



Research Article

Emerging Threats on Drinking Water Safety in Kathmandu: A Study of Physicochemical and Bacterial Parameters

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Abstract

Municipal tap and Jar water are the common drinking water sources for the residents of Kathmandu. This study was conducted to analyze the water quality of jar and tap water of Kathmandu valley between November 2023 and April 2024. Altogether 30 water samples; 15 tap water and 15 jar water were assessed during this study. Based on analysis, physicochemical parameters such as temperature, conductivity, TDS, chloride, alkalinity, hardness was found to be within drinking water quality guidelines of NDWQS, 2022. However, pH was out of recommended range. The bacteriological analysis revealed that only 5 tap waters were within the recommended limit whereas entire jar water exceeded recommended range. Four different enteric bacteria namely, *E. coli* (24%), *Klebsiella spp.* (29%), *Pseudomonas spp.* (33%), *Enterobacter spp.* (14%) was isolated from tap water samples. Likewise, five different enteric bacteria such as *E. coli* (38 %), *Klebsiella spp.* (22%), *Pseudomonas spp.* (16%), *Enterobacter spp.* (19%) and *Citrobacter spp.* (5%) were isolated from jar water samples. Antibiotic susceptibility test revealed that most of *E. coli*, *Pseudomonas spp.*, *Enterobacter spp.* isolates were found to be sensitive against Chloramphenicol, Norfloxacin, Gentamicin but resistant against Ampicillin. Most of *Klebsiella spp.* isolates were sensitive against, Azithromycin, but resistant against Ampicillin and cefoxitin. Similarly, isolated *Citrobacter spp.* were resistant to Ampicillin, Cefoxitin whereas sensitive against Norfloxacin. 23.8% of tap water isolates and 18.9% of jar water isolates were Multiple Drug Resistant. In conclusion, majority of the samples of drinking water were not safe for direct consumption and were found to be microbially unsafe and presence of multiple drug-resistant bacteria which may cause serious health hazards. Hence, regular monitoring of water quality, proper distribution system and treatment is necessary.

Introduction

Access to pure, abundant and safe drinking water is fundamental necessity for public health and human wellbeing. Thus, drinking water must be free of bacterial and physicochemical pollution (WHO, 2023). However, in case of Nepal, because of lack of basic water treatment facilities of supplied water, there have been a lot of reports of feces contamination in drinking water. Contaminated water, poor hygiene and unsanitary living circumstances continue to pose significant risks to public health resulting in waterborne illness such as dysentery, diarrhea, cholera and typhoid (Burlakoti et al., 2020)

Getting enough water is more important to most Nepalese than getting clean water (Maharjan et al., 2018). The valley now has less drinkable water available as a result of rapid and mostly unmanaged

urban and overpopulation. The valley now is known as by extreme water scarcity and declining water quality. The worsening water quality has a negative influence on public health (Burlakoti et al., 2020).

It is quite challenging to provide access to potable water for 1.7 million people living in Kathmandu valley. Even some have access to tap water but the municipal system is only occasionally operated. As a result, people began to use other alternative resources like as, deep wells, tube wells and stone spouts for drinking purposes. However, these sources are contaminated and unfit for consumption (Sarkar et al., 2022).

The expanding population of Kathmandu valley has resulted in a rise water usage and requirements. Kathmandu Upatyaka Khanepani Limited (KUKL) is in charge of running and managing the valley's water

and wastewater systems (Udmale et al., 2016). KUKL is the designated public entity in charge of providing drinkable water to the valley's inhabitants (Burlakoti et al., 2020). With a total demand of 470 MLD (million liters per day), the water supply in 2021 was 80 MLD during the dry season and 106 MLD during the rainy season (Shrestha et al., 2022; Thapa et al., 2018).

Because there is a shortage of drinking water and the piped water is of low-quality, jar water is one of the primary sources (Inoue et al., 2018). Jar water is widely used, as it is considered to have less contaminants (Subedi & Aryal, 2010). As, rising incidence of outbreaks of waterborne illness has been linked to problems with public water distribution system and shortage of drinkable tap water, residents are forced to use other alternative sources like bore wells and jar water. Many believe that jar water is safer for consumption and claims that taste and quality of jar water is comparatively better (Panthi Grishma & Shakya Bhushan, 2024). However, the most recent study conducted by the government's Epidemiology and Disease Control Division indicated that fecal coliform was present in over 25% of the drinking water used in Kathmandu (The Kathmandu Post). When stored, pathogenic microbes in bottled water can grow to dangerously high concentrations that could harm customers (Karki et al., 2022). According to the findings, the odds ratio of getting diarrhea were noticeably higher for consuming jar water and tanker water (Kobayashi et al., 2022). The research reveals a worrying scenario about quality of jar water being supplied. The cause of this might be attributed to either improper water treatment provided by commercial water providers or improper cleaning of the jar bottles used to fill water which is poorly explored in case of Kathmandu Valley (Subedi & Aryal, 2010).

Karki et.al examined tap water samples from 10 different sampling spots results showed that all tested physicochemical parameters were within the WHO (World Health Organization) and NDWQS (Nepal Drinking Water Quality Standards) range while ammonia, pH, iron was out of recommended range and many samples had coliform bacteria (Karki et al., 2022).

This study aims to analyze the quality of drinking water; tap water and jar water of different areas of Kathmandu valley. This research evaluated the physicochemical as well as bacteriological parameters by using standard procedures which can be useful for determining the current status of drinking water. Furthermore, the detection of antibiotic susceptibility patterns among bacterial isolates offers crucial information on emerging antimicrobial resistance, supporting public health monitoring efforts as the quality of drinking water has influential impact in human disease and infections, this research will ascertain the quality of drinking water being supplied

and help people to choose best source for drinking and domestic purposes.

Methodology

Study Area

This study was conducted to examine the quality of drinking water of Kathmandu district from November 2023 to April 2024. The convenient sampling method was applied for sample site selection and collection of water samples. A Total of 30 water samples were collected; 15 tap waters were collected from different places of Kathmandu and 15 jar waters were collected of different brands which were available in Kathmandu.

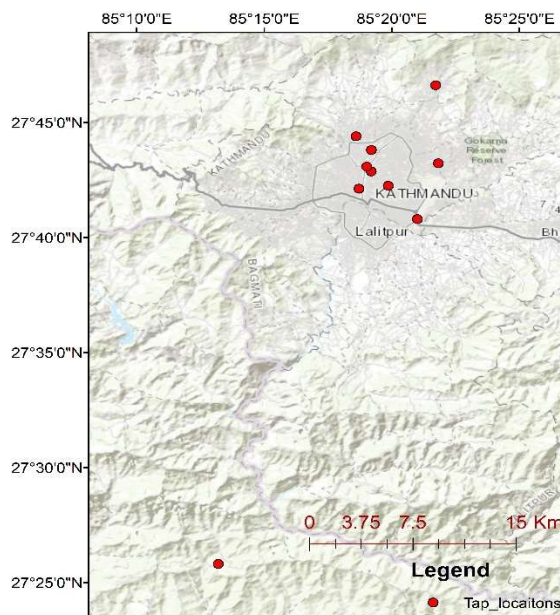


Figure 1: Map of the study area with marked sampling sites for tap water.

Physicochemical and bacteriological analysis of water samples

The assessment of physicochemical and bacteriological parameters of water was done in microbiology laboratory of Padmakanya Multiple Campus, Bagbazar, Kathmandu. Standard methods of APHA (2017) were followed to evaluate the quality of collected drinking water samples. Temperature and pH were measured immediately after the collection of samples. Other parameters were examined on the same day and always within the 6 hours of collection, if immediate examination was not feasible, samples were stored at 4°C. The physicochemical parameters like temperature, pH, conductivity and TDS (Total Dissolved Solids) were analyzed with the help of mercury thermometer, pH meter (HANNA instruments, Romania), conductivity meter (HANNA instruments, Romania) and TDS meter (HM Digital, India) respectively. The hardness, chloride and alkalinity were examined through EDTA (Ethylene Diamine Tetra acetic Acid) titration, argentometric method and acid-base titration respectively.

Bacteriological assessment of collected water samples was carried out by enumerating total coliforms using membrane filtration technique. After that, for the isolation of *E. coli*, pink colored bacteria with metallic sheen from filter paper were cultured in EMB (Eosin Methylene Blue) agar followed by subcultured in nutrient agar. Likewise, for isolation of *Klebsiella* spp., *Enterobacter* spp., *Citrobacter* spp., *Pseudomonas* spp., the pink colored bacteria from filter paper were subcultured in MacConkey agar and again subcultured in nutrient agar. Then, analyzing colony features, Gram's staining, and biochemical properties, white colonies from nutrient agar were identified.

Antibiotic susceptibility testing

The Kirby-Bauer disc diffusion technique using MHA (Muller Hilton Agar) was performed using different antibiotic discs to evaluate the antibiotic susceptibility of isolates. Using a sterile swab isolated colony was inoculated into Nutrient Broth tube and incubated at 37°C for 4 hours. After incubation turbidity of the suspension was matched with the turbidity standards of Mac- Farland No. 0.5. With the help of sterile swab, lawn culture of bacterial suspension was performed on sterile MHA plate. Then the plate was left for 5 minutes to inoculums dry. Appropriate antibiotic discs were placed on the inoculated plates using sterile forceps. Then the plate was incubated for 37°C for 24 hours. The zone of inhibition was measured and compared with an interpretive chart for standard zone sizes that the EUCAST (European Committee on Antimicrobial Susceptibility Testing) and Clinical and Laboratory Standards Institute (CLSI, 2007) supplied. After comparison with zone size interpretative chart isolated were selected as sensitive, intermediate or resistant for each antibiotic.

The antibiotics discs used for *E. coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp.,

Pseudomonas spp. was Ampicillin (10mcg), Chloramphenicol (30mcg), Gentamicin (30mcg),

Nitrofurantoin (300mcg), Norfloxacin (10mcg), Azithromycin (15mcg), Amikacin (30mcg), Cefoxitin (30mcg) and Ceftriaxone (30mcg).

Data analysis

All data were compiled and entered in MS-excel and analysis was done SPSS.19 (Statistical Package for the Social Sciences). For several physicochemical characteristics, descriptive statistics such as mean and standard deviation were computed. The two water sources (tap water and jar water) were compared using independent sample t-tests to see whether there were any statistically significant differences. The p-value was deemed statistically significant if it was less than 0.05. Accordingly, the results were evaluated and shown in tabular and text form.

Results

Analysis of physicochemical parameters of water samples

The temperature of tap water samples ranged from 10-19°C and jar water samples ranged from 11-22°C. The pH value of tap water samples ranged from 4.9 -8.8. Out of 15 tap water samples, only four water samples were within the (NDWQS, 2022) guidelines. One water sample exceeded the pH range whereas other remaining 10 water samples were below the recommended range. In case of jar water samples, the pH value ranged from 5.1-8.0. Out of 15 jar water samples, except four water samples, all other water samples were within the recommended range.

The conductivity range of tap water samples was 25.2-830 $\mu\text{S}/\text{cm}$ while in case of jar water the value ranges from 20-335 $\mu\text{S}/\text{cm}$. The hardness value of tap water ranged from 14-278 mg/l whereas jar water samples ranged from 4-62 mg/l. The alkalinity value ranged from 26-294 mg/l in case of tap water whereas the value ranged from 2-100 mg/l in jar water samples. The chloride values of tap water samples ranged from 2.2-113.6 mg/l and jar water ranged from 7.1-31.24 mg/l. Similarly, the TDS range of tap water samples ranged from 20-323 mg/l and jar water samples ranged from 4-220 mg/l. The physicochemical parameters such as conductivity, hardness, alkalinity, chloride and TDS of both tap and jar water samples were within the recommended range of NDWQS guidelines.

Table 1: Physicochemical analysis of water samples

Physicochemical parameters	NDWQS guidelines	Tap water			Jar water		
		Min	Max	Standard deviation	Min	Max	Standard deviation
Temperature (°C)	-	10	19	2.463	11	22	3.629
pH	6.5-8.5	4.9	8.8	0.924	5.1	8.0	0.756
Conductivity ($\mu\text{S}/\text{cm}$)	1500	25.2	725	241.383	20	335	83.633
Hardness (mg/l)	500	14	278	80.903	4	62	16.137
Alkalinity (mg/l)	500	26	294	91.093	2	100	29.164
Chloride (mg/l)	250	2.2	113.6	31.965	7.1	31.24	5.835
TDS (mg/l)	1000	20	323	104.893	4	220	129.646

Table 2: T-test comparing Physicochemical Parameters of tap water and jar water.

Physicochemical parameters	Mean		t-value	p-value
	Tap water	Jar water		
Temperature	14.933	18.2	-3.8532	0.001756
pH	6.404	6.682	-0.87413	0.396794
Conductivity	221.933	79.996	2.0393	0.06076
Hardness	87.866	21.133	3.287698	0.005393
Alkalinity	107.466	30.533	2.878132	0.012156
Chloride	29.014	15.336	1.663625	0.118402
TDS	102.93	62.733	1.760019	0.100226

(P < 0.05, df= 14). The values in bold represent significantly different between Jar water and Tap water.

Bacteriological analysis of water sample

Total coliform count

In this study out of 30 water sample (15 tap water samples and 15 jar water samples) only five tap water samples were within the recommended range of NDWQS (Fig 1).

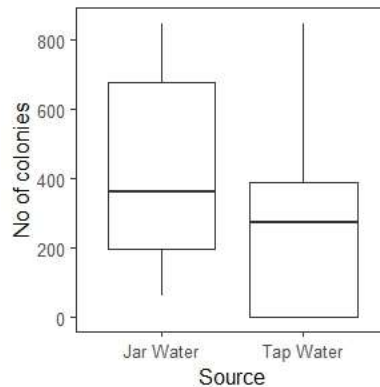


Figure 1: Distribution of total coliform count in tap and jar water samples.

Types of enteric bacterial isolates

Four different types of bacteria were isolated from tap water whereas five different bacteria were isolated from jar water. *Pseudomonas spp* was most the common organism in tap water samples (Fig. 2). *E. coli* was most common organism isolated from jar water samples (Fig. 3).

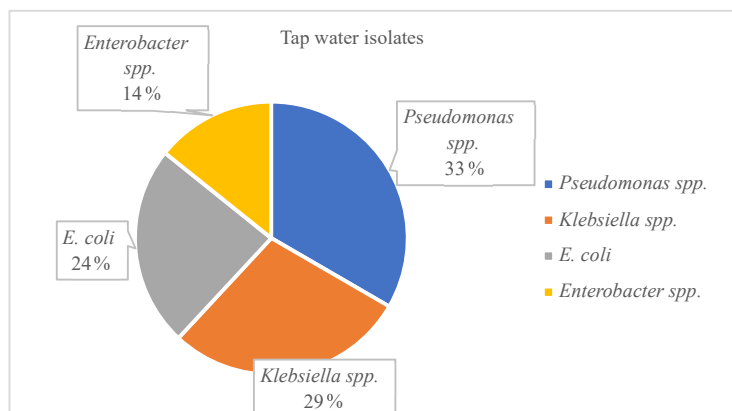


Figure 2: Types of enteric bacterial isolates on tap water.

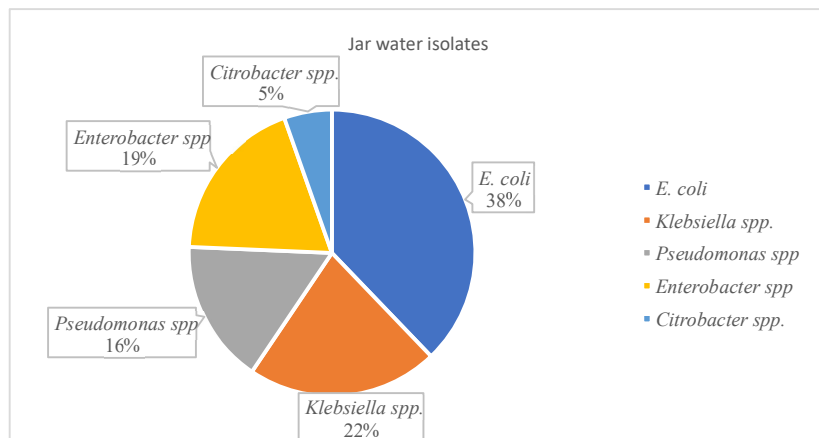


Figure 3: Types of enteric bacterial isolates on jar water.

Antibiotic Susceptibility Test (AST) of bacterial Isolates

The antibiotics susceptibility test is the technique to measure bacteria’s susceptibility to antibiotics. Result from sensitivity testing helps to choose the antibiotic against bacterial disease.

Antibiotic Susceptibility pattern of *E. coli*, *Enterobacter spp.*, *Pseudomonas spp.*

Antibiotic susceptibility test revealed that most of *E. coli*, *Pseudomonas spp.*, *Enterobacter spp.* isolates were found to be sensitive against Chloramphenicol, Norfloxacin, Gentamicin but resistant against Ampicillin (Table 3).

Table 3: Antibiotic susceptibility testing of *E. coli*, *Enterobacter spp.*, and *Pseudomonas spp.*

Antibiotics	C30			NIT300			NX10			AMP10			GEN30		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
<i>E. coli</i> isolates															
Tap water (n = 5)	3	2	0	2	1	2	3	0	2	0	1	4	3	0	2
Jar water (n = 14)	11	0	3	10	1	3	11	2	1	0	1	13	9	0	5
<i>Enterobacter spp.</i> isolates															
Tap water (n = 3)	2	1	0	2	0	1	3	0	0	0	0	3	2	0	1
Jar water (n=7)	6	0	1	4	3	0	3	2	2	1	0	6	5	0	2
<i>Pseudomonas spp.</i> isolates															
Tap water (n=7)	4	1	2	3	0	4	5	1	1	1	0	6	6	0	1
Jar water (n=6)	3	1	2	2	0	4	5	1	0	0	0	6	5	0	1

(n- number of isolates, S- Sensitive, I- Intermediate, R- Resistance, C30- Chloramphenicol, NIT300- Nitrofurantoin, NX10- Norfloxacin, AMP10- Ampicillin, GEN30- Gentamicin, CX30- Cefoxitin, AK30- Amikacin, AZM15- Azithromycin, CTR30- Ceftriaxone).

Antibiotic Susceptibility pattern of *Klebsiella spp.*

Most of *Klebsiella spp.* isolates were found to be sensitive against, azithromycin, but resistant against ampicillin and cefoxitin (Table 4).

Table 4: Antibiotic susceptibility testing of *Klebsiella spp.*

Antibiotics	C30			AMP10			AZM15			AK30			CX30		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Tap water (n=6)	4	2	0	0	0	6	6	0	0	3	3	0	0	0	6
Jar water (n=8)	5	2	1	0	0	6	7	0	1	4	3	1	1	0	7

Antibiotic Susceptibility pattern of *Citrobacter spp.*

The isolated *Citrobacter spp.* were resistant to Ampicillin, Cefoxitin whereas sensitive against Norfloxacin (Table 5).

in pH cause the toxicity of compounds in water bodies to either rise or fall (Shidiki et al., 2017).

The conductivity range of tap water samples was 25.2-830 $\mu\text{S}/\text{cm}$ while in case of jar water the value ranges from 20-335 $\mu\text{S}/\text{cm}$. All the water samples were found to be within the recommended range of NDWQS. It may be due to various dissolved salts and inorganic substances (Bajracharya et al., 2022). Shakya et al., (2013) found that 14.9% of tap water samples crossed recommended range while Karki et al., (2022) found that EC values of tap water samples were within the limit except sample from Swayambhu. Conductivity does not directly affect the human health but high conductivity might be the indicator for contamination in the water sample and is caused due to the concentration of conductive ions such as chlorides, alkalis, carbonate, sulphide and dissolved salts present in water (Karki et al., 2022).

The hardness value of tap water ranged from 14-278 mg/l whereas jar water samples ranged from 4-62 mg/l. All the water samples including tap and jar water were found to be within the recommended range of NDWQS. Maharjan et al., (2018) also found that hardness of jar water samples was within the recommended range. B. Adhikari et al., (2023) found that tap water samples were within the permissible limit. Because of the dissolved calcium and magnesium in the water, total hardness (TH) is formed. High levels of TH in the water have been linked to health problems in humans, including cardiovascular illness, kidney stones, growth retardation, and reproductive failure (Karki et al., 2022). Water that has been processed and bottled may be naturally soft, demineralized, or mineralized. Thus, depending on location, treatment, and water supply, the amount of minerals consumed from drinking and cooking water can vary greatly (WHO 2017, Maharjan et al., 2018).

The alkalinity range of all tap and jar water samples were found to be within the NDWQS guidelines, value ranging from 26-294 mg/l in case of tap water whereas the value ranged from 2-100 mg/l in jar water samples. The major causes of alkalinity of water are carbonates, bicarbonates and hydroxide (Bajracharya et al., 2022). The primary cause of alkalinity is the dissolution of minerals in water from soil, rock, limestone and dolomite. Alkalinity is significant because it buffers against abrupt pH fluctuations in water and serves as a pH stabilizer. It must be determined, as water having high alkaline level is typically not appetizing (Adhikari, 2020).

The chloride values of tap water samples ranged from 2.2-113.6 mg/l and jar water ranged from 7.1-31.24 mg/l. The chloride concentration of both tap and jar water samples were within the recommended guidelines of NDWQS. Chloride in drinking water originates from natural sources, sewage and industrial effluents, resulting in salty taste of water. Chloride can be an indicator of pollution (Koju et al., 2015)

The TDS range of tap water samples ranged from 20-323 mg/l and jar water samples ranged from 4-220 mg/l. All tap and jar water samples were within the recommended guidelines of NDWQS. All the piped water samples and jar water samples were within the recommended range (Khanal et al., 2023). The sources of TDS in drinking water include sewage, urban runoff, industrial wastewater, natural sources, chemicals used in the water treatment process, and the kind of plumbing, or pipework, that is used to transport the water. The total dissolved solids concentration in water is typically equal to the sum of the positively charged cations and negatively charged anions ions (Vaidya Ram & Narayan Labh, 2017).

Bacteriological analysis revealed that out of 15 tap water samples, only five water samples were within the recommended range while other 10 water samples exceeded the recommended range of WHO and NDWQS. Likewise, out of 15 jar water samples, all water samples exceeded the recommended limits. The findings correlated with Koju et al., Subedi & Aryal., Bajracharya et al., Adhikari et al., and Karki et al., who also detected microbial contamination in tested water samples exceeding national as well as WHO guidelines. It may be due to poor sanitation, improper handling of drinking water, improper disposal of solid and liquid wastes. The presence of coliform bacteria in water signifies contamination, making it unsuitable for drinking.

Five different enteric bacteria such as *E. coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Enterobacter spp.* and *Citrobacter spp.* were isolated from jar water samples. Likewise, four different enteric bacteria such as *E. coli*, *Klebsiella spp.*, *Pseudomonas spp.*, *Enterobacter spp.* were isolated from jar water samples. The cause of this might be attributed to either improper water treatment provided by commercial water providers or improper cleaning of the jar bottles used to fill water (Subedi & Aryal, 2010). *E. coli*, *Klebsiella spp.*, *Citrobacter spp.*, were isolated from collected tap water samples (Shidiki et al., 2017). 91.2% of jar water were contaminated by total coliform and 59.6% by fecal coliform (Subedi & Aryal, 2010).

These isolated pathogenic organisms were forwarded for antibiotic susceptibility test against five different antibiotics using standard discs diffusion method. Antibiotic resistance and multiple drug resistance was also determined. According to this study it was found that, most of *E. coli*, *Pseudomonas spp.*, *Enterobacter spp.* isolates were found to be sensitive against Chloramphenicol, Norfloxacin, Gentamicin but resistant against Ampicillin. Most of *Klebsiella spp.* isolates were sensitive against Azithromycin, but resistant against Ampicillin and cefoxitin. Similarly, isolated *Citrobacter spp.* were resistant to Ampicillin, Cefoxitin whereas sensitive against Norfloxacin. 23.8% of tap water isolates and 18.9% of jar water isolates were found to be Multiple Drug Resistant. *E. coli*, *Klebsiella spp.*, *Enterobacter aerogens* were isolated and all isolates

were found to be sensitive against ciprofloxacin and resistant to Ampicillin (Subedi & Aryal, 2010). Antibiotic Susceptibility test showed that most of isolated were sensitive against Gentamicin and Ciprofloxacin. *Klebsiella* spp. were found to be sensitive against Gentamicin, Cotrimoxazole and Cefoxitin and resistance towards Ampicillin. Out of 263 isolates, 67 were Multiple Drug Resistant Adhikari *et al.*, 2022. It may be due to Antibiotics are often used and misused in aquaculture and human medicine, which leads to the acquisition and buildup of metabolites in water bodies and an increasing rate of antibiotic resistance. Antibiotic-resistant bacteria are also developed in the aquatic environment by prolonged exposure to antibiotics, abuse of drugs in husbandry, and sewage and agricultural runoff enriching microorganisms (Koju *et al.*, 2015).

Temperature, hardness and alkalinity differed significantly between jar water and tap water while other parameters such as pH, conductivity, total dissolved solids and chloride were similar. The difference in temperature might be because the water was stored in different places. For example, jar water is commonly stored in bottled form in unregulated circumstances, which can affect thermal profile as compared to direct municipal tap water. The significant difference in hardness was probably because of different concentrations of calcium and magnesium which can be affected by the source of the water or by treatment techniques. Different dissolved carbonate and bicarbonate ions can cause variations in alkalinity, which is a measure of water's buffering ability. These variations are frequently influenced by natural geochemical variables or treatment procedures. On other hand, the absence of significant difference in pH, conductivity, chloride and TDS suggests that the absence of notable changes in pH, conductivity, TDS, and chloride suggests that the ionic balance and salinity levels of jar and tap water are largely uniform.

The results of this investigation provide insight into possible issues with drinking water quality that, if ignored, might endanger public health. The study revealed that microbial content of both tap and jar water were significantly higher than recommended limit indicating the necessity of routine testing and appropriate treatment to guarantee safe drinking. Waterborne illnesses can spread as a result of contaminated or inadequate water, especially among susceptible groups. However, the study does have some limitations. The result was based on a one-time study in limited water samples from certain areas of Kathmandu valley in winter seasons. The study only involves the water quality of the existing two public drinking water sources (tap and jar water). So seasonal analysis and analysis of other water sources can also be performed. Plus, more physicochemical as well as bacteriological parameters can be assessed as limited parameters were assessed during this study.

Conclusion

The study showed that physicochemical and bacteriological parameters of both tap water and jar water did not meet NDWQS guidelines. The results clearly showed that most of the tap water samples and all jar water samples had microbial contamination with coliform bacteria which were higher than NDWQS guidelines. Four different enteric bacteria namely *Pseudomonas* spp. (33%), *Klebsiella* spp. (29%), *E. coli* (24%), and *Enterobacter* spp. (14%) were isolated from tap water and five different enteric bacteria namely *E. coli* (24%), *Klebsiella* spp. (22%), *Enterobacter* spp. (19%), *Pseudomonas* spp. (16%) and *Citrobacter* spp. (5%) were isolated from jar water. Besides that, Antibiotic susceptibility test revealed that 23.8% of tap water isolates and 18.9% of jar water isolates were Multiple Drug Resistant. In conclusion, jar water and tap water were not suitable for drinking purpose and other domestic uses. So proper treatment should be done before using for drinking and other domestic purposes and regular monitoring of water quality and proper distribution system is necessary to prevent serious health hazards.

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CRedit Author Statement

RH: Conceptualization, Methodology, Investigation, Analysis, Writing- original draft, Writing- Reviewing and Editing; **AM:** Conceptualization, Supervision, Writing – Reviewing and Editing

Conflict of Interest

The author declares that there is no conflict of interest.

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