Microbiological assessment for potable water

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Abstract: Drinking water quality assessment in Kathmandu valley has always been crucial with reference to public health importance. The objective of this study was to assess the quality of drinking water with respect to physiochemical and microbiological parameters. A total of 63 random water samples were collected from different sources like stone spout, tap, well, boring, hand pumps and jar from different wards of Kageshwori Manohara Municipality, Kathmandu. The study was carried out for 6 months from January to July, 2019. The pH of the samples was in the range of 6.5-7.2, temperature 5-19 °C, chloride 0-96.56 mg/L, nitrate 0-5.4 mg/L and iron 0-1.1 mg/L. The physicochemical parameters including pH, temperature, chloride, nitrate of the samples were found to lie within the WHO guideline value except iron where 4 (6.34%) samples including 2 tap, 1 boring and 1 well exceeded WHO guideline. The bacteriological analysis of water samples revealed the presence of total coliform in 35 (55.56%) out of 63 samples, among which 8 (12.69%) were found to have faecal coliform. Furthermore, *Salmonella* spp. was isolated from 1 out of 63 samples. However, all the samples were free of *Shigella* spp. and *Vibrio* spp. It was found that most of the water samples were non potable as total and faecal coliform exceeded the WHO guideline value of drinking water.

Keywords: Drinking water; Chloride; Nitrate; Total coliform; Faecal coliform.

Introduction

Water is the most vital resource for all kinds of life on this planet and essential for ensuring the integrity and sustainability of the earth's ecosystems[1]. The presence of safe and reliable source of water is thus an essential prerequisite for the establishment of stable community. Safe drinking water is defined as water with microbial, chemical and physical characteristics that meet WHO guidelines or national standards on drinking water quality[2]. Safe water quality is a major concern with reference to public health importance as health and wellbeing of the human race is closely tied up with the quality of water used[3].

Human diseases such as, diarrhea, cholera, dysentery, typhoid, hepatitis A, and polio are supposed to be caused by inadequate sanitation, pollution or contaminated water[4]. Globally, over 884 million people have no access to safe water and nearly two million children die every year due to diarrheal disease[5]. Similarly, consumption of water with high level of nitrate is deleterious to infants who may develop a disease called methaemoglobinaemia (Blue Baby Syndrome) in which decline of transportation of oxygen by blood will be occurred. This disease can even lead to death, as a result of prolonged consumption of nitrate-rich water. Thus, high nitrate-water should not be used in infant formulas or other infant foods[6].

Despite of campaign for safe drinking water, still waterborne diseases significantly contribute in health problems in the developing countries like Nepal[7]. In Nepal, diarrheal disease ranks second in the list of top-ten diseases[8]. The total diarrhoeal cases reported in Nepal are

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Received: 27 Feb 2022; First Review: 14 Mar 2022; Second Review: 26 Apr 2022; Accepted: 28 Apr 2022.
Doi: https://doi.org/10.3126/sw.v15i15.45647

10,13,002 on 2 to 59 months children, Similarly, it is also reported that total of 20,13,891 children are prone to diarrhoea. The case fatality rate due to diarrhoea is 0.15/1000 under 5 year's old children[8]. Besides it, water is considered as unsafe for human consumption when it contains pathogenic or disease-causing microorganisms[9]. The water contamination with faecal bacteria is a common and persistent problem that has a direct impact on the public health, and in economic and social aspects[10]. The ultimate objective of public water supply is to─determine the microbiological quality of water in order to identify and minimize the public health risk from consuming contaminated water and from exposure to recreational water[11]. During microbiological assessment, the coliform bacteria are used as microbiologic indicators for water quality. Pathogens such as *Salmonella* spp., *Shigella* spp., *Vibrio cholera* and *E. coli* generally shed in human and animal feaces ultimately find their way into water supply through seepage of improperly treated sewage into ground water[12.] It became vulnerable when faecal contaminants enter the water supply. Bacterial contamination in drinking water has been one of the major public health issues in Nepal. A survey in drinking water from different sources of Kathmandu reported presence of unsafe levels of total coliform as well as faecal coliform in all the sources tested[13]. The larger the population served, the longer is the distribution system and therefore the greater the risk of contamination[14]. The poor management system and lack of monitoring of water quality has created serious threat to public health and environment. Thus, this study aims to analyse the quality of drinking water of Kageshwori Manohara Municipality, Kathmandu with reference to physicochemical and microbiological parameters. Besides this, the present study also assesses the pathogenic and opportunistic bacteria along with the risk associated with the presence of pathogens.

Materials and methods

Sample collection site

The study was conducted in Kageshwori Manohara Municipality from January to July, 2019. The sample are shown in Figure 1.

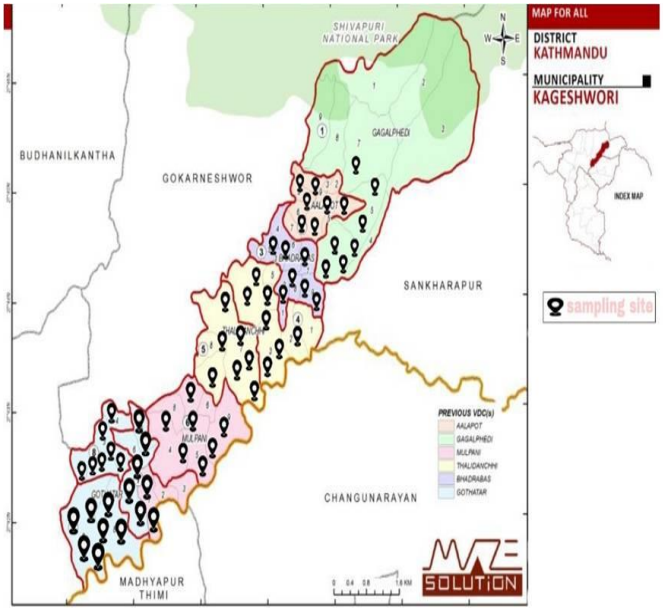

Figure 1: Map of Kageshwori Manohara Municipalityalong with sampling site

Samples were collected randomly from different sources like stone spout (dhunge dhara), well, tap and jar water which is the source of drinking water for the people of that Municipality. The list of water samples and their corresponding sources and locations are given in Table 1.

Sample collection

A stratified random sampling was conducted. Altogether 63 water samples were taken, 7 from each sample site. Sample site was specified on the basis of ward (Table 1).

Water samples were collected in sterile bottle for microbiological analysis and for physico-chemical analysis; plastic bottles which were washed thoroughly with water and rinsed 2-3 times with water to be tested were used. While taking samples, an external fitting from the tap was removed and the water was allowed to run for 2 min, after which sampling bottle was filled from gentle flow of water and the cap was replaced. A sample code and sampling date was written to each bottle. Temperature and pH of water samples were recorded at the site during sampling period. Samples were analysed on same day immediately after transported to lab. When the immediate analysis was not possible, the samples were preserved at 4 °C.

Table 1. Sample sites and sources of samples with sample code

Sample sites	Sample source	Sample code
Ward No1	Tap (T)	T_{19},T_{20},T_{21},T_{22},T_{23},T_{24},T_{25}
Ward No2	Tap (T), Spout (S)	T_2, T_3, T_4,T_{18}, S_{10},S_{11},S_{12}
Ward No3	Tap(T), Spout (S)	T_7,T_8,T_9,T_{10},T_{11},T_{17},S_{15}
Ward No4	Spout (S), Tap (T) ,Well (W)	S_7,S_8,S_9,S_{13},T_1,T_{16},W_7
Ward No5	Boring (B),Well (W), Spout (S)	B_1,W_2,S_2,W_4,W_5,S_{18},S_{29}
Ward No6	Well (W), Spout (S), Tap, Jar (J)	W_1, S_1,W_3,J_2,S_4,S_5,J_4
Ward No7	Spout (S) , Jar (J) , Tap (T) , Well (W) , Hand Pump (HP)	S_3,S_{16},J_3,T_{12},W_8,W_{10}, HP_1
Ward No8	Tap (T), Spout (S), Well (W)	T_5,T_{26},T_{27},T_{28},S_{14},S_{16},W_{11}
Ward No9	Jar (J), Hand Pump (HP), Tap (T), Well (W)	J_1,HP_2,W_9, T_6,T_{13},T_{14},T_{15}

Physicochemical examination of water sample

All together 5 physicochemical parameters namely: pH, temperature, chloride, iron and nitrate were selected for the purpose of analysis. Temperature and pH was measured using thermometer and pH meter (Hanna instrument, USA, Model-RIO2895) respectively.

Determination of chloride

Fifty milliliters of the water sample was taken in a conical flask and 2 mL of K_2CrO_4 reagent was added to it. The solution was titrated against 0.02 M $AgNO_3$ until a light red color appeared in the flask. The burette reading was noted and repeated until concurrent readings were obtained. The blank correction was done using distilled water (50 mL) which was titrated in the same way. A blank of 0.2 to 0.3 mL is usual. The concentration of chloride was then calculated using the following formula:

$$\text{Chloride (mg/L)} = \frac{\text{(A-B)} \times \text{N of } AgNO_3 \times 1000 \times 35.5}{\text{Volume of sample}}$$

Determination of nitrate

Ten milliliters of sample was taken in 50 mL test tube. Two milliliters of NaCl solution was added on it and was kept in cool water bath. 10 mL of H_2SO_4 was added and was mixed thoroughly. Brucine reagent of 0.5 mL was added and was mixed thoroughly. Tube was placed on hot water bath for 20 min. It was cooled and then absorbance was taken in colorimeter at 410 nm. From the standard curve the concentration of nitrate was calculated.

Determination of iron

The 50 mL sample was taken in 150 mL conical flask. Then 2 mL of conc. HCl and 1 mL of hydroxylamine hydrochloride was added on a sample. After that 10 mL of ammonium acetate buffer and 2 mL phenanthroline solution was added. Total volume 100 mL was made by adding distilled water. After 10 min, absorbance at 510 nm on a colorimeter (Esico International, India, Model- 312) was taken. From the standard curve the concentration of iron was calculated[15].

Microbiological examination of water sample

Total plate count

Total plate count was done by pour plate technique using Plate Count Agar (PCA) (Hi Media). For this sterile plate was taken and 1 mL of sample was placed on a plate. Autoclaved PCA media was then poured on a plate containing sample and was shaken gently in an aseptic condition. Plate was then allowed to solidify and was then incubated at 37 ˚C for 24 h.

Total coliform and faecal coliform count

Total coliform and faecal coliform bacteria were enumerated by pour plate technique using Violet Red Bile Agar (VRBA) (Hi Media). The 1mL of sample was placed in two sterile petri plates and the media was poured on

plates containing sample aseptically and were swirled to mix the inoculum evenly. Another thin layer of media was poured to create semi anaerobic condition. Media was allowed to solidify. Plates were incubated at 37 and 44.5 ˚C for total and faecal coliform respectively for 24 h. After incubation, the numbers of colonies were counted in CFU/mL.

Isolation and identification of *Salmonella* spp., *Shigella* spp. and *Vibrio cholerae*

For isolation of *Salmonella* spp. and *Shigella* spp., 1 mL of water was enriched in 9 mL Selenite F (SF) broth then sub-cultured on a selective enteric medium, Xylose Lysine Deoxycholate Agar (XLD, Hi Media). The plates were incubated at 37 °C for 24 h. Similarly, for *Vibrio* spp., 2 mL of water was enriched in 8 mL of 1% alkaline peptone water incubated at 37 °C for 6 to 8 h. Then a loop-full of the enrichment broth was streaked on Thiosulfate Citrate Bile Salts Sucrose (TCBS, Hi Media) agar medium plate incubated at 37 °C for 24 h. All colonies with different characteristics from XLD agar and TCBS agar was streaked onto Nutrient Agar (NA, Hi Media) to get pure culture. Bacteria isolated on respective selective or differential media were identified on the basis of their colonial, morphological characteristics and biochemical tests.

Results and discussion

Physicochemical and microbiological analysis of water

Five different physicochemical parameters were tested for drinking water samples collected from different wards of Kageshwori Manohara Municipality.

The pH of water samples varied from 6.5 to 7.2. None of the samples crossed the WHO guideline value for pH value of 6.5-8.5 (Table 2). Correlation between pH and number of bacteria was calculated by Karl Pearson's correlation coefficient method and the 'r' value was found to be 1.40 which shows positive correlation between them. Diwakar et al (2008) showed similar result where all water samples tested had their pH values within the permissible level[16]. The temperature of water samples varied from 5 to 17 °C. Correlation coefficient ‘r’ value was found to be 0.1 which is closer to 0 and that shows there is low degree of negative or positive correlation between them which signifies with increase or decrease in temperature, number of bacteria might increase or decrease. In the study, conducted in USA the coliform bacteria increased significantly in drinking water distribution system when the temperature increased to over 15 °C[17].

Determination of chloride, nitrate and iron content

The highest chloride content in water sample was 96.56 mg/L and the lowest chloride content recorded was 0 mg/L (Table 2). None of the samples crossed the WHO guideline value for chloride 200 mg/L. Therefore, the drinking water source of Kageshwori Manohara Municipality is safe to drink from chloride point of view. The study by Jayana et al (2009) showed the 2.85 % of sample crossed WHO guideline value while none of the sample crossed the Nepal guideline value for chloride content[18]. Chloride in drinking water may originate from natural sources, sewage and industrial effluents, urban runoff containing de-ionizing salts and saline intrusion[19]. Chloride in the form of chloride (Cl^-) ion is the major inorganic anion in water and wastewater. High concentration of chloride gives a salty taste to water and beverages. Excessive chloride concentrations increase rate of corrosion of metals in the distribution system, depending on the alkalinity of water.

All the water samples collected were within WHO guideline value for nitrate as 50 mg/L. The highest nitrate content of 5.4 mg/L was recorded and the lowest nitrate content of 0 mg/L was recorded (Table 2). Similar results were reported by Jayana et al in 2009, where all the water samples tested were within WHO value for Nitrate. Nitrate represents the highest oxidized form of nitrogen. The most important source of the nitrate is biological oxidation of organic nitrogenous substances, which come in sewage and industrial wastes or produced indigenous compounds.

Run-off from agricultural fields is also high in nitrate. Atmospheric nitrogen fixed into nitrates by the nitrogen fixing organisms is also a significant contributor to nitrates in water.

For iron 4 (6.34%) water samples exceeded WHO guideline value while other sample were within WHO permitted value as well as National guideline value. Maximum concentration being 1.1 mg/L whereas lowest being 0 mg/mL (Table 2). The exceeded values for iron were found in 2 taps, 1 well and 1 boring sample. Similarly, in 2009, study conducted by Jayana et al, also found all the samples tested was within WHO guideline value as well as National guideline. Whereas, Warner et al (2008), found 64 % of samples were above 0.3 mg/L[19]. In public health point of view, people are recommended to use filtration technique before consuming it. The occurrence of iron in water can also have an industrial origin; mining, iron and steel industry, metals corrosion, etc [20]. Iron in drinking water and water supplies can cause problems, such as giving reddish color and odor [21].

Analysis of total and faecal coliform count

Coliform bacteria have been recognized as a suitable microbial indicator of drinking water quality. They make up around 10% of the intestinal micro flora of the human and animal intestine. The level of coliform organisms present in the drinking water should not exceed the maximum permissible value of less than one cell per 100 mL of water set by the World Health Organization[22]. The microbiological analyses of water revealed the presence of total coliform. Out of total samples, 44.3% of samples were within the WHO guideline value for total coliform, among which 12.69% samples showed presence of faecal coliform (Table 2). Similarly, in the study conducted by Shakya (2012), 61.4% (70/114) of the water samples was found to have coliform count above the recommended level of WHO guideline[23]. However, Bajracharya (2007) and Aryal (2009) reported 73.7% and 86.2% samples respectively contaminated with total coliform[24, 25]. Chaidez (2008) detected 46% Total coliform and 26% faecal coliform in drinking water conducted in Mexico[26]. Jayana et al (2009) found 64.76% of all samples exceeding WHO permissible value for coliforms within which 28.88% were from tap water samples. The source of water contaminated with coliform may be due to infiltration of contaminated water (sewage) through cross connection and leakage points in case of tap, unhygienic handling of storage vessel, cross contamination of agricultural wastes. Comparatively high number of faecal coliform contamination was found in tap; stone spout, well and jar respectively. This relatively high number of faecal contamination could be attributed to direct faecal disposal near the source of water and lack of sanitation measures practiced by local people. Additionally, identification of organism showed that out of 63 samples, *Salmonella* was found in only one water sample collected from well. All other water samples were found to be free from *Shigella* spp. and *Vibrio* spp. The study done by Bhatta (2007), reported 14% samples to be positive for *Salmonella* and the organisms identified were *S. Typhi*, *S. Paratyphi A*, *S. Typhimurium* and *S. Enteritidis* [27]. Out of 68 samples tested, 4(4.65%) were positive for *Salmonella*[5]. Pathogens such as *Salmonella* spp., *Shigella* spp., *Vibrio*

Table 2. Physiochemical and microbiological characteristics of water samples

Category	Parameter	Unit	Range	WHO guideline value	Nepal standard value
Physical	Temperature	°C	5-19		
	pH		6-7.2	6.5-8.5	6.5-8.5
Chemical	Chloride	mg/l	0-96.56	250	250
	Nitrate	mg/l	0-5.4	50	50
	Iron	mg/l	0-1.1	0.3	0.3-3
Microbiological	Total Coliform count	CFU/100ml	0-328	0	0 in 95% samples
	Fecal coliform		0-88		

cholera and E. coli being shed in human and animal faeces ultimately find their way into water supply through seepage of improperly treated sewage into groundwater[12].

Conclusions

The water samples collected from different sources in Kageshwori Manohara Municipality were analyzed to assess the drinking water quality. The physicochemical parameters including temperature, pH, chloride, nitrate, iron were found to lie within the WHO guideline value except for iron. In case of iron, 6.34% of samples exceeded WHO guideline value. The study explored that most of the samples were found to be contaminated with coliform as 55.56% of the samples crossed the WHO guideline value. Out of total samples, (1.58%) of sample was contaminated with Salmonella. It can be recommended that the regular monitoring of quality of water and maintenance of source of water is needed in Kageshwori Manohara Municipality. Therefore, the consumers themselves should be aware of pollution of water, and its impact in their health. There should be regular programs regarding water treatment processes such as boiling, filtration, chlorination.

Acknowledgements

We would like to express our sincere gratitude to all the consumers of Kageswori Manohara Municipality for providing us with samples. We would also like to thank the supervisors and teachers from Trichandra Multiple Campus and Med Micro Lab for support and guidance throughout the work.

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