British Microbiology Research Journal
7(2): 81-92, 2015, Article no.BMRJ.2015.098
ISSN: 2231-0886

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Detection of Indicator Organisms in Drinking Water by Membrane Filtration Method in Open Defaecation Free VDCs of Kaski District, Nepal

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Authors' contributions

This work was carried out in collaboration between all authors. Author DRP designed the whole study, author NC wrote the manuscript. Authors DKK, NM and SS contributed in the patient enrollment, data collection and management. Author IS provided help in the statistical analysis and interpretation of the analyzed data. Authors CA and SU contributed in the critical reading, correction of English and improvement of the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2015/16162
Editor(s):
(1) Giuseppe Blaiotta, Department of Food Science, Via Università, Italy.
Reviewers:
(1) Anonymous, Pakistan.
(2) Vandré Barbosa Brião, School of Engineering, University of Passo Fundo, Brazil.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=989&id=8&aid=8340

Original Research Article

Received 13th January 2015
Accepted 10th February 2015
Published 4th March 2015

ABSTRACT

Aims: The aim of the project is to detect and enumerate the presence of indicator organisms in drinking water sources of open defaecation free (ODF) village development committees (VDCs) of Kaski district; to compare distribution of Total coliform (TC) and Faecal count (FC) among various water sources.
Study Design: Cross-sectional Study.
Place and Duration of Study: Department of Medical Laboratory Science, School of Health and Allied Sciences, Pokhara University, December 2013 to March, 2014.
Methodology: The study was conducted to detect and enumerate indicator organisms in ODF

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VDCs of Kaski district, Nepal. 44 water samples were collected from reservoir / distribution tank, tapstand and spring. The bacteriological water quality was analyzed by using membrane filter method to detect the presence and its risk level of fecal contamination to human health in study area. The study mainly focuses on two part of the study; laboratory analysis for water samples and questionnaire survey. Total water samples collected were 44, out of which 32 were from tap, 3 from reservoir tank and 9 from spring. Chlorine disinfection treatment was not done in any VDCs.

Results: All the drinking water samples from Bharatpokhari and Kalika VDCs (100%) were found to be fecally contaminated and the number of total coliforms and fecal coliform per 100ml water were above WHO guidelines for drinking water. None of the water sources from both the VDCs were potable for drinking. Mann-Whitney test was used to compare mean range between samples of two VDCs and mean rank of total coliforms of Bharatpokhari and Kalika VDC were found to be 29.18 and 13.17 and that of Bharatpokhari and Kalika VDCs were found to be 29.80 and 12.89 respectively. Tap water, reservoir/distribution tank and spring water were analyzed to detect indicator organisms and Kruskal Wallis test suggest no significant difference in fecal contamination level among various sources of water was found ($p>0.05$). The median concentration of fecal coliform of gravity water (spring) in Bharatpokhari was found to be 95 CFU/100 ml and that of Kalika is 40 CFU/100 ml. *E. coli* was detected in every water samples of both the VDCs.

Conclusion: The water bodies used for drinking purpose in Bharatpokhari and Kalika VDC are heavily contaminated with total and fecal coliform bacteria even though those VDCs have been declared ODF. The water bodies used for drinking purpose in Bharatpokhari is found to be more contaminated than Kalika VDC. The distribution of total coliforms and fecal coliforms are same across categories of source of sample. *E. coli* was isolated from every water sample under study.

Keywords: Chlorine disinfection; fecal contamination; indicator organisms; membrane filtration.

1. INTRODUCTION

Still one billion people do not have clean water, and 2.6 billion lack of basic sanitation [1,2]. Nepal faces a plethora of problem regarding both its drinking water quality and availability [3]. Only 66% of population has an access to piped water, and others are dependent on locally available water sources such as spring, stream and tube well for drinking and household uses. Throughout Nepal, people are exposed to severe health threats resulting from faecal contamination of drinking water by sewage, agriculture and industry [4]. Owing to the impact of sewage, typhoid, dysentery, and cholera are endemic every summer [5]. The diseases are transmitted particularly through human and animal excreta, especially faeces [6]. Kaski district was the first open defecation free district of Nepal declared in 18 July 2011 after construction of 18,035 households and 634 schools (Community 435 and Institutional 289) toilets with financial ODF campaign support from the government [7]. Chlorine disinfection treatment was not done in both Bharatpokhari and Kalika VDC. The water bodies used for drinking purpose in Bharatpokhari and Kalika VDC are heavily contaminated with total and fecal coliform bacteria even though those VDCs have been declared ODF. To prevent contamination of drinking water sources, appropriate treatment of contaminated sources, and monitoring to identify contamination as areas of emphasis in the quest to safeguard drinking water sources [8]. Bacterial indicator organisms used to assess the microbiological quality of water has been practiced for almost a century [9]. The primary objective for using indicator organisms and methods commonly related to their examination is to indicate the degree of water contaminated by faecal wastes [10]. *Escherichia coli (E. coli)* is considered indirect indicator of fecal contamination since it is widely distributed in the intestine as an essential intestinal flora that maintains the physiology of the healthy host and not usually found in other niches since, E. coli could be easily detected by its ability to ferment lactose and since other enteric bacteria like *Citrobacter, Klebsiella* and *Enterobacter* that can also ferment lactose and are similar to E. coli in phenotypic characteristics, so that they are not easily distinguished [11,12]. Public Health Service adopted the enumeration of coliforms as a more convenient standard of sanitary significance [13]. Total coliforms (TC) and Faecal coliforms (FC) are widely used to evaluate the quality of drinking water [14]. Fecal coliform is a subset of total coliforms that grows and ferments lactose at elevated incubation temperature (45.5ºC), hence also referred to as

thermotolerant coliforms [15]. Standard faecal coliform detection by membrane filtration procedure is considered best method of selection [16]. This method consists of filtering a water sample on a sterile filter with a 0.45-mm pore size which retains bacteria, incubating this filter on a selective medium and enumerating typical colonies on the filter. In this study we are detecting and enumerating total and fecal coliforms in water sources in order to know the particular status of drinking water and suggest local authorities make basic complacence of providing safe drinking water to community.

2. METHODOLOGY

This study was designed to detect the presence of indicator organisms in drinking water of ODF VDCs of Kaski district. The study was a cross sectional study conducted in School of Health and Allied science, Pokhara University, Nepal from December 2013 to March 2014. The study encompassed of 44 drinking water samples: 25 from Bharatpokhari VDC and 19 from Kalika VDC. All water sources that are used for drinking water purposes; tap water, reservoir tank and spring were included while those water sources that are not used for drinking purposes were excluded for study. Each water sample was given a code number and the following information was collected; Name and Ward number of the VDC, location of water source, chlorinated or non-chlorinated, date and time of sample collection. The research was carried out accommodating all principles of Helsinki declaration and ethical clearance was given by the research board of the School of Health and Allied Sciences.

2.1 Collection from Taps and Water Reservoir Tank

The external fittings from the tap were removed and allowed to run to waste for 1 minute. The tap was sterilized by igniting a piece of a cotton wool soaked in spirit and holding it with a pair of tongs close to the nozzle until the whole tap is unbearably hot to touch then tap is allowed to cool by running the water to waste for a few seconds. Sample bottle was filled from a gentle flow of water and the cap was replaced. Water proof marker was used to number the bottle with sample code number.

2.2 Collection of Sample from Spring

The cap of the bottle was aseptically removed and dipped into the spring. When no more air bubbles rise to the surface, the bottle was raised out of the spring and cap was replaced. The bottle was then labeled.

2.3 Transportation to Laboratory

Immediately after collection, samples were placed in an insulated cold box and then transported to microbiology laboratory. Water samples were processed within 6 hours.

2.4 Procedure for Membrane Filtration

20 ml sample was diluted to 100ml distilled water, shaken for 25 times and then delivered to filtration apparatus using graduated cylinder. Sterile filter was placed on filtration apparatus using sterile forceps. Vacuum was applied and afterwards, filtration apparatus was flame sterilized. Cylinder was rinsed twice with distilled water. Forceps were sterilized and filter paper was removed. Funnel was replaced on filtration apparatus. Filter paper was rolled onto media in petri dish. Inverted petri dish was then placed in incubator.

2.5 Identification and Enumeration Method

Indicator bacteria for presumptive identification and enumeration were cultured on selective media (m-Endo media and mFC agar media) after filtration of sample volumes onto gridded membrane filters. Detailed confirmation, identification *of E. coli* was done by biochemical tests; the total coliform bacteria are defined as the organisms that produce red colonies with a golden-green metallic sheen within 24±2 hours when incubated at 35.0±0.5°C on m-Endo medium while fecal coliform bacteria are defined as the organisms that produce blue colonies in whole or part within 24±2 hours when incubated at 44.5±0.2°C on m-FC medium.

2.6 Calculating and Reporting Fecal Indicator Bacteria Densities

Density per 100 ml is calculated by dividing the colony count for the sample by the volume filtered, then multiplying by 100.

2.7 Biochemical Identification of *E. coli*

Specific characteristic were oxidase negative, Indole positive, Methyl red positive and Voges-proskauer negative, Catalase positive, H_2S positive, Nitrate-reduction positive, β-galactosidase positive, Phenylalanine deaminase negative and Malonate negative and also able to solubilize gelatin, unable to utilize citrate, hydrolyzed urea, produced gas and acid in sucrose, glucose, lactose broths, produced acid in Mannitol broth while produced acid in litmus milk.

2.8 Statistical Analysis

All statistical analyses were performed using Statistical Package for Social Science (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA) and Microsoft excel 2007. The SPSS version 24 was used to carry out descriptive statistics. The results were expressed in the forms of pie-charts, bar diagrams, Tables etc.

3. RESULTS AND DISCUSSION

According to the data presented in Figure, highest total coliform and fecal coliform median concentration were found in spring water source and lowest total coliform and fecal coliform median concentration were found in reservoir/distribution tank of Bharatpokhari village development committee.

The Figure shows that there was highest median concentration of total coliform and fecal coliform in spring water and lowest median concentration in tank water in Kalika village development committee.

The Table shows that of out of 25 total samples from Bharatpokhari VDC, 16/25 (64%) were found to be under high risk group and 9/25 (36%) were found to be under very high risk group. While out of 19 samples from Kalika VDC, 2/19 (10.5%) were found to be under low risk group and 17/19 (89.5%) under high risk group.

The Table shows that out of 32 water samples collected from tap source, 1/32 (3%) was found to be under low risk, 25/32 (78%) under high risk and 6/32 (19%) under high risk group. Out of 3 samples collected from reservoir tank, 1/3 (33.3%) was found to be under low risk, 1/3 (33.3%) under high risk and 1/3 (33.3%) under very high risk group. Out of 9 samples collected from spring water source, 7/9 (77.7%) were found to be under high risk and 2/9 (22.3%) under very high group.

The Table shows that there is significant difference in TC & FC between two VDCs ($p=0.00$). The mean rank of TC & FC in Bharatpokhari VDC are 29.18 & 29.80. However, the mean rank of TC & FC in Kalika VDC are 13.71 & 12.89. Those data show that the water quality of Kalika VDC is better than Bharatpokhari VDC.

The Table shows that there is no any significant difference in TC & FC among various sources of water under study ($p>0.05$). The distribution of TC & FC among various water sources under study between Bharatpokhari & Kalika VDCs were similar.

Over the past ten years effective efforts have been made to detect and enumerate target organisms in clinical and environmental samples rapidly and with sensitivity [17]. Total coliforms and fecal coliforms are being used as indicator organisms for drinking water contamination. Within the coliforms family fecal coliform is of the prime interest and reliable indicator for fecal contamination rather than total coliform [18], because its presence indicates recent fecal contamination with the possibility of enteric pathogens [19]. Assessment of water quality depends on detection of indicators in water samples [20]. All the drinking water samples from Bharatpokhari and Kalika VDCs (100%) (Figs. 1,2) were found to be fecally contaminated which was a comparable result to previous studies concluded by Rai SK et al. [21]. The study done by Shakya S et al. [22] in WDR of Nepal revealed water supplied in rural drinking water schemes do not meet the WHO drinking water standard and maximum population was under high risk of fecal contamination. Most of research indicated analogous pattern of contamination; water samples were positive for TC and *E. coli.* Rai SK et al. (2009) in Sankhuwasabha and Rasuwa district [23], Karen Harpp et al. [24] and Andrea N.C. Wolfe [25] in Kathmandu valley. Contamination of all the drinking water sources of study VDCs were probably due to human or animal excreta. The factors responsible for contaminating drinking water at source points in Nepal included the lack of protection and proper treatment of water, leakage in pipe distribution system [26]. Due to leakage of pipes, biofilm formation in the distribution system, intermittent water supply, and human activities biological contamination is likely to occur [27-29]. In our

present study too, all samples from both VDCs were fecally contaminated and the number of total coliforms and fecal coliform per 100 ml water (Figs. 3, 6) were above WHO guidelines for drinking water (Figs. 4, 7). None of the water sources from both the VDCs were potable for drinking (Figs. 5, 8) (Tables 1, 2). Chlorine disinfection treatment was not done in both Bharatpokhari and Kalika VDC. Mann-Whitney test was used to compare mean range between samples of two VDCs. The mean rank of total coliforms of Bharatpokhari and Kalika VDC were found to be 29.18 and 13.17 respectively. This is supported by mean rank of fecal coliforms. The mean rank of fecal coliforms of Bharatpokhari and Kalika VDCs were found to be 29.80 and 12.89 respectively (Table 3). Thus, comparatively, water samples from Kalika VDC were found better than Bharatpokhari VDC. In our study, tap water, reservoir/distribution tank and spring water were analyzed to detect indicator organisms and Kruskal Wallis test suggest no significant difference in fecal contamination level among various sources of water was found ($p>0.05$) (Table 4). The median concentration of fecal coliform of gravity water (spring) in Bharatpokhari was found to be 95 CFU/100 ml and that of Kalika is 40 CFU/100ml less than that of Pandey B and Shakya S (2009) [30], In our research, *E. coli* was detected in every water samples of both the VDCs. Studies have been reported that *E. coli* is the only coliform which is mostly associated with a fecal source [31,32]. It was also reported that the other thermo-tolerant coliform and total coliform were also capable of growth in non-polluted water. This supplements a recommendation for *E. coli* to be used as the prime indicator bacteria for recent fecal contamination [33]. *E. coli* is typically sensitive to environmental stress.

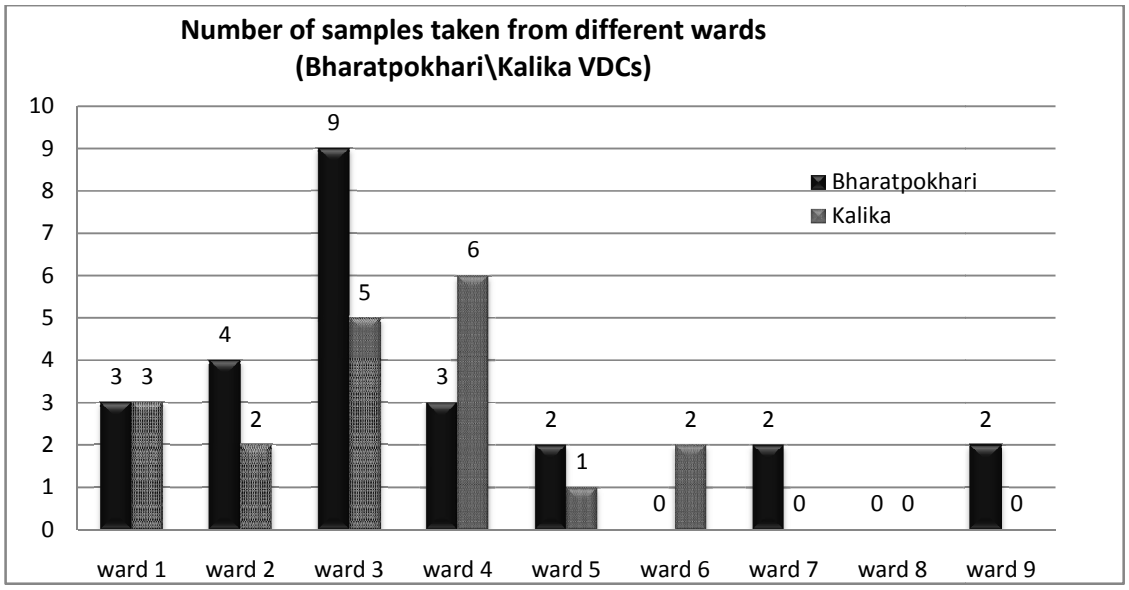

Fig. 1. Sample size taken from different wards of Bharatpokhari and Kalika

Table 1. WHO Risk measurement and bacteriological risk grade related to fecal coliform count in VDCs

FC count/ 100 ml	Bacteriological risk grade (BRG)	Risk grade	Bharatpokhari VDC	Kalika VDC	Total samples (44)
0	1	No Risk	0	0	0
1-10	2	Low Risk	0	2	2
11-100	3	High Risk	16	17	33
101-1,000	4	Very High Risk	9	0	9

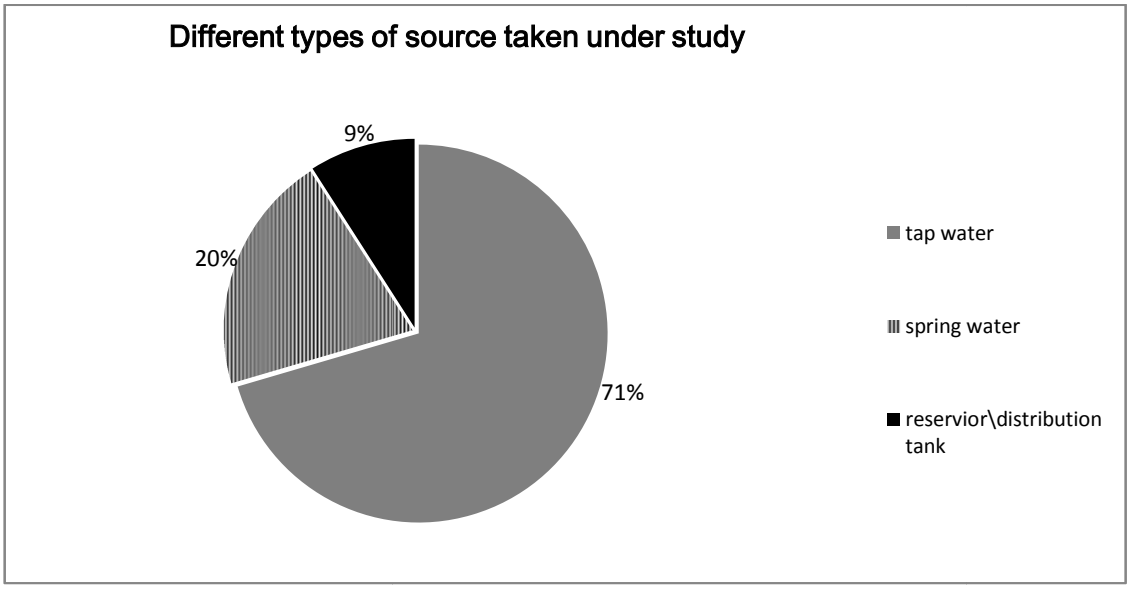

Fig. 2. Different type of water sources taken under study

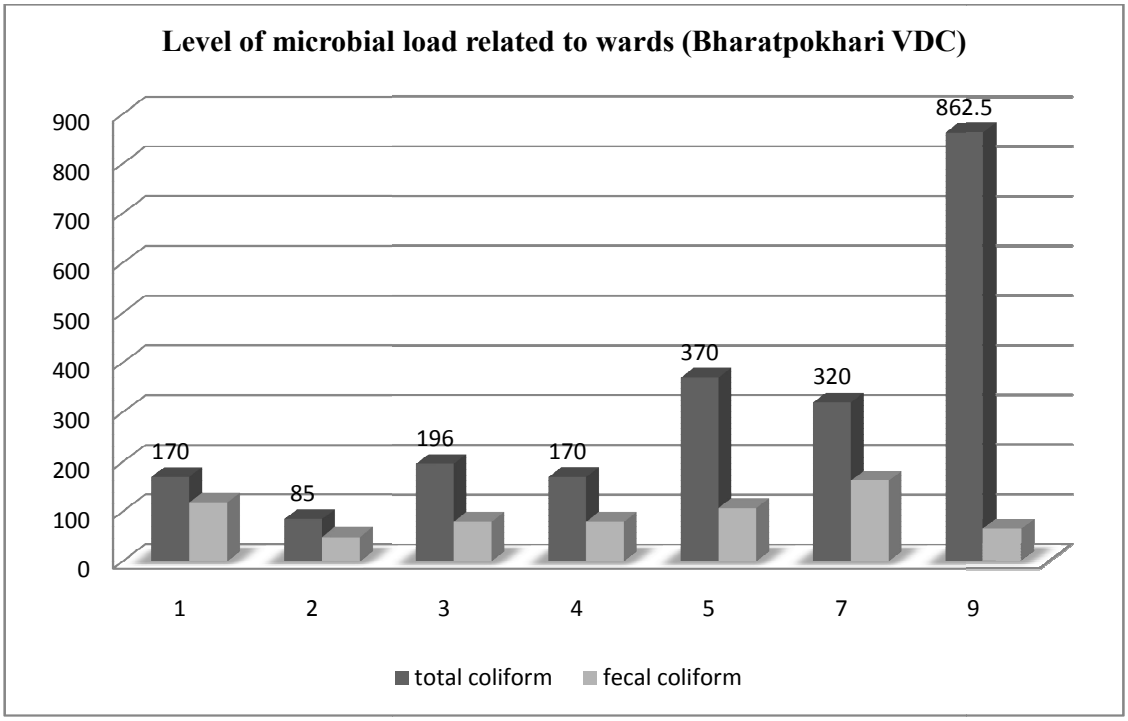

Fig. 3. Median concentration of total coliform and fecal coliform on various wards of Bharatpokhari village development committee

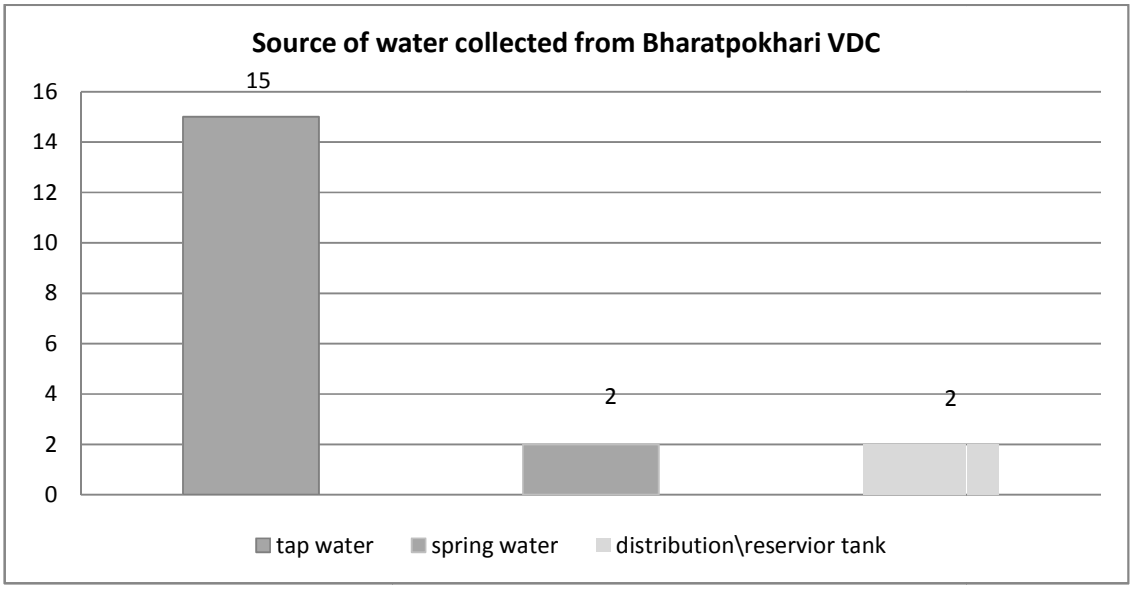

Fig. 4. Different water sources collected from Bharatpokhari village development committee

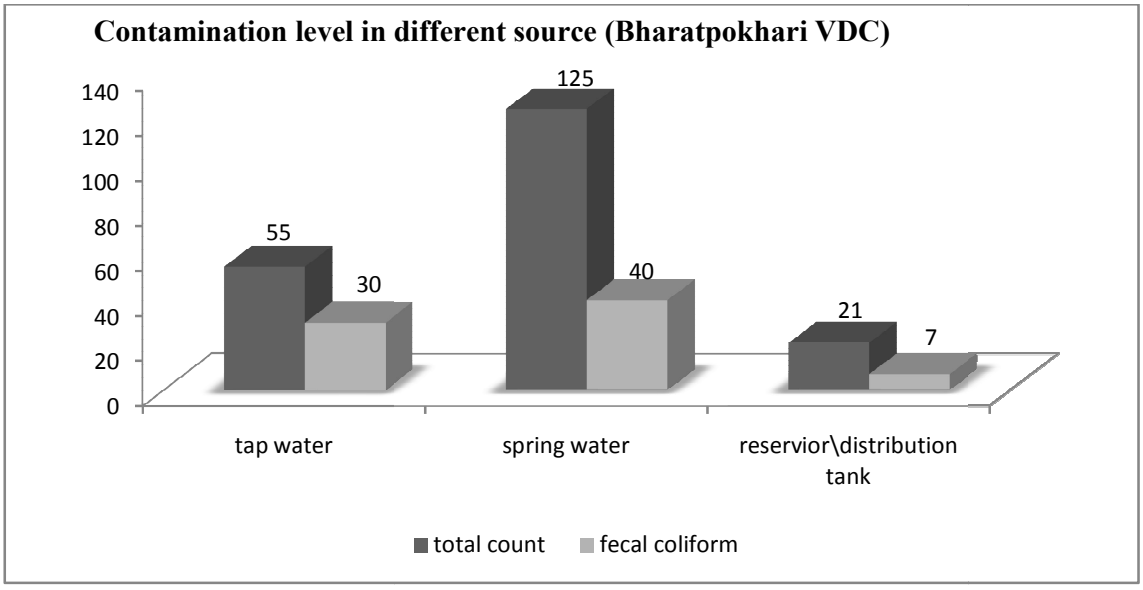

Fig. 5. Median concentration of total coliform and fecal coliform in different sources of Bharatpokhari

Table 2. WHO Risk measurement and bacteriological risk grade related to fecal coliform count on water sources

Bacteriological risk grade	Risk group	Fc \100 ml	Tap water	Reservoir\tank	Spring water	Total
1	No risk	0	0	0	0	0
2	Low risk	1-10	1	1	0	2
3	High risk	10-100	25	1	7	33
4	Very high risk	100-1000	6	1	2	9
Total			32	3	9	44

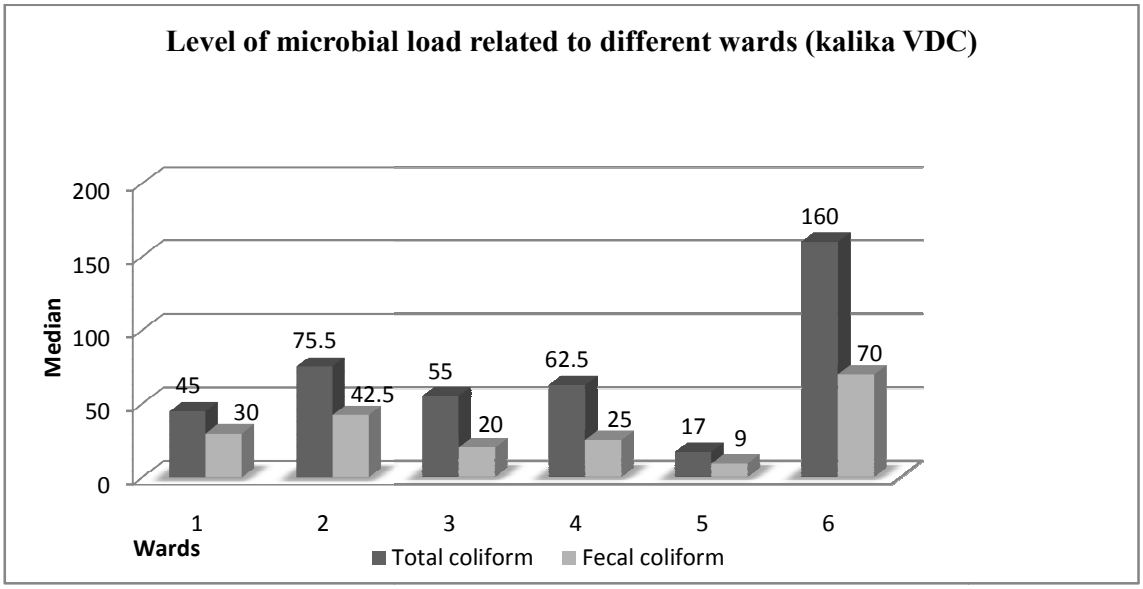

Fig. 6. Median concentration of total coliform and fecal coliform on different wards of Kalika village development committee

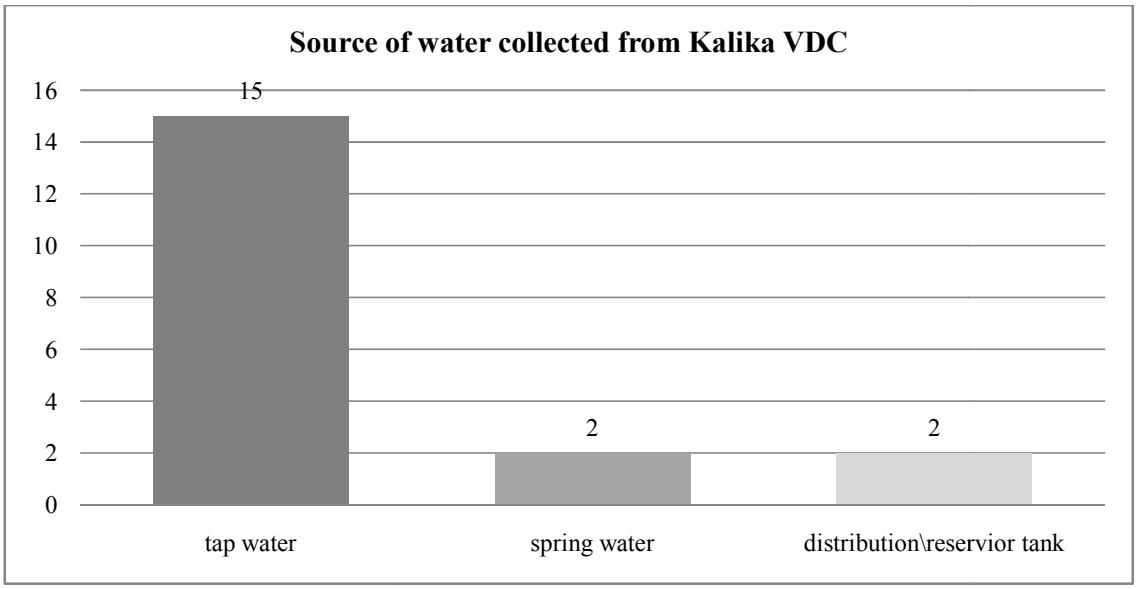

Fig. 7. Different water samples based on source collected from Kalika village development committee

Table 3. Mann-Whitney test comparing mean rank of Bharatpokhari & Kalika VDC

VDCs from where samples were collected		N	Mean rank	P value
Total coliform count	Bharatpokhari	25	29.18	0.00
	Kalika	19	13.71	
	Total	44		
Fecal coliform count	Bharatpokhari	25	29.80	0.00
	Kalika	19	12.89	
	Total	44		

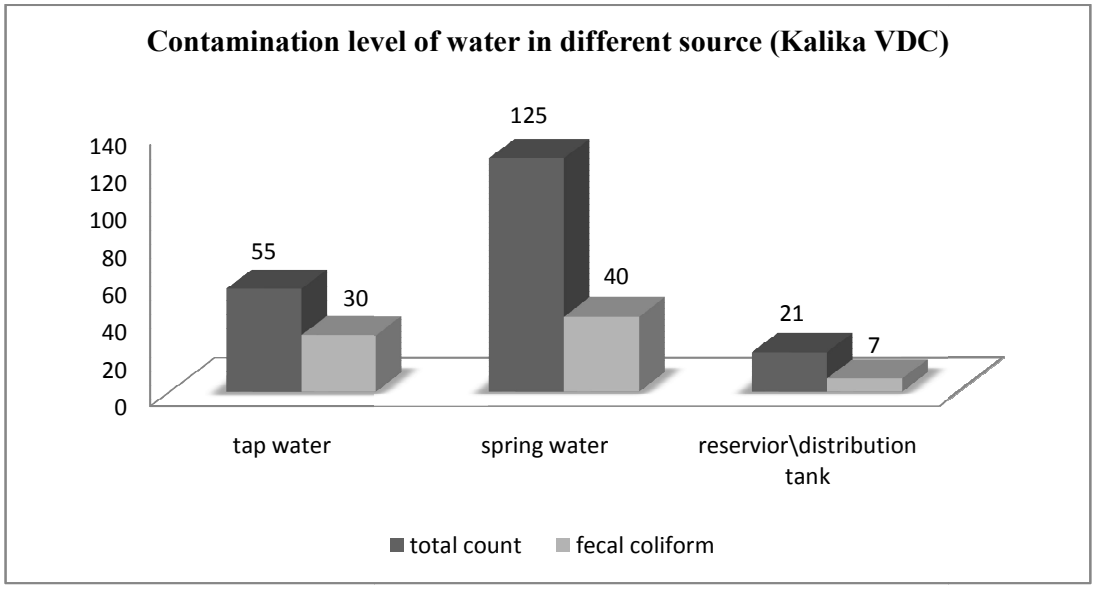

Fig. 8. Median concentration of total coliform and fecal coliform in different water sources of Kalika village development committee

Table 4. Kruskal Wallis test to compare TC & FC of various water sources under study

Indicator organism	source	N	Mean rank	P value
TC	Tap	31	20.48	
	Spring	9	31.39	
	reservoir	4	18.13	0.063
FC	Tap	31	22.15	
	Spring	9	25.44	
	Reservoir	4	18.63	0.65

4. CONCLUSION

The factors responsible for contaminating drinking water sources include the lack of protection and proper treatment of water, leakage in pipe distribution system, intermittent supply of water, poor drainage system and poor environment surroundings of water source. Chlorine disinfection treatment was not done in both Bharatpokhari and Kalika VDC. The water bodies used for drinking purpose in Bharatpokhari and Kalika VDC are heavily contaminated with total and fecal coliform bacteria even though those VDCs have been declared ODF. The water bodies used for drinking purpose in Bharatpokhari VDC was found to be more contaminated than Kalika VDC. The distribution of total coliforms and fecal coliforms are same across categories of source of sample. *E. coli* was isolated from every water sample under study. The drinking water of area under study should be boiled before drinking it. Government should fulfill its basic complacence of providing safe drinking water to community. The regular chlorine disinfection treatment of drinking water must be ensured in order to make drinking water sources potable for drinking.

5. LIMITATIONS

Only two ODF VDCs were taken under study. Sample size was small. Sampling period was short because of time limit. More fruitful result would have been obtained if sampling was done throughout the year including all the seasons.

6. RECOMMENDATIONS

Keeping in view the quality of drinking water of area under study following recommendations have been made:

- The drinking water of area under study should be boiled before drinking it.

- Government should fulfill its basic complacence of providing safe drinking water to community.
- The regular chlorine disinfection treatment of drinking water should be ensured.
- The quality of drinking water should be checked in light of drinking water guideline established by WHO and Nepal.
- The source of drinking water should be protected from un-necessary human and animal access.
- The general cleanliness and hygiene of water main storage reservoirs should be maintained.
- Sewage water should be treated and disinfected before disposing it.

CONSENT

The research is carried out accommodating all principles of Helsinki declaration and ethical clearance was given by the research board of the School of Health and Allied Sciences.

ACKNOWLEDGEMENTS

We wish to acknowledge to all writers, editors and publishers of reviewed literature, we express our gratefulness to Associate Professor Dr. NirmalaJamarkattel, Director, School of Health and Allied Sciences, Pokhara University and Niraj Chaudhary, program coordinator, Department of medical lab technology for their consistent support to conduct this research project. I appreciate the help from faculty and staff of Department of Medical Lab Technology, School of Health and Allied Sciences, Pokhara University and I also grateful to all the participating those who are involve directly and indirectly in this research work.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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