use of pesticides in agricultural fields are the major causes of polluting water sources (Lapworth et al., 2012).

With the increasing demand for water supply and considering health safety, the global market of bottled water increased drastically. With the increased production and import of bottled water from bottled water companies, the perception of bottled water as clean and safe for drinking began to proliferate as well. Nowadays, most of tourists, hotels, restaurants, and even households prefer bottled water for drinking. However, the safety of consuming bottled water is still questionable, as most of the bottled water available on the market was not within the standard as declared by bottled water companies (Patsiaouras et al., 2015).

According to the Department of Food Technology and Quality Control (2018)/19 (DoFTQC, 2018), out of 243 bottled drinking water samples collected, 43 samples were not feasible for drinking. Overall, 149 cases were filed by the department out of which 49 cases were against bottled drinking water. The laboratory analysis of bottled drinking water showed that 27.58% were beyond the standard. The treated drinking water had higher contamination of *Escherichia coli (E. coli)* and Total coliform bacteria (Gaihre et al., 2022; Maharjan et al., 2018).

The presence of fecal coliforms in water is a signal of water contamination, with *E. coli* serving as the primary indicator of fecal contamination in water (Singh et al., 2019). Drinking contaminated water has led to increased rates of waterborne diseases like diarrhea, typhoid, dysentery, and cholera (Haramoto, 2018). Microorganisms may grow in the water used in the manufacturing and handling processes if the conditions are right. Apart from the presence of repulsive electrostatic and hydrodynamic forces in water, these environments may favor the formation of biofilms (Sharma et al., 2016). An organized community of pathogenic cells called a "biofilm" is encased in an adhesive polymeric matrix that is self-produced (Sauer et al., 2022). Secretion of protective substances termed extracellular polymeric substances by the microbes present in the biofilm increases the durability of their existence (Sauer et al., 2022) which may cause chronic inflammation in the tissues and lungs (Hø et al., 2017). The infection may vary depending on the biofilms

produced by the microbes. The intestinal infections are mostly caused by *Escherichia coli* biofilms (Sharma et al., 2016).

The Codex Alimentarius of bottled water suggests hygienic procedures for the collection of natural mineral waters, their treatment, bottling, packing, storage, distribution, and sale for immediate consumption in order to ensure a secure, healthy, and complete product (WHO and FAO, 2001). It requires periodic monitoring of bottled water following basic parameters: Appearance, odor, and taste; Physical: temperature, electrical conductivity; Physicochemical: pH and Chemical: according to water characteristics, and content of carbon dioxide. It suggests adopting WHO guidelines for drinking water quality, 2017 (WHO, 2017).

Since water quality affects human health both directly and indirectly, it is important to test the quality of drinking water using physical, chemical, and microbial characteristics. Physicochemical parameters including pH, turbidity, conductivity, nitrate, iron, ammonia, and other parameters may have a direct or indirect influence on microbiological conditions (Duressa et al., 2019). In order to lessen the risk that microorganisms provide to human health, effective treatment and consistent monitoring of the water processing firms should be investigated. The aim of this study was to analyze the physicochemical and microbiological quality of bottled water sold in the Kathmandu Valley market, to evaluate the biofilm-formation in the bacterial isolates, and to detect the drug-resistant and biofilm-associated genes - *bcsA* and *csgD*.

Section snippets

Sample collection

This investigation focuses on Kathmandu Valley's bottled water for consumption. It is situated in Nepal's central region, between 27° 36′ and 27° 48′ N and 85° 12′ and 85° 31′ E. According to the Census Nepal, 2022 , the total population of Nepal has reached about 29 million, and assessing safe drinking water is very difficult for the huge population.

The investigation was carried out at the Environment Research Laboratory of the Nepal Academy of Science and Technology. Bottled water samples…

Physical and chemical properties of bottled water

The temperature of the bottled water was 25 °C at the time of analysis. The National Drinking Water Quality Standards, 2022 (NDWQS) and WHO Guideline 2017 (Table 2), were met by all samples evaluating physical characteristics, including turbidity, electrical conductivity, and total dissolved solids except for pH. Among all, 30.00% of bottled water samples were found to be slightly acidic (Fig. 1). The mean pH was 6.76±0.56 with minimum and maximum values of 5.28 and 8.27, respectively. While…

Conclusion

The study showed that bottled water exceeded the National Drinking Water Quality Standards in pH, iron, total and fecal coliform count, and *E. coli* counts. This study found 43.33% of bottled water samples with a very high risk of total coliform contamination. It identifies the presence of antibiotic resistant bacteria and also biofilm-forming genes in the isolated bacteria of bottled water. It concluded that 56.67% of bottled water is not safe for drinking. It exposed the bad handling, poor…

Author contributions

Sunita Shrestha: Laboratory works - data collection, curation, analysis; writing – original draft preparation, editing. Sayara Bista: Laboratory works - data collection. Naina Byanjankar: Laboratory works - data collection. Tista Prasai Joshi: Supervision, writing – review, editing, and finalization. All authors reviewed the manuscript.…

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.…

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