Nepal J Biotechnol. 2026 Jul; 14 (1):9-15 Research Article DOI: https://doi.org/10.54796/njb.v14i1.473

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: https://nepjb.com/index.php/NJB ISSN (Print): 2091-1130

Antibiotic susceptibility pattern of *Escherichia coli* and *Klebsiella* spp. Isolated from treated tap water and untreated water of Kathmandu

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Received: 27 Dec2025; Revised: 16 Jun 2024; Accepted: 20 Jun 2026; Published online: 31 Jul 2026

Abstract

The health of every living thing is directly related to water. However, pathogenic microorganisms are becoming more prevalent in water supplies these days, which is causing a number of health problems for people. This study was conducted to analyze the predominant coliform isolated from tap water and untreated water samples and their susceptibility pattern towards various antibiotics. Coliforms were enumerated by Most Probable Number method and conventional morphological and biochemical test was used for identification and antibiotic susceptibility test was done by modified kirby bauer disc diffusion method. Among total samples, 15 treated tap water samples and 17 untreated, well and bore water samples were collected. Coliform was present in 100% of the 17 untreated water samples. In this study, 9 (60%) of the treated tap water samples exceeded the WHO recommended limit. The total number of coliform in untreated water was found to be in the range of 9- 110 MPN/100ml while 0- 28 MPN/100 ml was found in treated water. Among total samples, 10 (58.8%) *Klebsiella* spp. and 7 (41.2%) *E. coli* were identified from untreated water samples (17) whereas total, 4 (44.4%) *E. coli* and 5(55.5%) *Klebsiella* spp. was identified from treated water samples (9). Antibiotic susceptibility test of isolates from untreated water showed, 3(42.8%) *E. coli* isolates were Ceftriaxone resistance and 1 (10%) *Klebsiella* spp. isolate was resistant to Tetracycline, Meropenem, and Ceftriaxone. From treated water, only 1 (20%) *Klebsiella* spp. showed resistance to Amikacin. There is no association between antibiotic susceptibility and types of water. Out of 17 isolated bacteria from untreated water sample, total 4 including 3 (17.64%) *E. coli* and 1 (5.88%) *Klebsiella* spp. produced Extended Spectrum β-Lactamase (ESBL). Presence of total coliform and ESBL producing bacteria in water indicates possible health risk to general public, suggesting regular monitoring of water sources for microbial quality.

Key words: Treated tap water, untreated water, Antibiotic susceptibility test, *E. coli*, *Klebsiella* spp.

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Introduction

Water is a vital component of all living things. Nearly half of the world's population lacks access to safe drinking water. In 2022, at least 1.7 billion people use faeces contaminated drinking water sources worldwide. Contaminated water with faeces may possess greater risk to public health as it becomes source of various waterborne diseases such as diarrhea dysentery, typhoid, polio, cholera and estimated to cause 505,000 deaths due to diarrhea each year [1]. Coliform bacteria have been utilized to access the microbial quality of water as they are considered as indicator of water pollution and are easy to detect and quantify. The term coliform refers to Gram-negative, rod-shaped bacteria that can grow in the presence of bile salts and are capable of fermenting lactose within 24 hours at 35–37 °C, producing both acid and gas [2].

Treated water is that water which is purified through filtration, chlorination or other purification method. Continuous monitoring of even treated water should be done as it might be contaminated with various pathogenic microorganisms. Untreated water is surface or ground water that has not been treated in any way. In Kathmandu, people primarily rely on groundwater, although it is believed that these sources are heavily contaminated with pathogenic microorganisms [2]. The overcrowding in Kathmandu is causing people donot have adequate water. The majority of people drink tap water, which is tainted with coliform bacteria. It is considered as primary indicator of faecal pollution and commonly found in water and waste water [3]. *Klebsiella* spp. also persists in water and sewage contaminated water. Several studies suggest that *E. coli* has ability to act as reservoir for antimicrobial genes [4].

Numerous antibiotics are ineffective against the microorganisms that were identified from the surrounding water sample [5]. Antibiotic resistant bacteria (ARB) in drinking water have been reported in different studies [6, 7, 8, 9] as well as in aquatic ecosystem, as reservoir of ARB and antibiotic resistant gene (ARG) [10, 11, 12]. In raw water or natural water, antibiotic resistant properties of bacteria usually come from wastewater and agricultural runoff. Presence of antibiotic resistant bacteria in untreated water and treated water can infect the human population. Although people often drink purified water, they use untreated water such as well water for other purposes, such as cleaning and bathing. There is also likelihood of cross contamination between treated and untreated water.

Despite these findings, there is limited data on comparative bacteriological quality and antibiotic susceptibility pattern of predominant *E. coli* and *Klebsiella* spp. isolated from treated and untreated sources of water. Such study will help in evaluating aquatic environment and the effectiveness of water treatment process and distribution system and assessing the possible health hazard associated with. Therefore the study aims to compare the difference in antibiotic resistant pattern of predominant isolate from treated and untreated water.

Materials and methods

Study Design: This was cross sectional study which was carried from February 2024 to April 2024. Both treated and untreated water samples were collected from several places of Kathmandu including Kirtipur, Tripureshwor, Sukedhara, Basundhara, Baudha, Baneshwor, Jadibuti, Kapan and other places. Altogether, 15 treated tap water sample and 17 untreated water sample (15 well water and 2 boring water) were collected from different place of Kathmandu.

Samples collection and transportation

Both untreated and treated tap water samples were collected from different locations in sterile plastic bottles. Treated water samples were collected in a 500 mL poly reagent plastic container to which 0.5 mL of 3% sodium thiosulphate was added then transported to the laboratory in less than an hour. The sample was taken without emerging from the bottle's neck, leaving the air gap and the securely fitting bottle cap contaminated [13].

Isolation and enumeration of coliform bacteria

The MPN method, also known as the multiple tube fermentation method, was used to isolate and count the coliform from the water sample. Three sets of three test tubes containing MacConkey broth in double strength (ds) and single strength (ss) culture media were used for this method. One set of test tubes containing ds medium was inoculated with 10 mL of sample after an inverted Durham's tube was transferred to each tube. To the two remaining tubes that contained the SS culture media, 1 mL and 0.1 mL of sample were added [14]. All the tubes were incubated at 37±0.5°C for 24-48 hours. After incubation change in color occur due to acid production and gas formation and was noted. Total positive tubes were counted and coliform was enumerated as MPN/100mL. The loopful of inoculum were taken from positive tubes and inoculate in Eosin methylene blue media (EMB) and incubated for 37^{o} C for 24 hours. After formation of colonies in EMB, it was inoculated in MacConkey broth for observation of acid and gas [15].

Identification of *E. coli* and *Klebsiella* spp. from water sample

Further Gram staining and several biochemical test including the sulfide indole motility test (SIM), the methyl red test, the voges proskauer test, the citrate utilization test, the triple sugar iron agar test, and the oxidative fermentative test (OF) were conducted [16, 17].

Antimicrobial susceptibility testing

Antimicrobial susceptibility test of isolated *E. coli* and *Klebsiella* spp. were done by Kirby Bauer Disk Diffusion method in which Meropenem (10 mcg), Cotrimoxazole (25 mcg), Amikacin (30 mcg), Ciprofloxacin (30 mcg), Tetracycline (30 mcg), Ceftriaxone (30 mcg), antibiotics were used. Antibiotic sensitivity and resistance were determined by measuring zone of inhibition [18].

Detection of Extended Spectrum β-Lactamase by combined disc assay

The screening test was carried out by identifying the zone of inhibition for the antibiotic ceftriaxone, which is a third-generation cephalosporin. Isolates that were resistant to ceftriaxone were classified as screening positive. The zone of inhibition of ceftriaxone or ceftazidime combined with and without clavulanic acid was measured as a confirmatory test. If the difference in the zone of inhibition between the Ceftazidime/Clavulanic acid combination on MHA was ≥5mm, the coliform isolates were considered to be ESBL producing isolates [18].

Results

Coliform estimation in treated and untreated water sample

From 17 untreated water samples from 15 different locations of Kathmandu, 17(100%) samples showed coliforms positive (**Table 1**). Out of 15 treated water samples from 13 different locations of Kathmandu, 9(60%) of the sample exceeded the WHO guideline value. The maximum and minimum total coliform count from 17 untreated water samples was 1100 and 9 MPN per 100

Table 1: Coliform estimation in treated and untreated water sample

Types of water sample	Number of Sample (n)	Sample within WHO guideline value		Sample exceeding WHO guideline		Minimum count MPN/100mL	Maximum count MPN/100mL
		Number	%	Number	%		
Untreated water	17	0	0	17	100	9	1100
Treated water	15	6	40	9	60	0	28

N=number, %=percentage

mL. Similarly, out of the 15 treated water samples, the highest and lowest count of total coliform was 28 and 0 MPN/100 (**Table 1**).

Table 2 Distribution of *E. coli* and *Klebsiella* spp. in treated and untreated water

Coliform bacteria	Untreated water		Treated water		Total	
	N	%	N	%	N	%
Escherichia coli	7	41.2	4	44.4	11	42.3
Klebsiella spp.	10	58.8	5	55.5	15	57.7
Total isolates	17	100	9	100	26	100

Distribution of *Klebsiella* spp. *and E. coli* according to types of water

Out of 17 coliform positive untreated water samples, total 17 isolates were recovered. Of them, 7(41.7%) were *E. coli* and 10(58.8%) were *Klebsiella* spp. Similarly, Out of 9 coliform positive treated water sample, total 9 isolates were obtained. Of them, 5(55.5%) were *Klebsiella* spp and 4(44.4%) were E coli (**Table 2**). In contrast to the majority of previous reports, which revealed that *E. coli* isolates were more common than *Klebsiella* spp., the current study found that *Klebsiella* spp. predominated in the water samples that were obtained.

Antibiotic susceptibility pattern of isolates from untreated water sample

All of the identified *E. coli* from untreated water were sensitive to Amikacin 7 (100%), Cotrimoxazole 7 (100%), and Meropenem 7 (100%) followed by Ciprofloxacin 6 (85.7%) and Tetracycline and 4 (51.1%) isolates were sensitive to ceftriaxone. However, 3 (42.8%) of the *E. coli* isolates showed Ceftriaxone resistance. All 10 (100%) *Klebsiella* species have been found to be sensitive to the Amikacin and Cotrimoxazole followed by 9 (90%) isolates were sensitive to Ceftriaxone, Meropenem, Ciprofloxacin, and 7 (70%) isolates were sensitive to Tetracycline, However, 1 (10%) showed resistance to Tetracycline, Meropenem, and Ceftriaxone (**Table 3**).

Antibiotic susceptibility pattern of isolates from treated water sample

From total treated water sample, out of total isolates of *E. coli*, 4 (100 %) were sensitive toward antibiotics Meropenem, Cotrimoxazole, Tetracycline followed by 3 (75 %) toward Amikacin and Ciprofloxacin and 2 (50 %) toward Ceftriaxone. Out of total isolates of *Klebsiella* spp., 5 (100 %) were sensitive toward antibiotics Meropenem, Cotrimoxazole, Tetracycline and Ciprofloxacin followed by Amikacin 4 (80 %), 2 (60 %) toward Ceftriaxone. 1 (20 %) of isolated were resistant toward Amikacin antibiotic (**Table 4**). There was no any association between antibiotic susceptibility with types of water (**Table 5**).

Table 3: Antibiotic susceptibility pattern of *E. coli* and *Klebsiella* spp. from untreated water.

	E. coli (7)			*Klebsiella* spp (10)		
Antibiotics	Sensitive Number(%)	Intermediate Number (%)	Resistant Number(%)	Sensitive Number(%)	Intermediate Number(%)	Resistant Number(%)
Meropenum (10mcg)	7(100%)	0(0%)	0(0%)	9(90%)	0(0%)	1(10%)
Cotrimoxazole(25mcg)	7(100%)	0(0%)	0(0%)	10(100%)	0(0%)	0(0%)
Amikacin(30mcg)	7(100%)	0(0%)	0(0%)	10(100%)	0(0%)	0(0%)
Ciprofloxacin(30mcg)	6(85.7%)	1 (14.3%)	0(0%)	9(90%)	1(10%)	0(0%)
Tetracyline(30mcg)	6(8.7%)	1(14.3%)	0(0%)	7(70%)	2(20%)	1(10%)
Ceftriaxone(30mcg)	4(57.1%)	0(3%)	3 (42.8%)	9(90%)	0(0%)	1(10%)

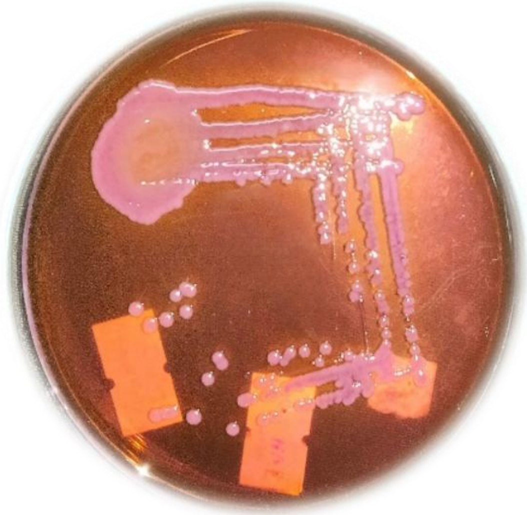
Table 4: Antibiotic susceptibility pattern of *E. coli and Klebsiella* spp. from treated water

	E. coli (4)			*Klebsiella* spp (5)		
Antibiotics	Sensitive Number(%)	Intermediate Number (%)	Resistant Number(%)	Sensitive Number(%)	Intermediate Number(%)	Resistant Number(%)
Meropenum (10mcg)	4(100%)	0(0%)	0(0%)	5(100%)	0(0%)	0(0%)
Cotrimoxazole(25mcg)	4(100%)	0(0%)	0(0%)	5(100%)	0(0%)	0(0%)
Amikacin(30mcg)	3(75%)	1(25%)	0(0%)	4(80%)	0(0%)	1(20%)
Ciprofloxacin(30mcg)	3(75%)	1(25%)	0(0%)	5(100%)	0(0%)	0(0%)
Tetracyline(30mcg)	4(100%)	(0%)	0(0%)	5(100%)	0(0%)	0(0%)
Ceftriaxone(30mcg)	2(50%)	2(50%)	0(0%)	3(60.7%)	2(40%)	0(0%)

Table 5: Association of antibiotic susceptibility of isolates with types of water

Antibiotics	Water types	Antibiotic susceptibility pattern				P-Value
		S	I	R	Total	
Meropenom (MRP)	Treated	0	0	9	9	
	Untreated	1	0	16	17	0.551
	Total	1	0	25	26	
Cotrimoxazol (COT)	Treated	17	0	0	17	
	Untreated	9	0	0	9	
	Total	26	0	0	26	NA
Tetracycline (TE)	Treated	9	0	0	9	
	Untreated	15	1	1	17	1.147
	Total	24	1	1	26	
Ciprofloxacin (CIP)	Treated	7	0	2	9	
	Untreated	10	3	4	17	1.916
	Total	17	3	6	26	
Amikacin (AK)	Treated	8	1	0	9	
	Untreated	15	1	1	17	.739
	Total	23	2	1	26	
Ceftriaxone (CTX)	Treated	0	2	7	9	
	Untreated	5	0	12	17	6.466
	Total	5	2	19	26	

S: Sensative; R: Resistant; I: Intermediate
NA: Not applicable since no statistics are computed because Zone of inhibition (COT) is a constant



Photograph 1: *Klebsiella* spp. on Eosin methylene blue (EMB)

Photograph 2: Antibiotic susceptibility testing of *E. coli*

Extended Spectrum β-Lactamase (ESBL) producing bacteria in water sample

Out of 17 isolated bacteria from untreated water sample, 4 isolates were ESBL producing bacteria where 3 (17.64 %) of them were *E. coli* and 1 (5.88%) was *Klebsiella* spp. as shown in **Table 6.**

Table 6: Extended Spectrum β-Lactamase (ESBL) producing bacteria in untreated water sample

Total isolates	Screening positive	Coliform	Confirmed ESBL producing isolates	
			Number	Percentage
17	4	*Escherichia coli*	3	17.64%
		Klebsiella spp.	1	5.88%

Discussion

All collected untreated water samples (100%) exceed WHO guideline value. The coliform level of untreated water in the current investigation was comparable to the 100%coliform positive observed in the collected tube well water reported by Diwaker et al [19] and 100 % in collected stone spout water reported by Prasai et al. [20]. However, 96% coliform was reported in Kathmandu's irrigation water supply [21]. In this study, 15(60%) treated tap water were contaminated with coliform. Similar results were observed on a tap water in a study conducted by Roopavathi et al. [22]. Whereas, our result was in contrast from those of Maharjan et. al., [23] where 92% of Jar water and 77% of treated tanker water were contaminated with coliform.

Out of 17 isolates from untreated water sample, 7 (41.17 %) *E. coli* and 10 (58.8 %) *Klebsiella* spp. was isolated. Similar studies conducted reported lower number (19.77 %) *Klebsiella* spp. and (21.51 %) *E. coli* isolated from ground water [24]. Similarly, in a study conducted by Jayana et al. (20.4 %) *E. coli* and (5.6 %) *Klebsiella* were reported [25]. In case of treated one, 5 (55.5%) *Klebsiella* spp. and 4 (44.4 %) *E. coli* were isolated. The result was in contrast with Kumar & Kumari [26] where (28.44%) *E. coli* and (14.65%) *Klebsiella* spp. was seen from tap water. In our study, *Klebsiella* spp was predominating *E. coli*. Similar case was reported by [27, 28]. However, in similar studies in drinking water, *E. coli* was predominant than *Klebsiella* [26].

In the antibiotic susceptibility testing, most of isolates from untreated water were sensitive to antibiotics tested. Such as, all (100%) *E coli* isolates were sensitive to Meropenem, Cotrimoxazole, Amikacin, and 85.7% of isolates were sensitive to Ciprofloxacin, Tetracycline and 57.1% to Ceftriaxone. In a study conducted by Sarker et. al., [29], *E. coli* isolated from surface water is 100% susceptible to Ciprofloxacin and 60% susceptible to Tetracycline. *E. coli* isolated from rooftop rainwater showed 85% sensitivity to Ciprofloxacin which was similar to present study [30]. Similarly, *E. coli* isolated from raw water in the environment was sensitive to 73.7% Tetracycline, 79.7% Ciprofloxacin, and 93.2% Meropenem [31]. Abbas [27] reported that *E. coli* and *Klebsiella* spp. isolates from river water showed a 35.82% resistance to Ceftriaxone during an antimicrobial susceptibility test which was higher in comparison to present study (23.53%). Another study revealed that 27.8% of *E. coli* isolates from river and wastewater were resistant to Ceftriaxone [32].

Similarly, in treated sample also, less number of microorganisms were found resistant such that 0% resistant given by all *E coli* to tested antibiotics and only 1(20 %) *Klebsiella* were resistant to Amikacin only. Whereas, according to research conducted by Talukdar, et al., [33], *E. coli* isolates from domestic water samples showed resistance to tetracycline (45%), followed by ciprofloxacin (17%) and ceftriaxone (9%). However, Islam and Nili [29] revealed that 90% of *E. coli* isolates from bottled and tap water were resistant to Tetracycline Ciprofloxacin (40%). Similarly, Adefisoye and Okoh [35] revealed that 60.1% of the *E. coli* isolates from the treated water sample were resistant to Tetracycline which showed higher resistant rate than to present study. In another study, out of 24 *Klebsiella* spp., 4(16.67 %) isolates showed resistant to Ceftriaxone [36], whereas Ceftrixone resistant bacteria was absent in treated water sample in this study. The statistical analysis revealed that there was no signficant association between Antibiotic susceptibility pattern in treated and untreated water (Table 5).

Present study showed that out of 17 isolated bacteria from untreated water sample, 4 isolates showed ESBL positive where were 3 (17.64 %) *E. coli* and 1 (5.88 %) *Klebsiella* spp. As compare to several previous studies, Sharma, et al., [37] reported, 12.5% *E. coli* isolated from ground water produced ESBL which was less compared to present study. In a study conducted by Blaak, et al., [38], 44 % *E. coli* were ESBL producers. Similarly, Riaz, et al., [39] reported that 39.27% of isolated *E. coli* and 26.10 % of isolated *Klebsiella* spp. from environmental water sources were ESBL producers. In another study, Atta, et al., [40] revealed that 27.2% of the coliform isolated from hospital wastewater and recreational water contained ESBL-producer.

Some previous studies showed ESBL producing coliform even in treated water in contrast to present study, such that, Mahmud, et al., [41] revealed that 17% of the 384 isolates of *E. coli* from the drinking water sample were ESBL producing bacteria. Abera, et al., [42] showed that 9.4% of the 64 Enterobacteriaceae (*E. coli* and *Klebsiella* spp.) isolated from drinking water were ESBL producers. In another study, 8 (19.5%) out of 41 *E. coli* from drinking water were ESBL producers [43]. So, previous studies found varying percentages of ESBL producing coliforms in treated water samples which indicate a potential difference in the prevalence of these antibiotic-resistant bacteria in treated water.

Hence this study showed substantial bacterial contamination in both treated and untreated water sample in Kathmandu with untreated water showing higher number of coliform. Presence of ESBL producing isolates in untreated water possess potential public health risk associated with water borne transmission. Even treated water showed presence of coliform in considerable number. Thus this finding suggests need of continuous surveillance of water quality and antibiotic resistance.

Conclusion

Since coliform is a strong indicator of pathogenic microorganisms, the presence of coliform bacteria in 60% of the water sample collected suggests that pathogenic microorganisms may still be present in the treated water. Additionally, it implies that the water treatment methods now in use are still insufficient. None of the untreated water samples such as well and boring water was free from coliforms. Most of the isolates from both water types were sensitive to antibiotics used. ESBL producers were found from untreated water. In conclusion, microorganisms do not significantly differ in their pattern of antibiotic resistance in treated and untreated water, however, every household's tap water along with untreated water such as well and boring water should be regularly monitored for microbial quality and screened for antibiotic resistant pattern.

Author's contribution

RM conceptualized the research and edited the manuscript. SK, BY and SKD drafted the manuscript and carried out the laboratory work. RK edited the manuscript.

Competing interest

The authors declare that there is no competing interest.

Funding

The research work was funded by University grant commission, Sanothimi, Bhaktapur, Nepal as a part of Small Research, Development and Innovation (Small RDI) Grant (Grant number- SRDIG 2079/80 S&T 08).

Acknowledgement

We were thankful to University grant commission, Sanothimi, Bhaktapur, Nepal for providing grant as a part of Small Research, Development and Innovation (Small RDI) Grant. I would also like to express my gratitude to the teachers and staff of Department of Microbiology, Tri-Chandra Multiple campus for laboratory facilities and invaluable guidance and support throughout the course of this project.

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