Evaluation of Ground Water Quality of Kathmandu Valley and Antibiotic Susceptibility Test against *Klebsiella pneumoniae*

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ABSTRACT

Objectives: The aim of this study was to assess quality status of ground water in Kathmandu valley and describe the antibiotic susceptibility pattern of the isolated *Klebsiella pneumoniae.*

Methods: A total of 100 samples were collected from different places of Kathmandu valley with 50 each from two different groundwater sources namely boring and well. This study was conducted from June to September, 2019 at Environment and Climate Study Laboratory, Nepal Academy of Science and Technology (NAST). The physicochemical analysis of the samples was done according to standard methodology. Membrane filtration technique was performed for the enumeration of total coliform and different biochemical tests were performed for isolation and identification of *Klebsiella pneumoniae* followed by Kirby-Bauer disc diffusion method for antibiotic susceptibility test.

Results: This study reveals the poor microbiological aspects of ground water sources as 98% of total water samples crossed the standard value for total coliform count. The pH, turbidity, ammonia, nitrate and iron content were found to be higher than Nepal Drinking Water Quality Standard (NDWQS 2005) in 15%, 26%, 34%, 7% and 26% of total water samples respectively. The chloride and arsenic content in all the water samples were within the NDWQS, 2005. The 12 out of 18 isolates of *Klebsiella pneumoniae* from ground water source were highly resistant against first generation Cefazolin however, 15 out of 18 isolates were sensitive to Chloramphenicol. Additionally, four isolates showed zone of inhibition in intermediate range provided by Clinical and Laboratory Standard Institute (CLSI) guideline towards Imipenem and Meropenem.

Conclusion: This study concludes that ground water sources were heavily contaminated by coliform bacteria and most of the physicochemical aspects were under standard limit. Although *Klebsiella pneumoniae* isolated from ground water were not multidrug resistant, one isolate was recorded to be resistant to Meropenem. These results alarm for circulation of antibiotic resistance in environmental bacterial isolates. Therefore, the appropriate water purification methods should be applied before consumption and should be examined periodically.

Keywords: Total coliform count, Antibiotic resistance, ground water, *Klebsiella pneumoniae*

INTRODUCTION

Groundwater resources are generally considered a reliable source of water for multi-purpose uses. Kathmandu valley, a largest urban center in the Nepal has experienced rapid growth in population in recent years and nearly half of the valley's water supply is derived from groundwater (Khatiwada et al. 2002). Due to huge gap in demand and supply of water in the valley, majority of households own either bore hole or well to extract ground water. This implies increased stress on quality and quantity of groundwater in the valley.

Date of Submission: August 25, 2020 **Date of Acceptance:** October 13, 2020

Published Online: December, 2020 **DOI:** https://doi.org/10.3126/tujm.v7i0.33850

Because of heavy dependence on ground water sources for drinking water and domestic uses, chemical and microbial contamination of ground water is a serious problem in Kathmandu valley. The contamination is mainly due to anthropogenic causes of pollution. It is assumed that ground water in Kathmandu is polluted due to sewage line, septic failures, open pit latrines, leaching from landfill sites, and direct disposal of domestic and industrial wastes to the surface water (Shrestha et al. 2012). It is notable that nearly 70% households are not connected to sewer system in Kathmandu discharging in open drains and river systems (Shrestha et al. 2017). Globally, over 2 billion people still rely on unsafe water and 4.2 billion rely on sanitation facilities, where their excreta is leaked untreated into the environment (WHO 2019). It is speculated that degradation of surface and shallow groundwater quality has encouraged people to extract of deep groundwater in search of alternative, safe, and reliable source (Shrestha et al. 2016). It is important to note that many studies of shown substandard quality of Kathmandu's groundwater in terms of high concentration of chemical pollutants (Emerman et al. 2010, Koju et al. 2014, Gwachha et al. 2020, Chapagain et al. 2009) and presence of microbial indicators and water-borne pathogens (Diwakar et al. 2008, Manandhar et al. 2010, Shrestha et al. 2018). However, the chemical and microbial quality of private non-piped groundwater has not been viewed separately. In this context it is noteworthy to understand the quality situation of such water so that the necessary mitigation approaches could be recommended to the users.

The pollution of water resources and unsafe drinking water increases the risk of mortalities due to water borne diseases like diarrhea, dysentery, hepatitis as well as many protozoan and helminths infection (WHO 1997). The presence of coliform in drinking water is considered as a possible threat or indicative of microbiological water quality deterioration (Rompre, et al. 2002). We reported various types of gram negative enteric bacteria including *Escherichia coli, Enterobacter* spp, *Citrobacter* spp, *Klebsiella* spp, in the ground water of Kathmandu valley (Bajracharya et al. 2007, Jayana et al. 2009, Prasai et al. 2007). In this period of antibiotic resistance, *Klebsiella pneumoniae* belonging to Enterobacteriaceae family, one of the most concerning pathogens involved in antibiotic resistance and together with other important multi-drug resistant pathogens, it has been classified as an ESBL organism (Navon-Venezia, et al. 2017). According to WHO, the occurrence of Extended Spectrum β Lactamase-producing *K. pneumoniae* has reached now endemic rates of up to 50% in many parts of the world, and up to 30% resistance rates in the community demonstrating its widespread nature (WHO, 2014). It is now well understood that water environment is reservoir of antibiotic resistant bacteria and their resistant genes (Joshi 2017).

In this study, we examined private non-piped groundwater sources to assess the selected chemical and bacteriological quality indicators; and analyzed the statistical correlation among quality indicators. In view point of increasing environmental antibiotic resistance; we also tested resistivity of *K. pneumoniae* against certain antibiotics including carbapenems. This study provides a picture of quality issues of non-piped groundwater sources in the valley.

MATERIALS AND METHODS

All chemicals used were of analytical grade. All aqueous solutions were prepared using 18.2 MΩ water (Millipore, Milli-Q). All glassware was soaked in nitric acid solution (10%) for at least 24.0 hours followed by three times rinsed with distilled water and dried at 60 °C for 4.0 hours before use. The water samples were collected from Kathmandu, Bhaktapur and Lalitpur. All the experiments were conducted in Environment and Climate Study Laboratory of Nepal Academy of Science and Technology (NAST). The water samples were analyzed for physicochemical and microbiological quality according to Standard method for the examination of water and wastewater (APHA 2005). The water samples were tested immediately on arrival to the laboratory. In case when the immediate analysis was not possible, the samples were preserved at 4 °C (WHO 2006). The temperature and the pH of the water samples were analyzed by Thermo Scientific Orion Star A111 pH meter. The electrical conductivity was measured by the Mettler Toledo conductivity meter. Turbidity was measured by using HANNA nephelometer. Similarly, for the chemical analysis, the concentration of chloride and total hardness were analyzed by Argentometric titration and EDTA titration, respectively. The iron content was analyzed phenanthroline method by using Agilent Technology Cary UV-Vis spectrophotometer at 510 nm. The standard test kits were used for the determination of ammonia (Standard Visocolor alpha kit), arsenic (Standard Quantofix kit) and nitrate (Standard Visocolor alpha kit) detection through indication of different color range.

The total coliform count was performed by using standard membrane filter technique in which 100 mL of water sample was filtered through sterile membrane filters having 0.45 µm pore size and 47 mm diameter. The membrane filter retained with microbial biomass was aseptically transferred to M-Endo agar and incubated at 37 °C. The bacterial colonies were enumerated after 24 hours. The pinkish colonies with golden green metallic sheen from M-Endo agar were sub-cultured on MacConkey agar and Nutrient agar. *Klebsiella pneumoniae* was presumptively identified based on results gram staining and different biochemical tests including Indole test, Methyl Red test, Voges-Proskauer test, Citrate utilization test, Urease test, Triple Sugar Iron test, Catalase test and Oxidase test.

The identified *K. pneumoniae* isolates were taken for the antibiotic susceptibility test by disc diffusion method, also known as Kirby Bauer disc diffusion. The inoculum was prepared by suspending the organisms into 2 mL of sterile saline (0.9% w/v NaCl) and the turbidity of this inoculum was adjusted to 0.5 McFarland standards. The inoculum was cultured at 37 °C on Mueller Hinton agar (MHA) media with sterile cotton swab. *K. pneumoniae* isolates were tested against five antibiotics - Cefazoline (30 µg), Cefepime (30 µg), Chloramphenicol (30 µg), Imipenem (10 µg), and Meropenem (10 µg). The zones of inhibition (mm) were measured at 18-24 hours of incubation. The antibiotic susceptibility was interpreted based on CLSI guidelines (CLSI, 2018).

In order to estimate statistical relationship among ground water quality parameters, Pearson's correlation coeeficient was calculated using OriginPRO 2018 software.

RESULTS

A total of one hundred ground water samples were collected for assessment from June to September, 2019. The collected samples were differentiated as well water 50 and boring water 50. The result values for individual parameters were compared with NDWQS, 2005 as shown in Figure 1.

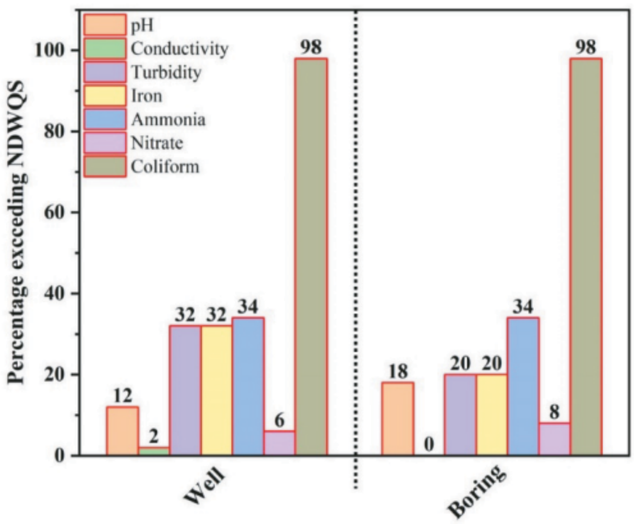

Figure 1: Percentage of water samples exceeding NDWQS, 2005 for given parameters

The temperature for both the sources ranged from 27 ˚C to 28.2 ˚C. The pH value varied from 5.7 to 8.8 as shown in Figure 2. The physical parameters such as pH, conductivity and turbidity of 12%, 2% and 32% respectively of well water samples exceeded the value of NDWQS, 2005. The chemical parameters as iron, ammonia and nitrate of 32%, 34% and 6% respectively of well water samples exceeded standard limit. Similarly, pH of 18% and turbidity of 20% boring water samples were crossed the standard limit. The chemical parameters like iron, ammonia and nitrate of 20%, 34% and 8% respectively of boring water samples exceeded NDWQS, 2005. However, hardness, chloride and arsenic were found within limit for both the ground water sources.

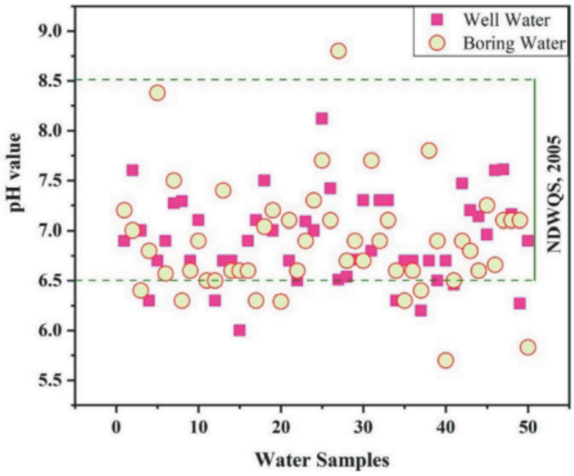

Figure 2: The pH value of ground water samples

Pearson's correlation coeeficient was determined to explore relationship among ground water quality parameters as given in Table 1. In this study, conductivity was positively correlated with hardness, chloride, ammonia and nitrate significantly (*p<0.05*). Likewise, turbidity was positively correlated with hardness and iron (*r=0.72*) significantly. Furthermore, hardness was positively correlated with chloride (*r=0.40*), ammonia (*r=0.44*) and (*r=0.33*) nitrate significantly. Interstingly, chloride showed significat negaive correlation with arsenic though coefficient value was low (*r= -0.20*). However, none of physicochemical parameters were significatly correlated with biological (coliform count) parameter.

Table 1: Pearson's correlation coefficient (*r*) among quality parameters of ground water samples (*n=100*)

Parameters	Conductivity	Turbidity	Hardness	Chloride	Iron	Arsenic	Ammonia	Nitrate	Coliform
pH	-0.06	-0.02	-0.05	0.07	-0.09	0.13	-0.04	0.00	-0.11
Conductivity		0.09	**0.61***	**0.23***	0.10	0.08	**0.41***	**0.26***	0.10
Turbidity			**0.21***	0.08	**0.72***	0.12	0.17	-0.07	0.10
Hardness				**0.40***	0.10	0.06	**0.44***	**0.33***	0.12
Chloride					0.06	**-0.20***	0.11	**0.27***	-0.04
Iron						0.11	0.13	0.02	0.07
Arsenic							**0.21***	0.10	0.09
Ammonia								0.02	-0.01
Nitrate									0.14

The values indicate Pearson's correlation coefficient (*r*). Bold face *indcates statistcally significant (p<0.05) correlation.

As for the microbiological test, the total coliform count in 98% of both the sources exceeded the NDWQS limits. We selectively targeted *K. pneumoniae* isolates for further study. *K. pneumoniae* was recovered from 18 ground water samples. Antibiogram of *K. pneumoniae* revealed higher degree of susceptibility towards the tested antibiotics. The frequency of the isolates susceptible to most of tested antibiotics ranged in between 72.2 to 83.3% with susceptibility to Cefepime (83.3%), Imipenem (77.8%), Meropenem (72.2%), and Chloramphenicol (77.8%) as demonstrated in Figure 3(a). However, in contrary Cefazoline was highly resisted by the *K. pneumoniae* isolates (83.3%). As shown in Figure 3(b), *K. pneumoniae* isolated from well water were more resistant to Carbapenem group including Imipenem (22.2%) and Meropenem (16.7%). Contrary to this isolates from boring water were resistant towards antibioltics - Cefepime (11.1%), Cefazoline (44.4%). Interestingly, none of the boring water isolates of *K. pneumoniae* were resistant towards Imipenem.

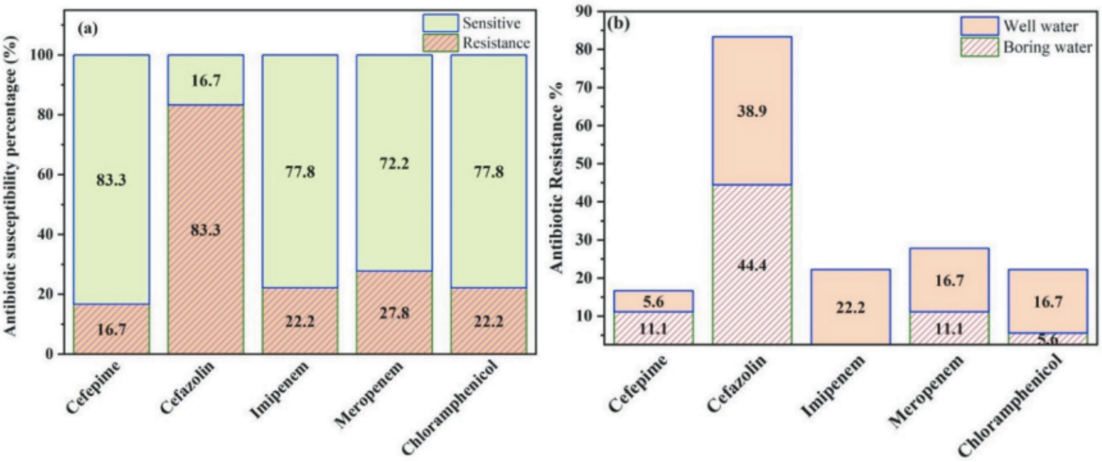

Figure 3: (a) Susceptibility pattern against *K. pneumoniae* isolates towards selected antibiotics (b) Antibiotic resistance pattern in well and boring water

DISCUSSION

This study was focused to assess the quality of private non-piped ground water sources in Kathmandu valley. The quality was compared with drinking water quality standards of Nepal (NDWQS 2005) to elucidate the results for physiochemical and microbiological parameters in ground water samples. The water samples were analyzed for physical (pH, turbidity, conductivity, and temperature), chemical (hardness, chlorine, iron, arsenic, nitrate, ammonia) and microbiological quality parameters (Total Coliform Count and identification of bacteria).

We found moderate temperature ranged (27 - 28.2 °C) for all the ground water samples, though water temperature may vary with seasonal variation. High water temperature enhances the growth of microorganisms and may increase taste, color and corrosion problem (WHO 2004). This correlates with the fact that such water is likely to support the growth of bacteria, algae and other life forms. However, several other factors are also crucial for microbial life in water. The pH is an important water quality parameter. Even though pH is not directly related to health risk, it is very crucial in chlorination process. When the pH exceeds 8, disinfection is less effective, while low pH is acidic and cause corrosion of metal pipes (WHO 2017). Conductivity of well water (2%) is comparatively higher than boring water, which indicates that there may be higher presence of dissolved solids in well water. The specific conductance measures the presence of dissolved solids such as chloride, nitrate, sulfate, phosphate, sodium, magnesium, calcium, and iron which indicates water pollution (Murphy 2007). Nearly 20% of well water samples and 32% of boring water samples crossed the permissible limit for turbidity and iron content; this may be caused by the presence of suspended and colloidal matter. This proportional increase in concentration of turbidity and iron content indicates the correlation between them. In a similar study, water with high turbidity had offensive color, taste, odor and also correlates with iron content of water sample and inhibits chlorination (Dietrich and Burlingame 2015). Turbidity is also considered as indirect indicator for the presence of microbes (WHO 2006). Hardness and chloride of most of all water samples were found within the limit proposed by NDWQS, 2005, however, only one (2%) water sample from well contained hardness above the standard. In a previous study done for the treated water in Kathmandu valley, similar results for hardness and chloride were observed (Maharjan et al. 2019). Arsenic concentration in ground water samples was within limit of NDWQS, 2005, except one well-water sample and four boring water samples which contained higher concentration of arsenic (0.025 mg/L). It is not surprising as previous studies have reported even higher concentration of arsenic (max. 2.8 mg/L) in certain deep ground water sources from Kathmandu valley (Emerman et al. 2010, Gwachha et al. 2020).

Most of the tested water samples contained nitrate within the standard value, however, its concentration in 6% of well and 8% of boring water samples exceeded the standard value. Nitrate itself is not toxic but the effects are hazardous as it is converted to nitrite by microbial action which may cause Blue baby Syndrome in infants (WHO 1997). The result showed that 34% of well water and 34% of boring water samples exceeded the standard limit for ammonia concentration. This implies higher concentration of ammonia in the ground water of the valley. Therefore, the household treatment options for ammonia removal are highly recommended. For instance, simple aeration of groundwater can also significantly reduce the level of ammonia and iron (Pacini et al. 2005, Zhang et al. 2019).

Pearson's correlation analysis revealed that hardness of the groundwater had significant ($p<0.05$) positive correlation with conductivity, turbidity, chloride, ammonia and nitrate. Similarly, conductivity itself was positively correlated with chloride, ammonia and nitrate along with hardness. This is important for water quality testing laboratories that higher conductivity in groundwater may predict higher values for aforementioned chemical parameters. It may help to reduce the number of parameters for testing. However, none of the tested chemical parameters were significantly correlated with coliform count. This indicates widespread contamination of coliforms in the environment and their unpredictability with chemical factors.

As a second focus of this study, the bacteriological water quality was assessed by enumerating total coliforms bacteria, and detecting *K. pneumoniae* in ground water samples. The results revealed that tested ground water samples were loaded with considerable numbers of total coliforms, most of them having too numerous to count (>300 CFU/mL). Considering detection of coliforms in ground water since long time (Koju et al. 2014, Bajracharya et al. 2007) and increased contamination level in this study, it can be anticipated that fecal bacteria might be well adapted in deep aquifers. The bacterial pollution of ground water might be mostly due to sewage infiltration and seepage from the polluted river flowing, unhygienic practices such as unsanitary septic tank constructed near the water sources (WHO 1997). Previous studies evaluating different water sources revealed that the samples were highly contaminated by total coliform bacteria (Maskey et al. 2020, Maharjan et al. 2018, Bishankha et al. 2012, Acharya et al. 2019, Shakya et al. 2012, Ghaju Shrestha et al. 2017). Therefore, fecal coliforms or other bacteriophage indicators should be adopted for water quality testing. This is one of limitation of this study. Coliform count may not represent fecal contamination accurately.

We could recover *K. pneumoniae* from 18 ground water samples. *K. pneumoniae* is important pathogen in clinical settings in particular causing lower respiratory infections. Antibiotic resistant *K. pneumoniae* have garnered increasing concern. In this study, we found *Klebsiella pneumoniae* was highly sensitive to Chloramphenicol as 15 isolates were sensitive and it was highly resistance to Cefazolin, which is a first-generation Cephalosporin as 12 isolates showed resistivity. *K. pneumoniae* didn't show the resistivity towards Imipenem. However, four isolates showed intermediate range of zone of inhibition to Imipenem. Furthermore, four isolates were in intermediate range and one isolate was resistance to Meropenem. Imipenem and Meropenem belonging to Carbapenem family are the most effective drug. The study held to assess the ESBL producing *Enterobacteriaceae* isolates in packaged water bags sold as drinking water in Kinshasa, the capital of Democratic Republic of Congo, reports that 150 *Enterobacteriaceae* isolates were recovered out of which 56% isolates were *K. pneumoniae*, 30.6% were *Enterobacter spp*, 4.7% *Citrobacter spp* and 3.3% *E. coli*. Eight isolates (5.3%) were confirmed as ESBL producers (Boeck et al. 2012). Hence, proper treatment option and regular monitoring of the drinking water should be implemented as the poor water quality has direct effects on public health.

CONCLUSION

The present work evaluated the quality of ground water for domestic purposes. This study concludes that most of the physicochemical aspects were under standard limit, however none of the water samples had an unquestionable quality. There was heavy contamination by coliform bacteria in ground water, indicating higher level of fecal pollution. This implies the maximum possibility of residing water borne enteric pathogens in the ground water of Kathmandu. These results may predict the possible epidemic outbreak of water borne diseases in Kathmandu valley. Although, *Klebsiella pneumoniae* isolated from ground water were not multi drug resistant, however, majority of isolates were resistant to Cefazolin. During the study, one isolate was recorded to be resistant to carbapenem (meropenem). This results alarm for circulation antibiotic resistance in environmental bacterial isolates. Further studies are recommended to investigate environmental (water) circulation of antibiotic resistance in Kathmandu. For the human consumption, it is very important to apply proper treatment options before using the ground water for drinking purpose. The awareness among people and proper sanitation practice can help to reduce the risk of epidemic outbreak.

ACKNOWLEDGEMENTS

The authors are grateful to Nepal Academy of Science and Technology (NAST) for providing all the laboratory facilities.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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