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Bacterial Assay of Drinking Water Commercially Marketed in Bharatpur Metropolitan City, Chitwan, Nepal

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Abstract
The bacterial assay of Drinking Water Commercially Marketed in Bharatpur Metropolitan City, Chitwan, Nepal is still not understood clearly. Out of twenty samples, ten samples were collected from 1 L bottles and ten samples were collected from 20 L water jars. The pH of the sample solution was evaluated using pH meter (AUTO DEULEX METERMODEL; LT-10). The most probable number (MPN) method was used to analyze Salmonella, yeast, and mould while the membrane filtration technique was used to evaluate coliforms and fecal-coliforms. Yeast and mold was cultured in Sabouraud Dextrose Broth while M-Lauryl Sulphate Broth solution was used for total coliform and fecal coliform. Salmonella was cultured in Rappaport Vassiliadis Salmonella Enrichment Broth media. The pH of most of samples lies between 6.5–8.5 which is very close to the National Drinking Water Quality standard, Nepal and WHO while some samples ranges below the National Drinking Water Quality Standard, Nepal. There is no any growth of the Fecal coliform bacteria in all the samples of one 1 L bottles where as 50% of the samples of 1 L water bottle showed the abundance of total coliform. The result revealed that the 20 L jar water was contaminated with total and faecal coliform bacteria. Out of the ten samples of one 1 L water bottle, nine samples were contaminated with total coliform whereas samples from 20 L jar showed 70% contamination with fecal coliform. Neither of the samples spotted Salmonella, mould and yeast. This study reveals the importance of monitoring the quality of drinking water production system..

Keywords Fecal coliform, total coliform, salmonella, yeast mold, water qulaity

1. Introduction
Drinking water should be free from any microbial contamination to prevent the transmission of waterborne diseases which measures the safety for public health (Milanovic et al., 2014). The presence of any kind of micro-organism in the drinking water measures the quality of water. Bacteriological testing gauze the presence of micro-organisms such as total coliform, fecal coliform, heterotrophic bacteria and salmonella which indicates the standard of the drinking water (Islam et al., 1970). Coliform bacterial phylogeny is frequently found in soil, vegetation, environment, and the intestines of warm-blooded animals. These are used as indicators of water quality and possible fecal contamination. Total coliform bacteria are not necessarily

harmful themselves but their presence in drinking water increases the probability of having other pathogenic viruses, bacteria, or parasites to enter the water supply chain (Milanovic et al., 2014). The presence of coliform bacteria in drinking water is a cause of concern as it indicates the possible existence of pathogenic enteric microorganisms, including Shigella, Vibrio, and Salmonella species. Health related issues are alarmed due to the contamination of fecal coliform in drinking water while all coliform are not directly associated with fecal contamination even though their presence in drinking water indicates the possibility of having harmful pathogens. Therefore, measurement of coliform bacteria in the drinking water is one of the major drinking water safety parameter (Zamberlan da Silva et al., 2008).

Water sources and treatment processes may possess an impact in the microbiological contamination of drinking water. Water sources that are contaminated with fecal matter or other pollutants can introduce bacteria into the water supply chain.

The water purification techniques in the production system also matters the contamination of micro-organism. Different treatment techniques, such as chlorination, disinfection and filtration are used to get rid of bacteria and other microorganisms. Filtration can physically remove bacteria present in water but disinfection techniques such as chlorination can kill them. It is crucial to remember that bacteria may remain in the water even though how efficient treatment procedures are used at place. The pivotal role is to check the presence of bacteria periodically to insure the safety of drinking water. Continuous monitoring in water resources and production system will help to prevent the possible bacterial contamination and can take appropriate corrective action to avoid for the same (Mulamattathil et al., 2015).

The most common nonpathogenic facultative flora in the human intestine is Escherichia coli. Even in the healthy people, certain E. coli strains have the capacity to cause gastrointestinal, urinary and central nervous system illnesses. In the Kathmandu Valley, different drinking water production industry used different variety of water sources in different combinations. Ito et al. (2020) disclosed that the estimated risk of contracting diarrheal illness is not only dependent on the type of water used for bathing but also dependent on drinking water and its production system. World Health Organisation (WHO) estimated that water and sanitation-related issues cause 80% of diseases in developing nations. E. coli strains that causes diarrhea can be categorised at least into six groups, each of which has a unique pathogenic scheme. When combined, these microorganisms definitely constitute the most prevalent cause of diarrhoea in children worldwide. Diarrhea genic E. coli infections can cause a number of different clinical syndromes, such as hemorrhagic colitis and hemolytic-uremic syndrome (enterohemorrhagic E. Coli), persistent diarrhea (enteroaggregative E. Coli), and infant watery diarrhea (enterotoxigenic E. Coli) on human health (Nataro & Kaper, 1998).

Globally, there is believe in the people that the water marketed in bottle and jar is safe for drinking purpose. Study reveals that drinking water marketed in bottle and jar contains coliforms and heterotrophic bacteria more than the National and International

Standards (Pant et al., 2016). Bharatpur is one of the fast growing cities of Nepal. It includes boreholes, wells, and piped water sources for drinking water (Eni, 1967). There is growing concern of the quality of drinking water. Therefore the main objective of this study is to measure the bacterial contamination and pH of the marketed water in bottles and jars within Bharatpur Metropolitian city for the safety measurement of its resident. Besides this, it is also helpful to make public awareness with experimental evidence generated from the trusted lab of Water Quality Testing Laboratory Bharatpur, 10, Chitwan, Nepal.

2. Materials and methods.

The study was carried out in the following stages

2.1 Study area and sampling

This was a cross-sectional study carried out in Bharatpur Metropolitian City. It is located 27º 32' 58'' to 27º 45' 40'' latitude and 84º 9' 41'' to 84º 29' 5'' longitude. It is the district head quarter of the Chitwan district and located in the central-southern region of Nepal. Bharatpur is Nepal's fifth-largest city having population of 199,867 as well (Sorrel, 2015).

2.2 Sample collection

Treated drinking water of ten different brands having capacity of 1 L bottle and 20 L jars of representing sample were taken on October 2023 for the study. All of the samples were collected at random from a number of stores and supermarkets in the selected area. Samples were collected in triplicate at the outlet stores (3 x 10 1 L bottle samples & 3 x 10 20 L Jar samples). Every sample was appropriately labeled for identification and kept in its original, sealed container. The samples were then taken to the laboratory for their analyses.

3. Analysis of the samples in the laboratory.

3.1. Test of pH

Electrometric method was used to determine pH. It was measured by a pH meter (AUTO DELUXE PH METER MODEL: LT-10). pH meter was calibrated using 100mL buffer solution having pH 7.0±0.05 and 4.0±0.05 (Merck, Vikhroli Mumbai).

3.2 Test of total and fecal coliform bacteria.

The tests of total and fecal coliform bacteria were carried out according to the method 9222B and 9222D APHA 23rd edition (Solutions et al., 1990). The culture medium applied for the bacteriological analysis was Lauryl Sulphate Broth and the membrane filtration method was used. A membrane filter was used to filter 100 mL of water from each sample (Whatman filter paper, pore size-0.47µm, diameter -47mm) with a vacuum speed 5.0 to 15.0 mmHg. Organisms get concentrated on the surface of the membranes. These membrane filters were then placed on the M-Lauryl Sulphate Broth surface and incubated (Wagtech™ Incubator) at 44^{o}C and 37^{o}C for 18 hours for fecal and total

coliforms, respectively. Three replicate samples were examined, and an average was recorded. It is well known that these temperatures are suitable for the growth of these bacteria. The unit of measurement for total and fecal coliform organisms per 100 mL is cfu/100 mL. For complete sterility, glassware materials were sterilized for 15 minutes at 121°C just after cleaning in distilled water. The water of 1 L bottle and 20 L jar were evaluated for bacteriological quality by comparing the results with the WHO and National Water Quality Standards, i.e., drinking water must not contain more than 0 coliforms per 100 mL (Halage et al., 2015).

3.3. Test of salmonella species.

The test of salmonella species was carried out according to the method introduced by Lotfy et al, (2011). Soyabean casein digest medium was prepared by adding the reverse osmosis (RO) water to 4.28 g of casein digest medium in the appropriate size of the conical flask and made final volume 100 mL (4.28 g/100 mL). The conical flask was cotten pluged, sealed with aluminium foil and sterilized in an autoclave for 15.0 minutes at 121°C and 15 pounds of pressure. After sterilizing, the medium was left to cool to approximately 50°C. After adding 10 mL of the water sample with the help of a micropipette, the conical flask was incubated for 24 hours at 37°C. The following day, 1 mL of prepared soyabean casein digest medium was added to Rappaport Vassiliadis Salmonella Enrichment Broth medium, which was prepared in accordance with the manufacturer's instructions. Conical flasks were then incubated for 24 hours at 37°C. Furthermore, Xylose- Lysine deoxycholate agar (XLD agar) was prepared by adding the RO water to 5.94 g in the appropriate size of the conical flask and made final volume 100 mL (5.94 g/100mL). After that, it was boiled with continuous shaking and autoclaved for 15 minutes at 121°C. After sterilizing, the medium was left to cool to about 50°C. They were divided among 90 mm diameter sterilized Petri plates at a ratio of 25 mL per plate with the addition of carefully prepared Rappaport Vassiliadis Salmonella Enrichment Broth and labelled appropriately. The plates were then left unaltered to solidify. At the end, Petri-plates were incubated for 24 hours at 37°C.

3.4 Test of yeast and mold species.

The test of yeast and mold species was carried out according to the method introduced by Alam (Alam, 2019). Sabouraud DextroseBroth solution was prepared according to the manufacturer’s recommendations. 5.5 g of Sabouraud Dextrose broth was completely dissolved in 100 mL RO water in a conical flask by warming on the hot plate. After sealing the conical flask with aluminium foil and plugging it with cotton, it was autoclaved for 15 minutes at 121°C and 15 lb of pressure. 1mL of water sample was added, and the mixture was allowed to cool to room temperature before being incubated for a 24-hour period at 25°C. On the next day, the Sabouraud Dextrose agar medium was made in accordance with the manufacturer's instructions. On warming over hot plate 6.67 g of SDA was allowed to dissolve in 100 mL of RO water in a conical flask. After sealing the conical flask with aluminium foil and plugging it with

cotton, it was autoclaved for 15 minutes at 121°C and 15 lb of pressure. After allowing the media to reach room temperature, it was roughly spread among the Petri dishes that had been dried, cleaned, and autoclaved. After that, the media was allowed to set at room temperature before 1 mL of the prepared sabouraud dextrose broth was added. After the agar solidified, the plates were covered with parafilm and kept in the incubator for 5 days at 25°C

Table 1. Drinking water specifications as per WHO & NDWQS

Microbiological contaminants	Unit	WHO (Herschy,2012), (WHO, 2018)	National Drinking Water Quality Standard (NDWQS, 2005)
Total Coliform +E. coli	cfu /100 mL	0	0 in 95% samples
Fecal coliform	Cfu/100 mL	0	0
Salmonella	Cfu/100 mL	0	0
pH	-	6.5-8.5	6.5-8.5

4. Results

4.1 pH

The pH of different samples of water contained in 1 L bottle and 20 L jar are mentioned in the table 2 and 3, respectively. The mean pH value of water contained in 1 L bottle is calculated 6.26 ± 0.27 whereas those contained in 20 L jars has mean pH of 6.53 ± 0.29. All water sample are acidic in nature and lies below the WHO and Nepal National Drinking Water Quality Standard (6.5 to 8.5) recommendation. Measured pH values of water samples of 1 L bottle & 20 L jar are mentioned in the table 2 & 3 below.

Table 2. Measured pH values of different water samples of 1 L bottles.

Sample ID	pH (Average ± SD)	Nepal Standard/ WHO Guideline for Drinking Water
AB	6.16 ± 0.27	6.5-8.5
AZ	6.14 ± 0.32	
GJ	5.77 ±0.30	
KL	6.39 ±0.20	
ID	6.40 ±0.29	
AK	6.56 ±0.39	
AG	6.32 ± 0.36	
AD	6.46 ± 0.09	
BH	6.22 ± 0.20	
RF	6.22 ± 0.07	

Table 3. Measured pH values of different water samples of 20 L jars.

Sample ID	pH (Average ± S.D)	Nepal Standard /WHO Guideline for Drinking Water
H	7.42±0.09	6.5 - 8.5
J	7.04±0.06	
TA	5.51 ±0.11	
P	6.46 ±0.32	
I	6.84±0.46	
A	6.72±0.33	
AGA	6.29 ±0.06	
D	6.57±0.34	
B	6.05 ±0.01	
R	6.47 ±0.10	

As per Nepal National Drinking Water Quality Standard and WHO guidelines for drinking water, the pH of drinking water should be 6.5 to 8.5. Table 2 shows that the pH values varied from 5.77 ± 0.30 to 6.56 ± 0.39. Out of the 10 samples, only one sample, i.e., AK has pH 6.56 ± 0.39 which lies within the pH limit of drinking water. Table 3 shows that the pH varies from 5.51 ±0.11 to 7.42 ± 0.09. The pH of other five samples, i.e., H, J, I, A & D lies within the limits of drinking water. In general, the pH scale can be used to determine the hardness or softness of water. Pure water has a pH of 7.0. Water is generally classified as basic if its pH is greater than 7.0 and acidic if it is less than 7.0. In surface water systems, the pH typically ranges from 6.5 to 8.5.
From the above result it shows that the water contained in 20 L jar is much more stable than the water contained in 1 L bottle. In reference to the pH data, the water contained 20 L jar is more suitable for drinking purpose than in the water contained in 1 L bottle

4.2 Bacterial analysis of the sample taken from 1 L bottle

Out of the ten samples of 1 L bottle, five of them are contaminated with bacteria having total coliform count of 1^{+}cfu/100 mL where as fecal coliform, Salmonella and Yeast/Mold are not seen in any samples, fig. 1 & 2 respectively. The total count of bacterial contamination of each sample is mentioned in the table 4 below.

Table 4. Total coliform, Fecal coliform, Salmonella and Yeast/Mold in water of 1L bottle.

S.No.	Sample ID	Total coliform (cfu/100 mL)	Fecal coliform (cfu/100 mL)	Salmomella (cfu/100 mL)	Yeast/Mold
1	AB	1	0	0	0
2	AZ	2	0	0	0

3	GJ	1	0	0	0
4	KL	0	0	0	0
5	ID	0	0	0	0
6	AK	0	0	0	0
7	AG	1	0	0	0
8	AD	1	0	0	0
9	BH	0	0	0	0
10	RF	0	0	0	0

Figure 1. The colnies of sammonella are not seen in the water sample taken from 1 L bottle (sample AB)

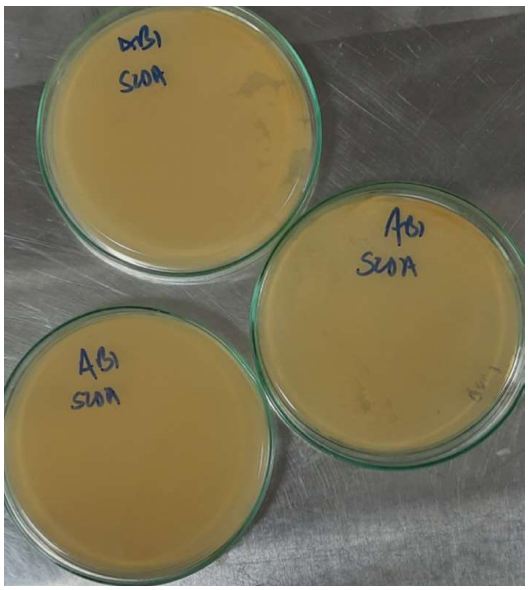

Figure 2. Colonies of Yeast/ Mold are not present in water sample of 1L bottle (sample AB)

4.3 Bacterial analysis of water sample taken from 20 L jar

The examination of water sample taken from 20 L jar showed that all the samples are free from salmonella, yeast and mold. However, total coliform count of 5^{+}cfu/100 mL is found in all samples except one. The fecal coliform is present in seven samples out ten, i.e. samples ID, H, P, I, A, AGA, B and R, fig. 3. The bacterial status in each sample is mentioned in table 5 below.

Table 5. Bacterial status of sample.

S.N.	Sample ID	Total coliform (cfu/100 mL)	Fecal coliform (cfu/100 mL)	salmonella (cfu/100 mL)	Yeast/Mold
1	H	16	5	0	0
2	J	3	0	0	0
3	TA	0	0	0	0
4	P	TNTC	4	0	0
5	I	TNTC	72	0	0
6	A	TNTC	8	0	0
7	AGA	4	2	0	0
8	D	3	0	0	0
9	B	9	1	0	0
10	R	2	1	0	0

TNTC:- Too numerous to count.

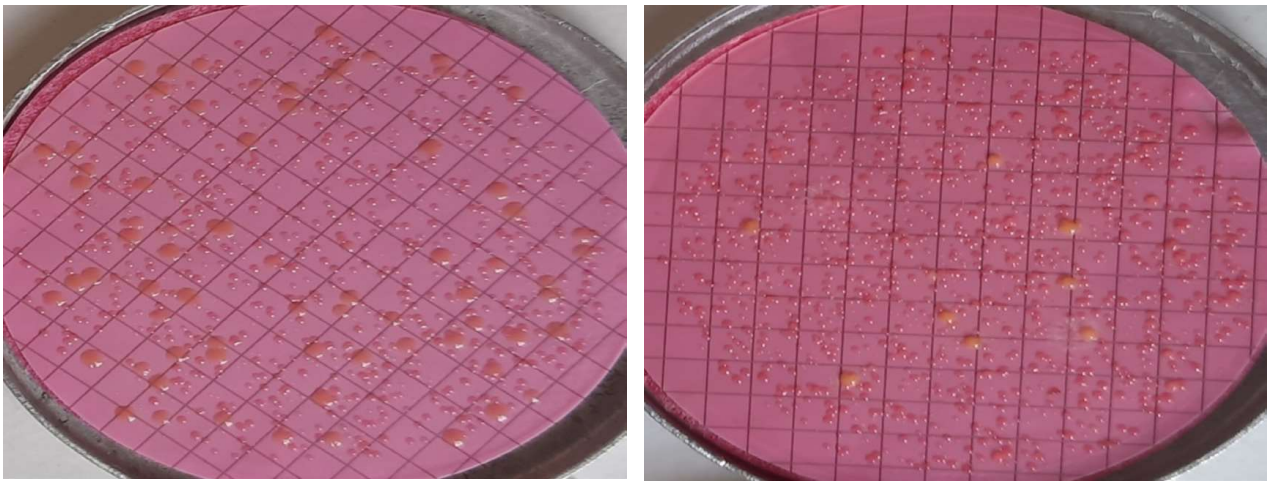

Figure 3. Colonies Fecal coliform in water sample of 20 L jar (samples I and A)

5. Discussion

The pH value of nine different brand of water contained in 1 L bottle and 5 different brands of water contained in 20 L jar lies below the WHO/ National Drinking Water Quality Standard. In total 70% of the available brands have pH below the specified limit of drinking water. Wright's (2015) reported that the pH of beverages and bottled waters found predominantly acidic. Ten out of the 14 beverages tested were acidic. The majority of drinking water are acidic (Wright, 2015). Schmidt & Huang (2022) examined the pH of various bottled waters and found that some of them were acidic as juices or soft drinks soda. Some spring/artesian waters, sparkling waters, and flavor-infused waters can lead to tooth erosion. Manufacturing process leads bottled waters to become slightly acidic to check the growth of micro-organism. There should be

awareness to maintain the pH in the appropriate level due to oral health and healthy hydration (Schmidt & Huang, 2022).

Total coliform is the indicator organism for other pathogenic bacteria. The presence of coliform in water increases possibility of having fecal pollution. This study reveals that the presence of total coliform is higher than the fecal coliform. This investigation shows that 70% samples of 20 L jar is contaminated with facial coliform whereas total coliform is present in 90% samples. Fecal coliform is potential to cause cholera, gastroenteritis, dysentery and typhoid fever (Zha et al., 2019).

Comparison of water present in two different capacity water container shows that water present 1L bottle have lower concentration of total coliform. Water having low concentration of micro-organism is relatively safe for the consumption. Similar results were reported in the study of Pant et al. (2016); Yousaf & Chaudhry, (2013) and Zamberlan da Silva et al.(2008). Timilshina et al. (2013) examined thirty distinct commercially available brands of treated water and revealed that 63.3% of the samples was found contaminated with TC counts which was higher than recommended by the World Health Organisation (WHO). Along side 90% of the samples were found to have had heterotrophic bacterial counts that were higher than the acceptable limits (Timilshina et al., 2013).

This study shows that neither of the samples contains mold, yeast, and Salmonella. Islam et al. (1970) reported that the tap water of Dhaka city is contaminated with Salmonella (0%), Escherichia coli (60%) and other microorganisms. Study also reported that 50% of treated water of the bottle, 87.5% of filtered water, and 100% of tap water samples exceeds the WHO guide lines for drinking water (Islam et al., 1970). As reported by Zamberlan da Silva et al.(2008), the bacteriological quality of treated water contained in the bottle and tap water was 36.4% which has crossed the the specified limit. one pathogenic bacteria and one coliform or indicator bacteria were found in 76.6% of the treated water samples contained in 20 L jars of water dispenser. It is challanging job to make water to be free from all kinds of bacteria. Almost all studies shows the presence of at least one indicator bacterium and one pathogenic bacterium in municipal water systems.

6. Conclusion

This research divulges that fourteen samples out of twenty are contaminated with total coliform bacteria while others are devoid of such organism. Specifically, total coliform colonies in samples A, I, AP, and H exceeded 15 cfu/100 mL, whereas samples AD, AG, GJ, and AB exhibited only 1 cfu/100 mL. Furthermore, seven samples had fecal coliform counts surpassing permissible limits, while the rest of the sample meets WHO and National Water Quality Standards. The pH of almost all water samples lie slightly below the acceptable range, i.e. acidic. Majority of the sample of 20 L jar is contaminated with fecal coliform bacteria which posses the threat for consumption.

Therefore, there should be continuous monitoring from the local authority as well as Water Quality Testing Laboratory of the Government to ensure for the safety of drinking water. Besides this public awareness campaign should be held in the local level to maintain the quality as per the WHO and National standard.

Declaration of competing interest: The authors have declared no conflict of interest.

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