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## **STUDY ON RISK OF BACTERIAL CONTAMINATION IN BUBBLE TOP DRINKING WATER USED BY COMMUNITY**

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### **ABSTRACT**

To assess the bacterial load in bubble top water and check its compliance with Indian and international standards. Safe drinking water is a fundamental need of human life. It is generally believed that bottled water is safe for usage without any additional processing methods. For long-distance travelers, it serves as the only source of reliable drinking water. Several authors have reported that bottled water does not always meet the acceptability standards. In this study 15 samples of different brands of bubble top drinking water were collected in a sterile disposable plastic container. From each sample collected 5 ml of water was centrifuged and the supernatant was discarded. From the sediment 50 µl was transferred to Nutrient agar and MacConkey agar. Using a sterile inoculation loop, the drop was spread on the entire plate on both the agar. The Nutrient agar and MacConkey agar plates were incubated at 37°C, aerobically for 24 hours. In this study, 15 water samples were analyzed. In all the 15 samples the bacterial load is found to be very high and shockingly above than any prescribed standard. The quantity of bacteria in Nutrient agar was to the extreme while the MacConkey agar indicates the presence of coliform

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bacteria in the bubble top water. According to this study, none of the bottled drinking water manufacturers are maintaining any standards in providing clean and safe drinking water for human consumption. Surveillance of bottled water manufacturing industries in the settings on a regular basis needs to be done by regulatory agencies. These measures will ensure safe and wholesome bottled water for public usage.

**Keywords: Bottled drinking water, bacterial load, Human consumption, Surveillance**

## INTRODUCTION

In a country where the major population depends on rain water for all the utility and drinking, water becomes an issue. When the river connectivity is poor and the groundwater recharge is insufficient, the ground water quality will be poor and will not be potable. In this situation, a major population depends on pre-treated water, which is convenient for consumption (**El-Harouny et al., 2008**). There are standards set for manufacture, storage and distribution of ready to use drinking water. But in developing countries, the regulatory mechanism and the monitoring systems are seldom evident or implemented. This becomes a major health problem even in day to day living in the community (**Belkouteb et al., 2020**). This study is done to explore the major risk factor, the bacterial load in such bottled drinking water.

Bubble top water is usually regarded as safe for usage by people. It serves as the only reliable source of drinking water available for long-distance travelers (**Edema, Atayese**

**and Bankole, 2011**). Bubble top water production companies are one of the fastest growing industrial sectors in this part of the world. Recently, there are more than 3000 bottling plants in India. Mostly, the manufacturing companies of bubble top water in India according to previous study are located in unhygienic locations like agricultural fields or estates and most companies use bore wells or pump or canal water as source of water. Here, water is pumped out from depths varying from 80 to 500 feet below from the ground which would contain more contaminants (**Finlayson, 2011**) (**Ashwin and Muralidharan, 2015**). Few sources of bubble top water are collected from public drinking water systems such as Municipality supply water (**Dege, 2011**). Ground water would have quality problems due to salinity and contaminants like agrochemicals, nitrates, fluoride, iron etc (**Goncharuk, 2014; Girija As and Priyadharsini J, 2019**). The ground water available in half of the districts in our

country was found to be unfit for drinking. This was because of the presence of contaminants exceeding the limiting levels of the Indian or international standards (Saravanan, 2013) (Gangil *et al.*, 2013).

Studies done in India and other parts of the world have reported that bottled water was contaminated or polluted with harmful disease-causing microorganisms at various stages of its production (Kassenga, 2007; Semerjian, 2011). Consumption of bubble top contaminated drinking water has led to frequent outbreaks of waterborne diseases such as cholera, typhoid, and hepatitis etc. (Ahmed *et al.*, 2020) (Girija *et al.*, 2019).

The microbiological qualities of bubble top drinking water of some manufacturers have been found to be in greater violation with Indian and international standards. According to the Central Ground Water Board, BIS (IS\_10500 and revised module IS 10500:2012) has specifications in Uniform Drinking Water Quality Monitoring Protocol. This standard has two limits i.e. acceptable limits and permissible limits in the absence of an alternate source. If any parameter exceeds the limit, the water is considered unfit for human consumption (Selvakumar and Np, 2017). According to BIS, E-coli or thermotolerant coliform bacteria and total bacterial count shall not be detectable in any

100 ml of sample ('A response to the Institute of Food Technology Annual Meeting in New Orleans 16–20 July, 2005', 2006).

Water is a very sensitive product. One of the main challenges of bubble top water processing is to produce a quality product free of pathogenic organisms and protozoa (Shahana and Muralidharan, 2016) (Marickar, Geetha and Neelakantan, 2014). Strict production control is critical to avoid any contamination of pathogenic bacteria or protozoa. As per International standards of drinking water by WHO, the drinking water should possess a TDS of less than 500 mg/l, zero toxic elements and disease causing bacterial population. Bubble top water processing includes Water filtration, Tank Venting, Carbonation, Bottle Blower and Bottle Washer & Bottle Filler. Water is also treated with Chlorine, which kills harmful bacteria and some viruses and also protects the water from recontamination while storing. EPA mandates that chlorine level in drinking water should be not higher than 4 milligrams per litre. EPA says fluoridation also provides some protection against skeletal fluorosis, a painful joint condition (Schweisfurth and Schweisfurth, 2013). Significant levels of pesticides like organochlorine compounds and

organophosphorus compounds have been reported in freshwater systems and in the bubble top water samples collected from some major cities in India (**Louie and Reuschlein, 2011**).

One of the most common flocculants used in water purification is aluminum sulfate which is also known as alum. Alum is a coagulant that helps to settle down all floating suspended solids and colloidal substances present in water (**Huser, Brezonik and Newman, 2011**) (**Pratha, Ashwatha Pratha and Geetha, 2017**). Alum is added to drinking water to remove the microorganisms like viruses, bacteria and protozoa present in the water. At low levels, these organisms can cause sickness and diseases. In contrast, aluminum sulphate in water can be absorbed by humans because after water treatment the aluminum is largely in an unbound form. At low levels aluminum in food, air and water is not harmful to our health, but with long term regular exposure, there is evidence linking aluminum to effects on the nervous system, with possible connections to several diseases, such as Parkinson's, Alzheimer's and Lou Gehrig's disease (**Vaishali and Geetha, 2018**) (**M, Geetha and Thangavelu, 2019**). Patients suffering from these diseases tend to have high levels of aluminum in some areas of their brains. There is no evidence to

suggest that aluminum affects reproduction or it causes cancer (**Mohamed et al., 2020**).

Despite the common belief that bubble water is safer to drink and has better taste than tap water, scientific studies have shown that the belief is not necessarily true (**Raj, 2005**). Research also shows that the sales and consumption of bubble water can have environmental and social impacts whose consequences are yet to be fully understood (**Opel, 1999**) (**Paramasivam, Vijayashree Priyadharsini and Raghunandhakumar, 2020**). We store water in all sorts of plastic bottles, jugs or containers. It can be below grade or high grade, but plastic is plastic (**Priyadharsini et al., 2018a**). This is a very harmful daily practice as plastic containers carry a lot of chemicals and bacteria that would result in Stunted growth, Early puberty, Premature birth, Infertility, Early menopause, Diabetes, Heart disease, Cancer. Previously our team has a rich experience in working on various research projects across multiple disciplines. (**Muthukrishnan and Warnakulasuriya, 2018**), (**Govindaraju, Neelakantan and Gutmann, 2017**), (**Chen et al., 2019**), (**Priyanka et al., 2017**), (**Sitharthan et al., 2019**), (**Priyadharsini et al., 2018b**), (**Azeem and Sureshbabu, 2018**), (**Wu et al., 2019**), (**Abitha and Santhanam, 2019**), (**Manohar and**

Abilasha, 2019), (Venu, Dhana Raju and Subramani, 2019), (Wang *et al.*, 2019), (S. A. Girija, Jayaseelan and Arumugam, 2018), (Sheriff, Ahmed Hilal Sheriff and Santhanam, 2018), (Dhinesh *et al.*, 2017) Now the growing trend in this area motivated us to pursue this project.

These observations imply that bubble top drinking water is insufficient to have safe water for consumption. Hence, periodic surveillance of packaged drinking water like bubble top water is very much essential. This will serve the dual purpose of monitoring the standards of bottled water production industries as well as help in giving reassurance of quality to users. Hence, To assess the bacteriological quality of bubble top water and check its compliance with Indian and international standards.

## MATERIALS AND METHOD

In this study, 15 samples of different brands of bubble top drinking water from different

places were collected in a sterile disposable closed plastic container. As soon as the water samples are collected in the disposable plastic container, it is stored in the refrigerator till the next step of the centrifugation process is done. From each sample collected, 5 ml of water was centrifuged and the supernatant was discarded. From the sediment 50 µl each was transferred to Nutrient agar and MacConkey agar. Using a sterile inoculation loop, the drop was spread on the entire plate on both the nutrient and Mac Conkey agar. Thereafter both the plates were incubated at 37 °C aerobically for 24 hours.



## RESULTS AND DISCUSSION

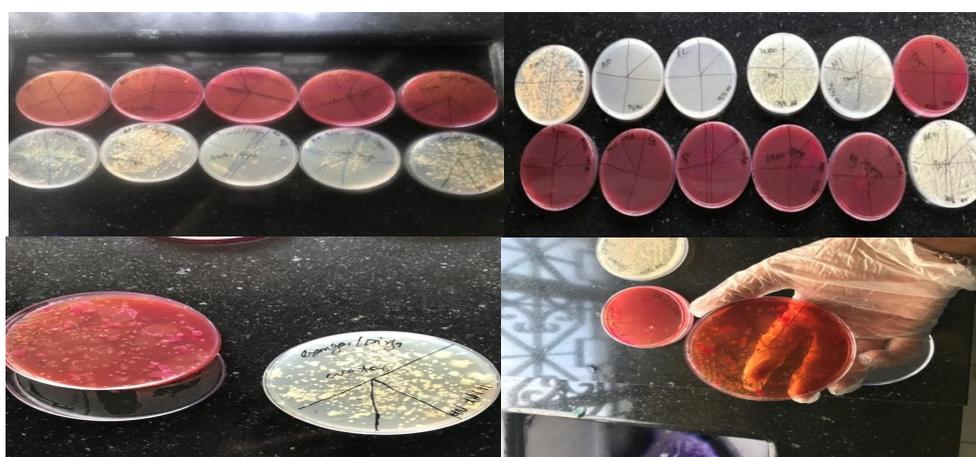


Figure 1: Presence of total bacterial count in Nutrient agar and presence of Coliform bacteria in Mac Conkey agar

Table 1: Total number of bacterial counts in both the agar per 100ml of different 15 samples of bubble top water

Sample No.	Total count/ 100 ml	Coliform/ 100 ml
1	8000	2400
2	40000	16800
3	24000	22500
4	68000	24800
5	74000	70000
6	7500	5000
7	36000	32500
8	72000	40000
9	51200	25000
10	21000	18000
11	15000	800
12	25000	1000
13	12800	1600
14	8000	4000
15	72000	1600

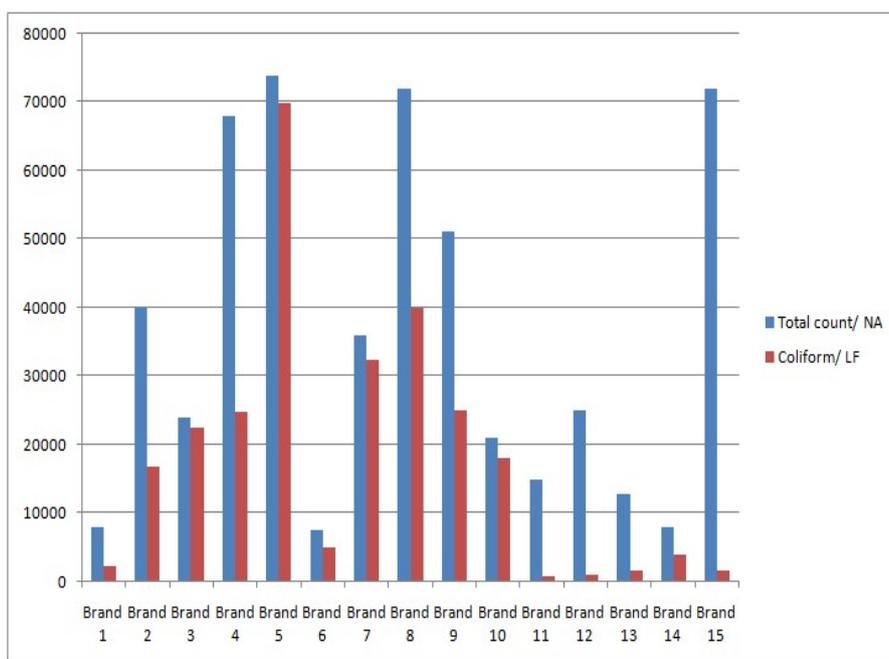


Figure 2: This bar graph represents the total number of CFU coliform bacterial count per 100ml of 15 different samples of bubble top water

Bubble top Drinking water was not upto the specific limit as specified by Indian and international standard of drinking water. There is a limit for number and kinds of organisms permissible in drinking water according to the WHO and international standards (Priyadharsini *et al.*, 2018b). The

acceptability of the water quality was determined based on the total bacterial load in the water and the presence of coliforms, indicator organisms and other physical and chemical parameters (S. A. S. Girija, Jayaseelan and Arumugam, 2018).

In this study, 15 water samples were analyzed. In all the 15 samples the bacterial load is found to be very high and shockingly above than any prescribed standard. The quantity of bacteria in Nutrient agar was to the extreme while the MacConkey agar indicates the presence of coliform bacteria in the bottled water, means that the pathogens are present. Most pathogens that can contaminate water come from human and animal feces (Shahzan *et al.*, 2019). The presence of these organisms, even when they are non pathogenic is also of greater risk in immunocompromised persons where it could cause opportunistic infections. Such persons include young children, elderly peoples, pregnant women, people with cancer, diabetes and people on medications as the corticosteroids which possess immunosuppressive properties (Bartram *et al.*, 2003). It has been pointed out that high numbers of heterotrophic bacteria in bubble top water might arise when unsterilized or uncapped plastic bottles are transported to bottling plants which would expose the interiors of the bottles to airborne contaminants (Smiline, Vijayashree and Paramasivam, 2018) and it is an indication of poor manufacturing practices involved in the processing of such water (Ibrahim, Mohammed and Hafez, 2014).

The observation from this study is an indication of the lack of required infrastructure and Requirements such as clean rooms, automatic bottle filling machines, clean water source, trained personnel and the need for staff supervision by personnel who are knowledgeable in water purification and dispensing must be met by companies who are involved in the production of packaged water for human consumption. This implies that the capital needed by a particular water bottling company in order to put all these things in place must be quite good. From the consumer side, Ensure whether it is properly sealed with details like manufactures name, BIS and FSSAI license number. These can be cross checked by the consumer on clean drinking water portal and the label on the seal and body should also carry the packing date and batch number. Our institution is passionate about high quality evidence based research and has excelled in various fields (Pc, Marimuthu and Devadoss, 2018; Ramesh *et al.*, 2018; Vijayashree Priyadharsini, Smiline Girija and Paramasivam, 2018; Ezhilarasan, Apoorva and Ashok Vardhan, 2019; Ramadurai *et al.*, 2019; Sridharan *et al.*, 2019; Vijayashree Priyadharsini, 2019; Chandrasekar *et al.*, 2020; Mathew *et al.*, 2020; R *et al.*, 2020;

Samuel, 2021). We hope this study adds to this rich legacy.

## CONCLUSION

According to this study, none of the bubble top drinking water manufacturers are maintaining any standards in providing clean and safe drinking water for human consumption. There is a need for a rigorous inspection and follow-up of water bottling facilities so that only those companies which consistently produce water with acceptable bacteriological index are allowed to produce water for public consumption. Surveillance of bubble top water manufacturing industries in the settings on a regular basis needs to be done by regulatory agencies. These measures will ensure safe and wholesome water for public usage.

## CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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