# BACTERIOLOGICAL EXAMINATION OF WATER SUPPLY AND DRINKING WATER AT HOUSEHOLD LEVEL IN MAKALBARI AREA BY THE MOST PROBABLE NUMBER METHOD

*Bharati Shakya, Supri Raj Shrestha and Vinutha Silvanus*

Department of Community Medicine, Nepal Medical College Teaching Hospital, Attarkhel, Gokarneshwor-8, Kathmandu, Nepal

## ABSTRACT

Water safety and quality are fundamental to human development and well-being. Yet, at least 2 billion people are using a drinking water source contaminated with feces worldwide. This study was conducted to assess the types of water supply, storage and methods of purification of drinking water and to examine the bacteriological quality of supplied and drinking water at household level by most probable number (MPN) test in Makalbari area in Gokarneshwor Municipality, Kathmandu. A total of 80 households were selected using simple random sampling technique and two samples (one from main water supply and another from drinking water, n=160 water samples) were collected from each household. Ethical clearance was taken from the Nepal Medical College Institutional Review Committee. SPSS version 16.0 was used for statistical analysis. Majority of water supply samples 65 (81.2%) were from tap, 11 (13.8%) were from well and 4 (5.0%) samples were from bore well. Bacteriologically, of the total water supply samples, 34 (42.5%) samples were excellent, 16 (20.0%) samples were suspicious and 30 (37.5%) samples were unsatisfactory by MPN index quality. Most of the households stored drinking water in the tanks 34 (42.5%). Most of the water samples 29 (36.3%) were directly consumed from the water supply without purification. Among the directly consumed drinking water samples, 13 (44.8%) samples showed unsatisfactory MPN index. Most commonly used methods of water purification was filtration 21 (26.2%). Of the total drinking water samples, 33 (41.25%) samples were positive for total coliform. Thirty-two (40.0%) of the households reported diarrhea in past one year. A highly significant association was found between MPN index of drinking water and occurrence of diarrhea in the households. The quality of the most of the water samples from water supply and drinking water were unsatisfactory. Inspite of this, drinking water was not purified in some of the households. Moreover, few of the drinking water samples were not potable even after purification. Therefore, community should be made aware to improve quality of drinking water in households.

## KEYWORDS

Coliform bacteria, drinking water, most probable number (MPN) method, Kathmandu

## CORRESPONDING AUTHOR

Dr. Bharati Shakya,
Lecturer
Department of Community Medicine,
Nepal Medical College Teaching Hospital,
Attarkhel, Gokarneshwor-8, Kathmandu, Nepal
Email: shakyabharati@hotmail.com
Orcid No: https://orcid.org/0000-0001-7834-168X
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# INTRODUCTION

Access to water and sanitation is an internationally recognized human right. Sustainable Development Goal target 6.1 calls for universal and equitable access to safe and affordable drinking water.[1] The human right to water entitles everyone, without discrimination, to sufficient, safe, acceptable, physically accessible and affordable water for personal and domestic use; which includes water for drinking, personal sanitation, washing of clothes, food preparation and personal and household hygiene.[2] Contaminated water can transmit diseases such as diarrhea, cholera, dysentery, typhoid and polio. Estimated 4,85,000 diarrheal deaths are caused due to contaminated drinking water every year.[1] More than 700 children under five years of age die every day from diarrhea linked to unsafe water and poor sanitation.[3]

Although Nepal is one of the richest countries in terms of per individual water availability, it is the poorest country in terms of use of water.[4] Even when water sources are free from contamination, household storage pots, tanks and insanitation around household may be sources of contamination if not properly cleaned and maintained.[5] A nationwide study has shown the contamination rate of drinking water ranging from 53% to 100%.[6] Diarrheal diseases are a major public health threat[7] and have been ranked second in the national list of research priorities.[8] Cholera has been endemic in Nepal for many years as evidenced by several large outbreaks documented in and around Kathmandu and other regions of the country.[9] According to Department of Health Services annual report,[10] in 2017/18, a total of 1,148,238 diarrhoeal cases were reported and the incidence of diarrhoea per thousand under age 5 children was 385. Total diarrhoeal death in health facility and Primary Health Centre was 47 and this was an increase by 42% compared to the last fiscal year. Enteric fever is also a common disease leading to frequent hospital visits. In city areas including Kathmandu, lack of solid waste management and leakage of sewers are responsible for the contamination of groundwater.[11]

Safe water supplied together with good sanitation and hygiene practices is more effective for reducing diarrhea. Improving microbial quality of water at point-of-use could reduce 39% diarrhea risk; improving sanitation and hand washing promotion reduce the incidence of diarrhea by about 30%.[12,13] The effect of point-of-use chlorine treatment significantly improves the quality of stored water in households and also reduces the risk of diarrhea by 25%-58% as observed from studies.[14-17] Water is said to be contaminated when it contains infective agents. Most Probable Number (MPN) method of water quality detection is cost effective and rapid tool to evaluate water quality in a routine microbiological laboratory with public health significance.[18] This study aimed to assess the types of water supply, storage and methods of purification of drinking water and to examine the bacteriological quality of supplied and drinking water at household level by MPN test.

# MATERIALS AND METHODS

A descriptive cross sectional study was conducted among the residents of Makalbari area of Gokarneshwor Municipality in Kathmandu, Nepal from July 2019 to Nov 2019. Makalbari area is a part of 4, 6 and 8 wards of Gokarneshwor Municipality with estimated 800 households. Using simple random sampling technique, 10% of total households i.e, 80 households were selected which would be representative of all the households of Makalbari area. Participants were interviewed with self-constructed semi-structured questionnaire. Ethical clearance was taken from the Nepal Medical College Institutional Review Committee (NMC-IRC). Verbal consent was taken from each households enrolled in the study before interview. During the interview, the information regarding sources of water supply, type of drinking water, storage of water, method of water purification and total number of diarrhea in past one year in the family were obtained. Water supply may be river, stream, open/closed well, bore well or tap. After the interview, total 160 samples, one sample from main water supply and one sample from drinking water from each (n=80) household were collected in a sterile glass bottle with tight lid (250ml) and investigated for bacteriological quality by MPN test at laboratory of Community Medicine Department, Nepal Medical College.

The MPN technique was performed by inoculating water sample in MacConkey's lactose bile-salt broth with neutral red (indicator for acid production) containing Durham tube (indicator for gas production) and incubating at 37 ºC for 48 hrs. The MPN test was done by inoculating 10 ml of water sample into 3 tubes containing 10 ml double strength lactose broth with Durham's tube, 1ml and 0.1ml of water samples was inoculated into separate sets of 3 tubes of 5 ml of single strength lactose broth and incubated at 37 ºC for 48hrs. After incubation, the number of

test tubes showing colour change (from red to orange) or gas formation or both were taken as positives and compared with the Standard McCardey's table. The results were expressed as MPN index or presumptive coliform count per 100 ml of water. The results were interpreted as follow: no coliforms in 100 ml of water as excellent water, 1-3 coliforms in 100 ml of water as satisfactory water, 4-10 coliforms in 100 ml of water as suspicious water, more than 10 coliforms in 100 ml of water as unsatisfactory water for drinking.

Statistical analysis of the collected data was carried out using SPSS version 16.0. Frequency distribution of the information regarding sources of water supply, type of drinking water, storage of water, method of water purification, bacteriological examination results and total number of diarrhea in past one year in the family were calculated. Association of bacteriological analysis with independent variables was found by using Chi square test. P value less than 0.05 was taken as significant.

## RESULTS

A total of 160 samples, 80 samples from the main water supplies and 80 samples from the drinking water from households were collected. Majority of samples from water supply 65 (81.2%) were from tap, followed by 11 (13.8%) samples from well and 4 (5.0%) samples from bore well. Most of the drinking water samples 29 (36.3%) were consumed directly from the main source without purification. Filtration 21 (26.2%) was the most commonly used method of purification. Most of the households stored drinking water in the tanks 34 (42.5%) followed by 30 (37.5%) in commercially available jars (Table 1).

Of the total samples from water supply examined, 34 (42.5%) samples showed excellent, 16 (20.0%) samples showed suspicious and 30 (37.5%) samples showed unsatisfactory MPN index. Twenty nine (44.6%) of the tap water showed excellent, 10 (15.4%) showed suspicious and 26 (40.0%) showed unsatisfactory MPN

index. None of the water supply samples showed satisfactory MPN index (Table 2).

Table 3 revealed that 51 samples from drinking water were purified before drinking but 29 water samples were consumed without purification. Among total drinking water samples which were purified, 34 (42.5%) samples showed excellent, 5 (6.25%) samples showed satisfactory, 9 (11.2 %) samples showed

### Table 1: Types of water supply, methods of drinking water purification and storage of drinking water in the study community (n=80)

| Characteristics | | n (%) |
|---|---|---|
| **Types of water supply** | Tap | 65 (81.2) |
| | Well | 11 (13.8) |
| | Bore well | 4 (5.0) |
| **Methods of drinking water purification** | Directly from water supply without purification | 29 (36.3) |
| | Filtered | 21 (26.2) |
| | Filtered and boiled | 15 (18.8) |
| | Boiled | 14 (17.5) |
| | Euroguard | 1 (1.2) |
| **Storage of drinking water** | Tanks | 34 (42.5) |
| | Jars | 30 (37.5) |
| | Bottles | 8 (10.0) |
| | Buckets | 6 (7.5) |
| | Pots | 2 (2.5) |

suspicious and 3 (3.75%) showed unsatisfactory MPN index. Same number of the filtered 13 (61.9%) and filtered and boiled 13 (86.7%) drinking water showed excellent whereas 13 (44.8%) of the directly consumed water showed unsatisfactory MPN index.

About 32 (40.0%) of the households reported diarrhea in past one year (Table 4).

### Table 2: Bacteriological water quality of water supply by MPN test (n=80)

| Type of water supply | MPN index of water supply n (%) | | | |
|---|---|---|---|---|
| | Excellent | Satisfactory | Suspicious | Unsatisfactory |
| Tap | 29 (44.6) | 0 (0.0) | 10 (15.4) | 26 (40.0) |
| Well | 3 (27.3) | 0 (0.0) | 5 (45.5) | 3 (27.3) |
| Bore well | 2 (50.0) | 0 (0.0) | 1 (25.0) | 1 (25.0) |

| Table 3: Bacteriological water quality of drinking water (without and/or after purification) by MPN test (n=80) | | | | |
|---|---|---|---|---|
| **Type of drinking water** | **MPN index of drinking water n (%)** | | | |
| | **Excellent** | **Satisfactory** | **Suspicious** | **Unsatisfactory** |
| Directly from water supply without purification | 4 (13.8) | 4 (13.8) | 8 (27.6) | 13 (44.8) |
| Filtered | 13 (61.9) | 1 (4.8) | 4 (19.0) | 3 (14.3) |
| Boiled | 7 (50.0) | 3 (21.4) | 4 (28.6) | 0 (0.0) |
| Filtered and boiled | 13 (86.7) | 1 (6.7) | 1 (6.7) | 0 (0.0) |
| Euroguard | 1 (100.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

Table 5 showed that diarrhea was seen more in the households 22 (66.7%) where MPN index of drinking water was unsatisfactory. A highly significant association was found between MPN index of drinking water and occurrence of diarrhea in the households (p = 0.0001).

## DISCUSSION

During our survey, we found that the quality of most of the water supply (25.0 to 40.0%) and drinking water (0.0 to 44.8%) samples were unsatisfactory. Yet, most of the water samples were consumed directly from the water source without any purification. In a

| Table 4: Distribution of diarrhea reported in households in past 1 year (n=80) | |
|---|---|
| **Diarrhea cases in past one year** | **n (%)** |
| Yes | 32 (40.0) |
| No | 48 (60.0) |
| Total | 80 (100.0) |

counts were very high.[19] In Bangladesh, all of the waters samples (n=75) used in the study were heavily contaminated with lactose fermentation positive bacteria. Sixteen samples showed maximum counts of positive results as ≥2400 MPN/100 ml of sample and only 2 samples showed lowest count as 2 MPN/100 ml of sample.[20] In similar study by Parvez *et al*,[21] a total of 106 (tube well, deep well, surface and municipal supplied) water samples from 37 districts of Bangladesh were analyzed. Out of these, 80% samples were contaminated with coliform and 34% were with fecal coliform. Using MPN test, the highest coliform count was >1100 cfu/100 ml in surface water from pond. The results were lower in our study in which out of 80 samples from water supply, majority of samples 65 (81.2%) were from tap, followed by 11 (13.8%) samples from well and 4 (5.0%) samples from bore well. Bacteriologically, 34 (42.5%) samples were excellent, 16 (20.0%) samples were suspicious and 30 (37.5%) samples were unsatisfactory by MPN index quality. However, none of the samples showed satisfactory MPN index.

| Table 5. Association of MPN index of drinking water with diarrhea (n=80) | | | |
|---|---|---|---|
| **MPN index of drinking water** | **Diarrhea in past 1 year** | | **p Value** |
| | **Yes n (%)** | **No n (%)** | |
| Satisfactory  (0-3 coliforms /100ml) | 10  (21.3) | 37 (78.7) | |
| Unsatisfactory (≥4 coliforms/100ml) | 22 (66.7) | 11 (33.3) | **0.0001*** |

*significant p value < 0.05*

cross sectional study done in Nigeria, none of the samples collected from well, stream, and river complied with bacteriological standards as total coliform counts generally exceeded 1,600 MPN/ml, and pathogen count such as Salmonella-Shigella counts and *Vibrio cholerae*

In India, Malathy *et al*[22] found that out of the 20 water samples from three different sources tested, 3 were satisfactory and 17 were unsatisfactory. Out of 3 samples, which were graded as satisfactory, one was mineral water supplied in can and 2 were corporation

supplied drinking water to houses through taps/hand pumps. Of the 17 samples that were unsatisfactory, 1 was mineral water in cans, 5 were corporation supplied water and 2 were well water. MPN index of water samples which tested satisfactory was <2/100 ml and MPN index of water samples which were graded as unsatisfactory ranged from 38 to > 1600/100 ml. Ghimire et al[23] conducted a study in Kathmandu Valley in which 200 water samples were collected from different water sources were tested for the presence of coliform bacilli using MPN technique. Of the tested samples, 130 (65.0%) were unsatisfactory, 19 (9.5%) were suspicious, 30 (15.0%) were satisfactory and only 21 (10.5%) were excellent. In our study, out of 16 suspicious samples from different water supplies, 10 were from taps, 5 were from well and 1 was from bore well. Of the 30 unsatisfactory samples, 26 were from taps, 3 were from well and 1 was from bore well. None of the samples showed satisfactory MPN index. Out of 34 samples, which were graded as excellent, 29 were from taps, 3 were from well, and 2 were from bore well. MPN index of water samples which tested satisfactory was <3/100 ml and MPN index of water samples which tested unsatisfactory ranged from 23 to >1100 /100 ml.

Rai et al[6] conducted a large scale study on contamination of drinking water covering all five development regions of Nepal. Of the total 506 water samples, 88.5% (448) samples were positive for total coliform. In another study conducted in different places of Kathmandu Valley, total plate and coliform count revealed that 82.6% and 92.4% of drinking water samples were found to cross the WHO guideline value for drinking water.[24] As compared to these studies, lower finding was observed in our study where only 41.25% (33/80) samples were positive for total coliform.

Silvanus et al[25] carried out twenty-three school health surveys in Kathmandu Valley in which forty six water samples were collected and tested using the Multiple tube test method. It was observed that 65.09% of the schools had access to 'improved water supply' namely piped water and tube wells. Nine (81.81%) of the schools purified the water at end-use point. Only two methods of purification were found to be in practice, Euroguard (60%) and Ceramic (40%) but none of the schools carried out disinfection of the drinking water sources. MPN Index of the drinking water samples (n=23) examined for bacterial quality. The minimum value was 0 and the maximum value was 460, 56.18% of the drinking water samples were of unsatisfactory quality, 13.05%

were of suspicious quality and 34.78% were of satisfactory quality. It was observed that 88.88% of the source water samples (n=23) were of unsatisfactory quality. In a community-based matched case-control study conducted in Ethiopia, cases were children <5 years of age with diarrhea and the controls were children without diarrhea during the two weeks before the survey. Twenty-five percent matched pair samples of water were taken from households of cases and controls in which all sampled water analyzed for total coliforms were positive. Majority of cases (83.8%) and 76.8% of controls' family members informed that they didn't treat their drinking water to make it safe.[26] Similarly, in our study, most of the water samples 29 (36.3%) were consumed directly from the main source without any purification. Filtration 21 (26.2%) of drinking water was the most used method of purification. After purification also, 12 water samples showed unsatisfactory MPN index. This could be due to contamination during storage or handling of drinking water or due to insanitation.

In our study, 38 (47.5%) drinking water samples showed excellent, 9 (11.2%) samples showed satisfactory, 17 (21.2%) samples showed suspicious and 16 (20.0%) samples showed unsatisfactory MPN index. Diarrhea was seen more in the households 22 (66.7%) where MPN index of drinking water was unsatisfactory. A highly significant association was found between MPN index of drinking water and occurrence of diarrhea in the households (p =0.0001) in contrast to the study done in Pakistan[27] which showed that there was no association between the incidence of childhood diarrhoea and the number of Escherichia coli in the drinking water. Faecal contamination levels in household water containers were generally high even when the source water was of good quality. Similarly, Rai et al[28] investigated a diarrheal outbreak in Rautahat district for which stool samples were collected from individuals with acute diarrheal illness (n = 16) and healthy non-diarrheal children (n = 39), along with samples from local drinking water sources (n = 8) and their sewage system (n = 10). V. cholerae O1 Ogawa serotype was isolated in 18.7% of the diarrheic stool and 20.0% of the sewage. Coliforms were found in all drinking water samples, with 87.5% testing positive for fecal coliform. However, almost all parasites were found in non-diarrheal stool. Hence, these findings provide evidence that the diarrheal outbreak was associated with V. cholerae O1 Ogawa serotype, possibly transmitted through the drinking water sources contaminated with fecal matters from their sewage (drainage)

system. According to WHO, some 8,29,000 people are estimated to die each year from diarrhoea as a result of unsafe drinking-water, sanitation, and hand hygiene[29] and diarrhoea is largely preventable, and the deaths of 2,97,000 children aged under 5 years could be avoided each year if these risk factors were addressed.

The results clearly showed that the quality of most of the water samples from water supplies and drinking water were not satisfactory. Moreover, most of the water samples were consumed directly from the main source without purification. After purification also, few of the drinking water samples were not potable. Diarrhea was seen more in the households where MPN index of drinking water was unsatisfactory. Hence, a regular monitoring of the water quality for improvement should be carried out to prevent water borne diseases

and also to check the water sources from being further polluted. Also, increasing awareness of community and promotion for behavior change should be recommended on the water source selection, safe handling and storage of water, hand washing practices before water drawing from storage, purification of water, latrine use and sanitary disposal of domestic waste to prevent water borne diseases in future.

## ACKNOWLEDGEMENTS

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