Microbiological and Physico-Chemical Assessment of Drinking Water in Bharatpur Metropolis

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ABSTRACT

Objectives: To understand microbiological and physico-chemical quality of drinking water in Bharatpur metropolis.

Methods: In a cross-sectional study, a total of 500 water samples (200 Municipal-supplied tap water, 200 hand-pump water and 100 open-well water) were collected from Bharatpur and processed for the investigation of physico-chemical and microbiological parameters. Physico-chemical parameters were analyzed by using an ENPHO water kit. The pH, arsenic and free residual chlorine (FRC) values of all the samples were within Nepal Drinking Water Quality Standard (NDWQS) 2062, whereas the concentrations of total hardness, ammonia, iron and nitrite didn't meet the recommended limit in some of the samples. For bacteriological testing, membrane filtration (MF) technique was employed.

Results: Out of the total 500 samples, 42.8% (214/500) were coliform positive. Open well water (57%) showed the highest prevalence of coliforms followed by hand-pump water (43.0%) and municipal tap water (35.5%). The occurrence of coliform in water was found to be associated with the proximity of latrines/cowsheds from the source of water ($p<0.05$). On antibiotic sensitivity testing (AST) of the coliforms, Gentamicin was the most effective antibiotic whilst Ampicillin was the least effective. A higher incidence of multi-drug resistant (MDR) isolates was observed with *Citrobacter* spp. 46.5% (60/129) followed by *E. coli* 11.4 % (7/61).

Conclusion: None of the *Klebsiella* spp. isolates were MDR. Regular monitoring of water quality parameters should be done by the concerned authorities and stakeholders of the Metropolis to ensure the quality of drinking water to the city-dwellers.

Keywords: Drinking water, Bacteriological, Coliforms, Physicochemical, Nepal

INTRODUCTION

Drinking water is indispensable for human existence. It is the essential survival component of all life forms (Sehar et al. 2011). Water plays a significant role in maintaining the health and welfare of humans. Clean and safe drinking water is considered a fundamental right of human beings and an absolute need for health and productive life.

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Safe drinking water, as defined by the WHO Guidelines does not represent any significant risk to health over a lifetime of consumption, including different sensitivities that may occur between life stages. Safe drinking water is required for all usual domestic purposes, including drinking, food preparation and personal hygiene (WHO 2006). Deterioration in drinking-water quality may have severe impacts on food processing facilities and potentially upon public health (WHO 2008). The quality of the water supply is important in determining the health of individuals and whole communities. The problem is profound in developing countries like Nepal where the water treatment system does not exist in most of the places or is inadequate if available though (Aryal, Gautam & Sapkota 2012). In Nepal, various natural water sources such as wells, stone spouts and ponds are neither treated nor protected properly. Thus, the quality of water has been deteriorating due to poor management and no proper monitoring of water quality (Diwakar, Yami & Prasai 2008).

The greatest risk from microbes in water is associated with the consumption of drinking water that is contaminated with human and animal excreta, although other sources and routes of exposure may also be significant (WHO 2006). Over the past decades, there has been a marked increase in the consumption of water derived from different sources in place of tap water for drinking purposes in many regions of the world. In developing countries like Nepal, the majority of the people uses river, hand pump, well and more recently municipal water as the main sources of water for drinking and domestic purposes. A tube well (also called a borehole) consists of a polyvinyl chloride pipe, typically 37 mm in diameter, attached to a polyvinyl chloride screen placed in fine to coarse sands generally between 20 m and 300 m below the ground and simple suction hand pumps are typically used to lift the water(Luby et al. 2008). Underground water is clean but it depends upon the quality and quantity of materials dispersed and dissolved in it (Tambekar & Neware 2012). Municipal water is an important water resource and treatment facility in ensuring the supply of safe drinking water. However, continuous monitoring should be done in the distribution system to protect the quality of safe drinking water (Zuthi, Biswas & Bahar 2009).

The Government of Nepal has gazetted the National Drinking Water Quality Standards (NDWQS) in 2062 B.S. as an effort to take the first step towards assuring drinking water quality (NDWQS 2005). The NDWQS requires municipalities to meet the national standards within five years after gazetting. Rural communities have been given a further five years before they have to meet the standards (Aryal, Gautam & Sapkota 2010). According to World Health Organization (WHO), 80 % of the diseases in developing countries are either water or sanitation-related (Pant, Poudyal & Bhattacharya 2016). As of 2016/2017 report by the Department of Health Service (DoHS) showed 23,742 cases of water borne diseases among inpatients in Nepal and out of which 270 cases resulted in death. The leading water borne disease was Typhoid fever causing 115 fatalities (Department of Health Services (DoHs) 2017). The quality of drinking water has been deteriorated because of contaminants such as bacteria, viruses, heavy metals, nitrates and salts present in the water supply as a result of inadequate treatment of drinking water or cross contamination after treatment (Nkansah, Boadi & Badu 2010).

Coliform bacteria have been used to evaluate the microbiological quality of water since coliforms have been recognized as a suitable microbial indicator of drinking water quality, largely because they are easy to detect and enumerate in water (Tallon et al. 2005). The term coliform organisms refer to Gram negative, rod-shaped bacteria capable of growth in presence of bile salts or other surface active agents with similar growth inhibiting properties and able to ferment lactose within 24 h at 35-37ºC with the production of acid, gas. They are also catalase positive, oxidase negative and non-spore forming and display β-galactosidase activity. Coliform bacteria belong to genera *Escherichia, Citrobacter, Enterobacter* and *Klebsiella* spp. (Cabral 2010). In recent times, drug resistance among bacterial isolates is increasing day by day. The emergence of antibiotic resistance by bacteria and particularly, multiple antibiotic resistance is growing public health to mankind in recent years (Chaudhary et al. 2011). Besides microbial contaminants, contamination of water resources with heavy metals, nitrates, nitrites have received particular concern as they arenot easily biodegradable thus acts as toxic material even at a lower concentration. The presence of these toxic materials in drinking water and long exposure to such metals, ammonia, nitrates may have adverse health impacts (Yasin, Ketema & Bacha 2015).

Bharatpur is one of the fastest and rapidly growing city of the country and the people are still unaware of the consequences of unsafe water. Although, metropolitan supplies some level of treated water to most of the towns, some residents of the metropolis are out of reach from this water and they hinge on handpump water and also on open well as well. Also, the safety aspects of drinking water supplied by the Department of Drinking Water in Bharatpur havenot been assessed by any institution or researcher as of now. Considering these facts, this study was done to access the quality standards of different sources of water used by residents of Bharatpur Metropolitan City.

MATERIALS AND METHODS

Study design and setting

A cross-sectional study was conducted from January 1, 2019 to December 15, 2020 in order to investigate the physico-chemical and microbiological parameters of drinking water in Bharatpur Metropolis. Bharatpur is a rapidly growing fourth largest city in the country situated at the southern part in Bagmati province. The three most common sources of drinking water (Municipal supplied tap water, hand-pump water and open-well water) used by the residents of the Metropolis were included in the study. Altogether, a total of 500 samples comprising of 200 municipal tap water, 200 hand-pump water and 100 open-well water samples were collected.

Sample collection and transportation

Municipal (Tap) water, hand-pump and open well water samples were collected separately in sterile polyethylene bottles of 250 mL capacity. First of all, the mouth of tap and hand-pump was disinfected and water was passed for 2 min, then only the sample was collected in the bottle (Luby et al. 2008). In case of open well, cap of the collection bottle was opened inside of the water. For every sample, the temperature was noted at the site of sample collection with the help of a portable thermometer. The water samples were transported carefully to the Microbiology Laboratory of Birendra Multiple Campus for further analyses. The water samples were coded as “M” for municipal water, “H” for hand-pump water and “W” for open well water.

Sample analysis

Physico-chemical analysis

The samples were analyzed for physicochemical parameters and compared with NDWQS (NDWQS 2005). The temperature was measured at the site of sample collection. Other physicochemical parameters such as pH, arsenic, free residual chlorine, ammonia, iron, nitrate, phosphate, chloride, nitrite and hardness were analyzed in the laboratory using the ENPHO water test kit (ENPHO, Nepal). The procedures to analyze physicochemical parameters were followed as in the operational manual of the ENPHO water test kit (ENPHO 2001).

Microbiological analysis

Microbiological quality of drinking water was determined using the standard membrane filtration (MF) techniques for coliform counts. In this method, 100 mL of water sample was measured using a sterile measuring cylinder and filtration was performed using a sterile membrane filter of pore size 0.45 μm. The membrane filters were placed with grid side up in EMB agar and incubated at 37 ºC and 44.5 ºC for 24 hrs for total coliform and fecal coliform respectively. Suspected colonies were sub-cultured on MacConkey agar. Lactose fermenting pink colored colony was sub-cultured on Nutrient agar and further Gram staining and biochemical tests (Catalase, Oxidase, Indole, Citrate utilization, Methyl red and Voges-Proskauer tests, TSI test, SIM test) were performed (Dantebo, Furgasa & Beyene 2019). For preliminary identification of *E. coli* O157:H7, *E. coli* isolates were streaked on the MacConkey Sorbitol agar and incubated at 37 ºC for 24 hrs. Colorless colonies on sorbitol agar were confirmed as *E. coli* O157:H7 (Zareen, Sajid & Ali 2014; Sapkota et al. 2019).

Antibiotic susceptibility test

The antibiotic susceptibility pattern of the isolates was determined by following the Modified Kirby-Bauer disc diffusion method (CLSI 2016). Briefly, pure culture of the test organisms was transferred into sterile normal saline and the suspension was standardized by adjusting its turbidity equivalent to density of 0.5 McFarland solution.

Each suspension was streaked uniformly over the surface of Mueller Hinton Agar (MHA) using sterile cotton swab. Using sterile forceps, antimicrobial discs gentamicin (10 µg), ciprofloxacin (5 µg), ampicillin (10 µg), trimethoprim (5 µg), cotrimoxazole (25 µg), cefoxitin (30 µg) and ceftriaxone (30 µg) were placed widely spaced on the surface of MHA plate. The plates were incubated at 37ºC for 24 hrs.

Following incubation, the plates were examined for the zone of inhibition. The inhibition zone diameter was measured in mm and the organism was considered as susceptible or intermediate or resistant by comparing zone of inhibition with standard chart 21. Besides, isolates were observed for multi-drug resistance (MDR). Non-susceptibility to at least one agent in three or more antimicrobial categories was considered as MDR (Magiorakos et al. 2012).

Quality control

In the current study, the quality and accuracy of all the tests were maintained by following standard procedures of collection, isolation and identification. For quality control, media and reagents were prepared, stored and utilized as recommended by the manufacturing company. For each batch of a test, a positive and negative known culture was used for color reaction and biochemical test. In order to monitor the quality of antibiotic sensitivity test, a standard strain of *S. aureus* (ATCC-25923) and *E. coli* (ATCC-25922) were used as reference strains.

Data management and analysis

All raw data obtained from laboratory investigation were tabulated and presented in defined tables using the R-programming statistical analysis tool was used to evaluate all the obtained data (version 1.2.5033, https://cran.r-project.org/). The results were presented through tables. Chi-square test was performed to determine the associations between selective variables and p<0.05 was considered to have a significant association.

RESULTS

Source-wise distribution of coliforms

A total of 500 water samples (200 from hand-pump, 200 from municipal tap water and 100 from open well) were collected and analyzed, of which 214 (42.80%) samples showed the presence of coliforms. Out of 500 total samples, 43% (86/200) hand-pump water, 35.5% (71/200) municipal tap water and 57% (57/100) open well water showed the presence of coliforms (Figure 1)

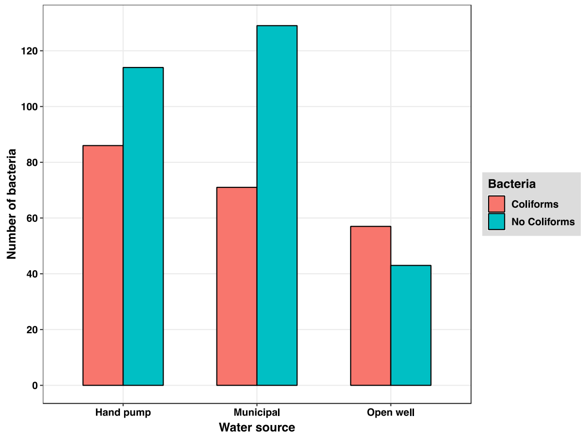

Figure 1: Source-wise distribution of coliforms

Distribution of coliforms based on proximity of water source from latrines/cowsheds

The distance of water source from the latrine/cowsheds at each household was also noted during sample collection. Hundred and fourteen samples collected were from the water sources which were very near to latrinse/cow sheds. Of those 114 samples, 82 (71.93%) samples showed the growth of coliforms. Remaining 143 and 243 samples lied at near and far distance with latrines/cow sheds respectively. Of the 143 and 243 samples, 61 (42.66%) and 71 (29.22%) samples were found to be contaminated with coliforms. A strong association was seen between contamination of water and distance of water sources from latrines/cow sheds sources (p<0.05) (Table 1).

Distribution of coliforms in water

Of the 263 coliform isolates, *Citrobacter freundii* 77 (29.28%) was the most predominant. *Klebsiella oxytoca* 32 (12.17%) was the least isolated coliform. Out of 263 isolates, only 56 (21.29%) isolates were fecal (thermo-tolerant) coliforms, of which *E. coli* accounted for 21 (37.50%) isolates. The preliminary test showed that 4.92% (3/61) of the *E. coli* isolates were *E. coli* O157:H7 (Figure 2).

Antibiotic susceptibility pattern of coliforms

All the colform isolates were subjected to antibiotic susceptibility testing. Majority of the coliform isolates were sensitive towards gentamicin and ciprofloxacin. All *Klebsiella* spp. were sensitive towards gentamicin, cotrimoxazole and cefoxitin, whereas resistant to ampicillin. Other coliform isolates also showed a high rate of resistance towards ampicillin (98.4% and 88.5%) (Table 2). Among 263 bacterial isolates, 67 (25.5%) were multidrug-resistant. Higher MDR isolates were observed with *Citrobacter* spp. 46.5% (60/129) followed by *E. coli* 11.4 % (7/61), while none of the *Klebsiella* spp. isolates was MDR (Table 3).

Physico-chemical analysis of water

Various physico-chemical parameters of the water were analyzed by using the ENPHO water kit and the values were compared with National Drinking Water Quality Standard (NDWQS) 2062. Temperature of water sample varied from 22-32˚C. The highest temperature was noted from the hand-pump water and the lowest was recorded from open-well water. The pH of the water varied from 6.5-8.5 all lying within NDWQS guideline 2062. Arsenic and free residual chlorine (FRC) of all the samples lied within the recommended limits whereas other parameters such as chloride, ammonia, iron, nitrite and total hardness weren't within the prescribed limits in some of the samples. Most notably, around 20% of samples didn't meet the criteria for nitrite. Details of physico-chemical parameters are presented in (Table 4).

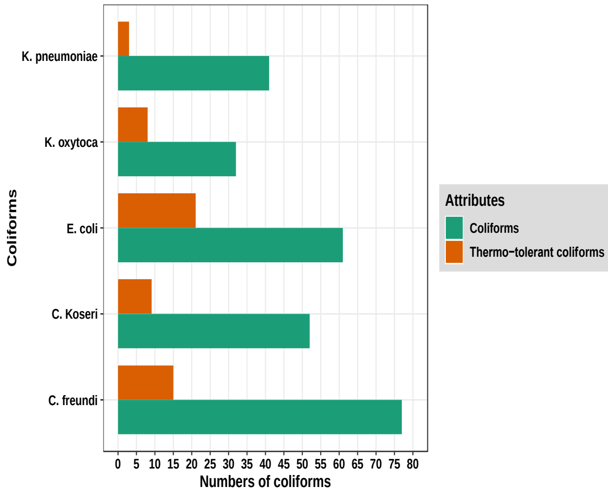

Figure 2: Distribution of coliforms and thermo-tolerant coliform

Table 1: Distribution of coliforms with respect to distance of water sources from latrines/cow sheds

Variables	Parameters	Coliforms		Total	p-value
		Present n (%)	Absent n (%)		
Distance from latrine	Far	71 (29.22)	172 (70.78)	243	< 0.05*
	Near	61 (42.66)	82 (57.34)	143	
	Very near	82 (71.93)	32 (28.07)	114	
	Total	**214**	**286**	**500**	

*significant at 5% level of significance. Very near : <10 m; Near : 10 m - 30 m; Far : >30 m (WHO 2009)

Table 2: Antibiotic resistance pattern of coliform isolates

SN	Antibiotics	Susceptibility pattern								
		Citrobacter spp. (n=129)			*Klebsiella* spp. (n=73)			*E. coli* (n=61)		
		S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
1	Gentamicin	95.35	3.10	1.55	100	0.00	0.00	77.05	0.00	22.95
2	Ciprofloxacin	84.50	6.20	9.30	79.45	20.55	0.00	67.21	11.48	21.31
3	Ampicillin	1.55	0.00	98.45	0.00	0.00	100	11.48	0.00	88.52
4	Trimethoprim	64.34	4.65	31.01	79.45	0.00	20.55	77.05	0.00	22.95
5	Cotrimoxazole	70.54	6.20	23.26	100	0.00	0.00	88.52	0.00	11.48
6	Cefoxitin	51.93	4.65	43.41	100	0.00	0.00	67.21	0.00	32.79
7	Ceftriazone	72.09	9.30	18.60	79.45	20.55	0.00	67.21	11.48	21.31

S= sensitive; I=intermediate; R=resistant

Table 3: Distribution of MDR and Non-MDR isolates

SN	Isolated coliforms	MDR n (%)	Non-MDR n (%)
1	*Citrobacter* spp. (n=129)	60 (46.51)	69 (53.49)
2	*E. coli* (n=61)	7 (11.48)	54 (88.52)
3	*Klebsiella* spp. (n=73)	0 (00)	73 (100)

Table 4: Source-wise distribution of physico-chemical parameters

Chemical parameters	Range	Sample Type			% within NDWQS
		Hand-pump	Municipal	Open well	
pH (6.5-8.5)	<6.5	0	0	0	100%
	6.5-8.5	200	200	100	
	>8.5	0	0	0	
Arsenic	≤0.05	200	200	100	100%
	>0.05	0	0	0	
FRC	<0.1	200	200	100	100%
	0.1-0.2	0	0	0	
	>0.2	0	0	0	
Ammonia	≤1.5	183	200	71	90.80%
	>1.5	17	0	29	
Iron	≤3	196	200	86	96.40%
	>3	4	0	14	
Nitrate	≤50	159	191	53	80.60%
	>50	41	9	47	
Phosphate	Absence	26	17	2	-
	0.05	169	181	41	
	0.2	5	2	57	
Chloride	≤250	200	200	89	97.80%
	>250	0	0	11	
Nitrite	≤3	0	0	2	-
	>3	200	200	98	
Total Hardness	≤500	197	200	86	96.60%
	>500	3	0	14	

DISCUSSION

Deterioration in water quality is reflected by its physical, biological and chemical conditions. Water pollution is one of the serious public health issues in developing countries. In recent years, water-borne diseases have gained due attention as the most emerging infectious diseases in developing countries like Nepal. Water contamination occurs due to human activities, including agriculture, recreational and industrial activities and disposal of human wastes (Aryal et al. 2012). Groundwater in Nepal is at the risk of contamination mostly from pathogenic bacteria, pesticides, nitrate and industrial effluents (Basnet & Bhattarai 2013).

The current study was undertaken with the major objective of assessing the quality of drinking water in Bharatpur Metropolis-one of the most rapidly growing cities of the country. Altogether, 500 water samples (200 each from hand-pump and municipal tap water and 100 from open well sources) were analyzed for the presence of coliforms and important physiochemical parameters. According to the guideline set by NDWQS-2062, pH of drinking water should be within 6.5-8.5 and all the water samples we investigated had a pH within the prescribed limits. pH is an important water quality parameter and a large variety of pollutants such as point and a non-point natural source of water pollutants from industry, agricultural and domestic practices affect the pH of receiving water. The water having pH <6.5 may cause corrosion of metal pipes thereby releasing toxic metals and >8.0 adversely affect the disinfection process (Aryal et al. 2012).

All of the collected water samples were within the NDWQS guideline-2062 value for arsenic concentration. Many studies have reported arsenic as a prime factor for the development of lung, kidney and bladder cancer, and since it is naturally present deep into the earth, it may contaminate the water sources (Aryal et al. 2012; Shrestha et al. 2017). It may also be present in the water due to the dissolution of naturally occurring minerals, ores, and industrial effluents (Jayana et al. 2009; Aryal et al. 2010; Aryal et al. 2012). In our study, ammonia concentration was below 1.5mg/L in all of the hand-pump water and municipal tap water, but 29 samples from open well water exceeded this value. Most of the studies done in Nepal have reported that the ammonia concentration of drinking water lied within NDWQS values, but these studies didn't consider the open well water (Aryal et al. 2012; Kumar Mishra & Acharya 2019). The reason behind the higher rate of ammonia concentration in open well water may be due to the openness of sources contributing to more chances of pollution (Aryal et al. 2012; Maharjan, Joshi & Shrestha 2018). A study done by Warner reported that 45% of water sources exceeded ammonia as per NDWQS value (Warner et al. 2007). The term free residual chlorine (FRC) is given to each one of the (HClO), (OCl^-), Cl^- (g) compounds. The present study reported the FRC concentration of all the samples was below 0.1 mg/L. A study carried out in Isfahan, Iran reported FRC concentration between 0.01mg/L to 0.24 mg/L in bottled drinking water (Farhadkhani et al. 2014). Chlorine in treated water is dangerous to human health and can cause allergic symptoms ranging from a skin rash to intestinal symptoms to arthritis. It destroys the human flora which protects against pathogens (Sheikhi et al. 2014).

A study performed by Diwakar found 48.28% of samples exceeded the NDWQS value for iron and nitrate concentrations. In contrast, our study showed that only 3.60% and 19.40% samples exceeded these values respectively (Diwakar et al. 2008). This difference may be due to the higher pollution in that area as sufficient amounts of phosphorus (due to the usage of fertilizers, untreated waste) results in high concentrations of nitrates that lead to phytoplankton (algae) and macrophyte (aquatic plant) production (Vaidya & Labh 2017). Also, an increase in nitrates is of health significance to pregnant women and infants under six months. It leads to disease in infants known as Blue baby syndrome (Jayana et al. 2009). Our study reported the absence of phosphate in only 9.00% samples and 78.20% samples were measured with 0.05mg/L phosphate. All the samples had nitrites concentration above >3mg/L except for 2 samples from open well. In a previous study, Warner found a similar rate of nitrite concentration where no sample crossed permissible limits (Warner et al. 2007). In case of hardness concentration, we found that 96.60% of samples were within the NDWQS limits. This result is similar to the study done by Diwakar in Bhaktapur, where the hardness content of all water samples tested was within the standard value (Diwakar et al. 2008).

Microbiological analysis revealed that 214 (42.80%) water samples harbored at least one type of coliform bacteria. This contamination rate seen in our study in Bharaptur is lower than a previous study done in Kathmandu which reported 63.00% samples has exceeded coliform count (Maharjan et al. 2018). Another study in the country done by Prasai reported 92.40% of drinking water was contaminated by coliforms (Prasai et al. 2007). A study done in different water sources of Dhaka city exhibited coliforms in almost all samples (Islam et al. 2010). In line with this study, various previous studies also indicate a similar prevalence of coliform in drinking water. A study done in Mexico reported coliform in only 46.00% of samples, whereas a study done in Bangladesh showed coliform in only 41.00% of water samples (Chaidez et al. 2008; Luby et al. 2008). Among the 263 coliform isolates, *C. freundii* was the dominant isolate as it was recovered in 29.27% water samples. The prevelence of *Citrobacter* spp. seen in this study was higher than the study done in Madhyapur Thimi, which reported the presence of 20.4% *Citrobacter* (Jayana et al. 2009).

Prevalence of other coliforms in the current study was *E. coli* (23.19%), *K. pneumoniae (*15.59%), *K. oxytoca (*12.16%) and *C. koseri* (19.77%). Several studies done by Nepalese researchers in the country are in commensuarate with this study as the rate of encountering *E. coli* and *Citrobacter* spp. is higher than the rate of other coliforms such as *Klebsiella* spp. (Prasai et al. 2007; Jayana et al. 2009; Islam et al. 2010; Pant et al. 2016). For instance, a study done in water samples of Kathmandu city reported 26.4% *E. coli* and 22.60% *Citrobacter* spp., while only 5.4% were *Klebsiella* spp. isolates (Prasai et al. 2007). Among the 263 isolates recovered in our study, 56 (21.29%) were thermo-tolerant coliforms, indicating that these microbes may have arisen due to fecal contamination. Similar result was observed in a work carried out by Ramteke where 30.32% isolates were thermotolerants (Ramteke & Tewari 2007). But a similar study from Kirtipur has higher rate of thermotolerant coliforms where they reported more than half the total (51.56%) isolates as thermotolerant (Subba, Joshi & Bhatta 2013). Of all *E. coli*, 4.92% were *E. coli* O157:H7. It was in tune with the other studies where significantly lower number of *E. coli* O157:H7 was reported (Johnson et al. 2003; Momba, Abong'o & Mwambakana 2008). Water sources such as wells and hand-pump water were found more contaminated with coliforms probably due to infiltration of various kinds of pollutants, biological wastes from hospitals, toxic wastes from chemical industries form plumes of pollutants that travel in the groundwater and contaminates the underground environment (Jayana et al. 2009). Moreover, domestic wastewater, industrial waste, increase in the use of agrochemicals and hazardous waste disposal sites add groundwater contamination. The reason behind the contamination of municipal water may be due to the cross-connection of the other waste pipeline below the surface and any leakage will result in microbial contamination (Onyango et al. 2018).

Among 500 water samples collected for laboratory investigation, 200 samples were from municipal tap water and hand pump water each and 100 samples were collected from open well sources. More than half, i.e., 57 (57%) of the open well water sources were found to be contaminated with coliforms, whilst only 71 (35.5%) and 86 (43%) samples from municipal tap water and hand-pump were contaminated with coliforms. The reason behind high the rate of contamination of open well sources may due to the fact that they are not fully covered and there is high chances of contamination. Most of the people who use open-well water also had their cow-shed near to their wells. Municipal water is distributed after disnfection process, yet there may be chances of leakages of the water pipes leading to their contamination. Hand-pump water is pipetted out deep from the earth's surface, yet there can be slim chances various substances leaching into the water source (Parvez, Liza & Marzan 2016). People residing far from city are reliant on hand-pumps and open well. These people are also engaged in cow/buffalo farming which lies near to the water sources and hence there is more chances of contamination of water sources. The highest percentage of coliforms (71.93%) were recovered from the water samples collected from the water sources lying very near to the latrines/cow sheds. This may be due to the less proximity between cow sheds/latrines and water source leading to fecal contamination of water (Onyango et al. 2018).

On antibiotic susceptibility testing of the coliform isolates, gentamicin and ciprofloxacin were found the most effective antibiotics. This finding is contrary to the study done in Nigeria, which showed all the coliform isolates from sachet and borehole waters were resistant to both drugs (Okeke et al. 2015). Ampicillin was found to be the least effective against coliform isolates as more than 80% of the isolates resisted it. A total of 67 (25.5%) isolates were multidrug-resistant (MDR). We observed higher multi-drug resistance with *Citrobacter* spp. 46.5% (60/129) followed by *E. coli* 11.4 % (7/61). None of the *Klebsiella* spp. isolates were MDR. The study conducted by Chaudhary and his colleagues in drinking water reported that 62.5% *Citrobacter* isolates, 72.2% *Klebsiella* isolates and 92.3% *E. coli* isolates as MDR (Chaudhary et al. 2011). The increased rate of antibiotic resistance can be attributed to their frequent use and misuse in human medicine and aquaculture, resulting in the acquisition and accumulation of metabolites in environmental natural waters (Ateba et al. 2020). Furthermore, overuse of antibiotics in husbandry and long term exposure of bacteria to the antibiotics enriched by sewage and agricultural runoff also develops antibiotic resistant bacteria in the aquatic environment (Xi et al. 2009).

CONCLUSION

The current study showed that most of the physico-chemical parameters of drinking water in Bharatpur metropolis were within the set criteria by NDWQS guidelines 2062, whilst the microbiological parameters were unsatisfactory. More worrisome is that still a few people in the metropolis use open-well as the source of drinking water from where fecal coliforms have been recovered. In addition, recovery of MDR isolates from drinking water is another serious issue as this leads to easy dissemination of MDR strains in the environment. The concerned metropolitan authorities seriously need to work on these issues to guarantee the health of the city dwellers.

Strengths and limitations of the study

This is the first comprehensive study exploring the quality of drinking water in Bharatpur metropolis. The result of the study may be useful to the metropolitan water supply office andother concerned authorities to devise plan and policies to ensure safe drinking water to all its citizens. On the downside, the current study has not encompaased other pathogenic bacteria besides coliforms. The study also lacks molecular characterization of the isolates. Moreover, the exact sources of contamination of drinking water were not assessed. Future research should address these limitations.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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