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Impact of Chlorine Treatment on Antibiotic Resistance Pattern of Coliforms in Drinking Water Supplied in Kathmandu Valley

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Chlorine is the most widely used disinfectant for treating drinking water. However, some evidences suggest that it leads to bacterial resistance to different antibiotics. A descriptive cross-sectional study was conducted from the duration of June 2023 to May 2024 with the primary aim of determining the antibiotic resistance patterns in coliforms isolated from water before and after chlorine treatment. The chlorine resistance was also studied in the isolated coliforms. A total of 60 (26 untreated and 34 treated) water samples were processed for the isolation of total coliforms by the membrane filtration technique, and the isolates were identified from morphological and biochemical characteristics. Antibiotic resistance patterns of the coliforms were obtained by performing antibiotic susceptibility tests using the Kirby-Bauer disc diffusion method. Additionally, the isolates were exposed to various concentrations of chlorine for 10 minutes, 20 minutes, and 30 minutes to detect chlorine resistance in coliforms. Of all the water samples tested, 50 (83.3%) samples contained coliforms. Coliform detection was greater in untreated (96.1%) than in treated (73.5%) samples. The number of total coliforms in untreated samples was higher (0 to 1160 CFU/100 mL) than in treated samples (0 to 328 CFU/100 mL). In this study, *Klebsiella* spp. (13 from untreated and 8 from treated samples) and *E. coli* (13 from untreated and 19 from treated samples) were isolated. Antibiotic susceptibility test of the coliforms revealed that 50% of the *Klebsiella* spp. and 36.8% of *E. coli* isolated from treated water, whereas 69.2% of *Klebsiella* and 53.8% of *E. coli* from untreated water were resistant to ceftriaxone. Upon exposure of coliforms to chlorine for different time periods, 4, 2, and 1 coliform obtained from treated water showed growth in 50 ppm chlorine concentration in 10 min, 20 min, and 30 min, respectively, whereas 3, 2, and 1 coliform bacteria from untreated water showed growth at 50 ppm in 10 min, 20 min and 30 min respectively. This study could not show the impact of chlorine treatment on the antibiotic resistance of coliforms. However, continuous monitoring might be necessary to determine the cause of the spread of antibiotic resistance in bacteria.

Keywords: Coliform, Antibiotic susceptibility test, Chlorine resistance, Untreated water, Treated water

INTRODUCTION

Coliform bacteria are an indicator of water pollution. They are Gram-negative, facultative anaerobic, non-spore forming, motile or non-motile bacteria that produce acid and gas within 24 to 48 hours (1). The existence of fecal coliform bacteria in the water body specifies that the water has been polluted with the fecal substance of warm-blooded animals. Therefore, the enumeration of fecal bacteria is important for basic as well as applied research in aquatic microbial ecology and for drinking water quality assessment (2, 3). According to an estimate, 80% of all infections and over one-third of deaths in the developing countries are caused by the intake of polluted drinking water. As per the report of the World Health Organization (WHO) about 600 million cases of diarrhea and dysentery, and 46000 infant deaths were stated per year because of polluted water and insufficiency of sanitation (4). The levels of coliform bacteria present in the drinking water should not exceed the maximum permissible value of less than one cell per 100 ml of water set by the World Health Organization (5). Coliform bacteria include *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., and *Enterobacter* spp. *E. coli* is an opportunistic pathogen that can survive well in aquatic environments, and it is highly adapted to horizontal gene transfer, which is seen as the vector for antibiotic resistance dissemination (6, 7). *Klebsiella* spp. can cause nosocomial infections and may be a potential source of the organisms in hospital environments and other healthcare facilities (8). Depending upon the predominant species of chlorine, hypochlorous acid, and/or hypochlorite ion, disinfection with chlorine can achieve greater than 99.9% destruction of bacteria. For example, more than 99.9% inactivation of *E. coli* was achieved at the free chlorine concentration of 0.2 mg/L at a contact time of 0.5 minutes (19). However, it was also found that bacteria can survive high chlorine concentrations, such as

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2.0 mg/l free chlorine for 1h (20). Bacteria from chlorinated systems were more resistant than those from the unchlorinated waters (21). Lichevallier (22) suggested that the efficacy of the treatment of drinking water should be regularly monitored, as water treatment plants could not eliminate all coliforms. It was described that the frequent and overuse of sodium hypochlorite has led to the development of chlorine-resistant bacteria (23). Cross-resistance between disinfectants and antibiotics is also becoming widespread for bacterial pathogens (24). For example, a higher level of chlorine tolerance in antibiotic-resistant *Escherichia coli* was found when compared to antibiotic-susceptible strains (25).

Kathmandu Valley is facing a rapid increase in urbanization, population growth, and unmanaged waste disposal in water bodies. Considering the source, water is directly related to human activities. Similarly, chlorine has been utilized as a disinfectant for treating water for a long period of time. It would be necessary to study the antibiotic-resistant properties as well as the chlorine-resistant properties of bacteria from chlorinated water. Hence, the study aimed to determine the association of chlorine treatment and antibiotic resistance in treated water.

MATERIALS AND METHODS

Study design, sampling site, and sample size: A cross-sectional descriptive study was carried out from June 2023 to May 2024. The untreated samples (pre-chlorinated) were collected from Kathmandu Upatyaka Khanepani Limited (KUKL), water/waste quality assurance division, treatment and distribution plant, Mahankalchaur. Treated samples (post-chlorinated) were collected from different places in the Kathmandu valley. A total of 60 (26 untreated and 34 treated) water samples were collected and processed using standard protocol by APHA (26).

Sample collection: Treated tap water was allowed to run for 2 minutes before filling the sterile bottle, then the cap was replaced. The chlorine concentration was measured immediately at the sampling site using the chlorotex reagent. Treated samples were neutralized by using a sodium thiosulfate solution for the isolation of coliform. All samples were transported in a cold chain box and processed within 4 hours using the standard method of APHA (26) at the Department of Microbiology, Tri-Chandra Multiple College, Ghantaghar, Kathmandu.

Isolation and enumeration of coliforms: For isolation and enumeration of coliforms, a 100 ml water sample was filtered through a membrane filter of pore size 0.45 µm, then placed in M-Endo agar. The plates were incubated at 37°C for 24 hours. After incubation, the total number of coliforms was enumerated (26). Coliforms were identified by using standard microbiological techniques, including colony morphology, Gram-staining, and different biochemical tests. A single colony was transferred to nutrient broth and incubated at 37°C for 4 hours. Biochemical tests such as catalase, oxidase, indole, methyl red, Voges-Proskauer, and citrate, oxidation-fermentation test, and triple sugar iron agar test, were performed from the inoculum in nutrient broth. For the quality control, a purity plate was done (27).

Antibiotic Susceptibility Test: The antibiotic susceptibility test was performed using the modified Kirby-Bauer method. Antibiotics used were ciprofloxacin 5 mcg, cotrimoxazole 25 mcg, tetracycline 30 mcg, ceftriaxone 30 mcg, amikacin 30 mcg, and meropenem 10 mcg (28).

Chlorine-resistant test: Isolated bacteria were inoculated in nutrient broth and incubated at 37°C for 24 hours so that the cultures remain in the exponential growth phase. Bacterial concentration was standardized at 10^6–10^8 with an optical density of 0.5 at 600 nm. A stock solution of 10% or 100000 ppm calcium hypochlorite was prepared. Dilutions of 10000, 5000, 1000, 100, 50, 1, 0.5, and 0.1 ppm were prepared. Bacterial culture was inoculated in tubes containing different chlorine concentrations, with the exposure times 10 min, 20 min, and 30 min. An aliquot of 100 µl of 1M sodium thiosulfate was added to each tube to neutralize the effect of calcium hypochlorite and incubated at 37°C for 24 hours. The presence of growth was considered a positive result (29).

Data analysis: All data were entered in Microsoft Excel (2016), and statistical analysis was done by SPSS version 21.

RESULTS

Coliforms in treated and untreated water: Among the total 60 water samples, coliform bacteria were found in 50 (83.3%) samples. Among 34 treated samples, 25 (73.5%) samples were found to be coliform positive. Similarly, among 26 untreated water samples, 25 (96.1%) samples contained coliforms. Among untreated samples, water contained 0 to 1160 CFU/ 100 ml of total coliform, while in treated water, water contained 0 to 328 CFU/ 100 ml of total coliform (Table 1). Among the total water samples (60), 53 isolates were identified as coliform bacteria, of which 32 isolates (2 isolates identified in mixed culture) were identified as *E. coli* (50.8%) and 21 isolates (1 isolate identified in mixed culture) were identified as *Klebsiella* spp. (33.3%) (Table 1).

Antibiotic resistance pattern in coliforms isolated from treated and untreated water: Out of 8 *Klebsiella* spp. isolated from treated water, 4 (50%) isolates were resistant to ceftriaxone, followed by amikacin 1 (12.5%). Out of 19 *E. coli* from treated water, 7 (36.8%) were resistant to ceftriaxone, followed by ciprofloxacin 2 (10.52%) and tetracycline 2 (10.52%) (Table 2). From untreated water samples, out of 13 *Klebsiella* spp., 9 (69.2%) isolates were resistant to ceftriaxone, followed by ciprofloxacin 3 (23%), respectively. Out of 13 *E. coli*, 7 (53.8%) were resistant to ceftriaxone, followed by meropenem 1 (7.6%) and tetracycline 1 (7.6%) (Table 3).

The study found no association between antibiotic resistance among the isolates and the variability of water types (*p>0.05 = not significant) (Table 4).

Chlorine resistance in coliforms: From treated water samples, 18 isolates were tested for chlorine treatment at different concentrations. Growth of coliform bacteria was found at 0.1 ppm, 0.5 ppm, 1 ppm, 10 ppm, and 50 ppm. The highest number of coliform bacterial growth was found at 0.1 ppm, which was 18, 17, and 17 for 10 min, 20 min, and 30 min, respectively. A total of 4, 2, 1 isolates were resistant to 50 ppm of chlorine for 10, 20, 30 minutes, respectively (Table 5).

From untreated water samples, 17 isolates were tested for chlorine treatment at different concentrations. Growth of coliform bacteria was found at 0.1 ppm, 0.5ppm, 1ppm, 10 ppm, and 50 ppm. All coliform bacterial growth was found in 0.1 ppm, in which one isolate was found resistant up to 100 ppm. A total number of 3, 2, 1 isolates were resistant to 50 ppm of chlorine for 10, 20, 30 minutes, respectively (Table 6).

Table 1: Number of water samples with coliform bacteria in treated and untreated water samples.

Types of samples	Total number	Coliform-positive sample (percentage)	Total coliform (CFU/100 ml)		Types of coliform bacteria	
			Minimum	Maximum	*Klebsiella* spp.	*E. coli*
Treated	34	25 (73.5%)	0	328	8	19
Untreated	26	25 (96.1%)	0	1160	13	13
Total	60	50 (83.3%)	0	1488	21	32

Table 2: Antibiotic susceptibility pattern of *Klebsiella* spp. and *E. coli* isolated from treated water.

Antibiotics	*Klebsiella* spp. (n=8, %)			*E. coli* (n=19, %)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Meropenem (10 mcg)	8 (100%) 8	0 (0%)	0 (0%)	17 (89.4%)	1 (5.2%)	1 (5.2%)
Cotrimoxazole (25 mcg)	7 (87.5%)	1 (12.5%)	0 (0%)	18 (94.7%)	0 (0%)	1 (5.2%)
Tetracycline (30 mcg)	8 (100%)	0 (0%)	0 (0%)	16 (84.2%)	1 (5.2%)	2 (10.52%)
Amikacin (30 mcg)	7 (87.5%)	0 (0%)	1 (12.5%)	18 (94.7%)	1 (5.2%)	0 (0%)
Ciprofloxacin (30 mcg)	8 (100%)	0 (0%)	0(0%)	15 (78.9%)	2 (10.52%)	2 (10.52%)
Ceftriaxone (30 mcg)	3 (37.5%)	1 (12.5%)	4 (50%)	10 (52.63%)	2 (10.52%)	7 (36.8%)

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

Antibiotics	*Klebsiella* spp. (n=13, %)			*E. coli* (n=13, %)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Meropenem (10 mcg)	11 (84.6%)	1 (7.6%)	1 (7.6%)	12 (92.3%)	0 (0%)	1 (7.6%)
Cotrimoxazole (25 mcg)	12 (92.3%)	0 (0%)	1 (7.6%)	13 (100%)	0 (0%)	0 (0%)
Tetracycline (30 mcg)	11 (84.6%)	0 (0%)	1 (7.6%)	12 (92.3%)	0 (0%)	1 (7.6%)
Amikacin (30 mcg)	13 (100%)	0 (0%)	0 (0%)	13 (100%)	0 (0%)	0 (0%)
Ciprofloxacin (30 mcg)	9 (69.2%)	1 (7.6%)	3 (23.0%)	11 (84.6%)	2 (15.38%)	0 (0%)
Ceftriaxone (30 mcg)	3 (23.0%)	1 (7.6%)	9 (69.2%)	4 (30.7%)	2 (15.38%)	7 (53.8%)

Table 4: Association of antibiotic susceptibility of isolates with the types of samples.

Antibiotics	Water	Antibiotic Susceptibility				Chi-square	*P*-Value
		Sensitive	Resistance	Intermediate	Total		
Meropenem	Treated	25	1	1	27	.398	.820*
	Untreated	23	1	2	26		
	Total	48	2	3	53		
Cotrimoxazole	Treated	25	1	1	27	.981	.612*
	Untreated	25	0	1	26		
	Total	50	1	2	53		
Tetracycline	Treated	24	1	2	27	.002	.999*
	Untreated	23	1	2	26		
	Total	47	2	4	53		
Ciprofloxacin	Treated	25	1	1	27	2.001	.368*
	Untreated	26	0	0	26		
	Total	51	1	1	53		
Amikacin	Treated	23	2	2	27	.591	.744*
	Untreated	20	3	3	26		
	Total	43	5	5	53		
Ceftriaxone	Treated	13	3	11	27	2.708	.258*
	Untreated	7	3	16	26		
	Total	20	6	27	53		

*Note: * p>0.05 = not Significant and p<0.05 = Significant.*

Table 5: Resistance of coliform bacteria in different concentrations in treated water.

Time	Growth of Coliform bacteria	Concentrations (ppm) / Time exposure (minutes)								
		0.1	0.5	1	10	50	100	500	1000	5000
10 min	Growth	18	16	15	4	4	0	0	0	0
	No growth	0	2	3	14	14	18	18	18	18
20 min	Growth	17	16	15	2	2	0	0	0	0
	No growth	1	2	3	16	16	18	18	18	18
30 min	Growth	17	16	14	1	1	0	0	0	0
	No growth	1	2	4	17	17	18	18	18	18

Table 6: Resistance of coliform bacteria in different concentrations in untreated water.

Time	Growth of Coliform bacteria	Concentrations (ppm) / Time exposure (minutes)								
		0.1	0.5	1	10	50	100	500	1000	5000
10 min	Growth	17	15	13	3	3	1	0	0	0
	No growth	0	2	4	14	14	16	17	17	17
20 min	Growth	17	16	16	2	2	0	0	0	0
	No growth	0	1	1	15	15	17	0	0	0
30 min	Growth	17	16	14	1	1	0	0	0	0
	No growth	0	1	3	16	16	0	0	0	0

DISCUSSION

Among the samples, 50 (83.3%) water samples were found to be coliform positive. In this study, 73.5% treated and 96.1% untreated samples were found to be coliform positive. Similarly, Koju *et al*. (30) and Diwakar *et al*. (31) found 86 % and 82.76 % of total coliform in the water samples, respectively. In contrast, the study conducted in Madhyapur, Thimi reported 64.76% of total coliform (32) in water samples. In some studies, Shakya *et al*. (33), Bishankha *et al*. (34), Prasai *et al*. (35) observed 61.4 %, 56.1%, 92.4% of coliform, respectively, in tap water, which cross the guideline value of WHO for drinking water.

In this study, the coliform count in untreated water was found to be 0 to 1160 CFU/ 100 ml. In contrast, Karimi *et al*. (36) reported 16 to 8850 MPN/100 ml of coliforms in groundwater in Kenya. Similarly, Sarker *et al*. (37) reported between 3.4 × 10 to 4.8 × 10^{13} CFU/100 ml in pond water. A study in Dhaka, Bangladesh, it was ranged from 33 to 1×10^{3} CFU/100 ml in tap water (38). Likewise, Ferro *et al*. (39) showed that total coliform varied from 0 to 200 CFU/100 ml in tap water from Bagua.

Out of a total of 60 samples, 32 isolates were identified as *E. coli*, and 21 isolates were identified as *Klebsiella* spp. in 50 water samples. Among the 53 coliform bacteria, 2 *E. coli* and 1 *Klebsiella* spp. were isolated as mixed cultures from treated and untreated water samples, respectively. Prasai *et al*. (40) reported 26.4% *Escherichia coli*, and 5.4% *Klebsiella* spp. In a similar study by Shakya *et al*. (33) observed 10 different enteric bacteria, with *E. coli* being the most predominant, followed by *Pseudomonas* spp., *Citrobacter*, *Klebsiella* spp., *Shigella* spp., *Proteus* spp., *Enterobacter* spp., non-typhoidal *Salmonella*, *Providencia* spp., and *Edwardsiella* spp. Ideally, drinking water should be free of any pathogenic microorganisms or bacteria indicative of fecal pollution. The detection of fecal indicator bacteria in drinking water serves as a sensitive method for quality assessment, but it is not possible to identify every potential pathogen that may be present (5). From treated water, 8 *Klebsiella* spp. and 19 *E. coli* were identified. Out of 8 *Klebsiella* spp. from treated water, 4 (50%) isolates were resistant to ceftriaxone, followed by amikacin 1 (12.5%). On the other hand, out of 19 *E. coli* from treated water samples, 7 (36.8%) were resistant to ceftriaxone, followed by ciprofloxacin 2 (10.52%), and meropenem 1 (5.2%). Similarly, low levels of resistance (<10%) were seen on antibiotics ciprofloxacin, cotrimoxazole, gentamycin, ceftrixone, and no resistance to amikacin, meropenem Daly (41). However, Larson et al. (42) observed that 21.4% of *E. coli* from drinking water were cotrimoxazole resistant. *Enterobacter* spp. was the predominant isolate, were resistance to erythromycin (79.5%), followed by 62.67% penicillin, 61.9% amoxycillin, 34.5% ampicillin, 21.1% tetracycline, 15.4% ceftriaxone, and 14.7% amikacin (32). In untreated water, a total of 13 *Klebsiella* spp. and 13 *E. coli* were identified. Among the *Klebsiella* spp. isolates, 9 (69.2%) isolates resistance to ceftriaxone, followed by 3 (23.1%) resistant to ciprofloxacin, and 2

(7.6%) resistant to meropenem, cotrimoxazole, and tetracycline. Similarly, 7 (53.8%) *E. coli* isolates were resistant to ceftriaxone, with 1 (7.6%) *E. coli* resistance to meropenem and tetracycline. The presence of various pollutants such as antibiotics, pesticides, and insecticides in the environment contributes to the spread of antibiotic resistance in drinking water sources (43). In a study of natural water sources, it was also found that 50% of *E. coli* isolates were resistant to ampicillin and cefixime, while 50% of *Klebsiella* spp. showed resistance to tetracycline and cotrimoxazole (44).

In this study, there are no significant differences in antibiotic-resistant patterns in chlorinated (treated) and unchlorinated water. However, Khan *et al.* (45) showed that chlorination can increase the abundance of antibiotic-resistant bacteria (ARB). They also explained that there is a correlation between the resistance of chlorine-resistant bacteria (CRB) to chlorine disinfectants and antibiotics, which indicates that the presence of CRB leads to an increase in the abundance of antibiotic resistance genes (ARGs) after disinfection. Most of the isolates in this study were resistant to ceftriaxone. In the survey of antimicrobial resistance (AMR) in *E. coli* in the Netherlands, 17.1% of Extended-Spectrum Beta-Lactamase (ESBL) *E. coli* isolated from river water and wastewater were reported as pathogenic, with around 84% of the isolates exhibiting resistance to three drug classes, including beta-lactams, tetracyclines, and aminoglycosides (46).

In the treated water samples, a total of 4, 2, and 1 coliform bacterium showed growth at 50 ppm chlorine concentration in 10 mins, 20 mins, and 30 mins, respectively (Table 6). On the other hand, a total of 3, 2, and 1 coliform bacterium showed growth at 50 ppm, in 10 mins, 20 mins, and 30 mins, respectively (Table 7). The research conducted by Najmuldeen (47) found 5 isolates of *Enterobacter* were resistant to 25 mg/l chlorine, 3 isolates to 50 mg/l, 4 isolates to 100 mg /l, 2 isolates to 200 mg/l, 1 isolate to 300 mg/l, and 2 isolates were fully resistant to 400 mg/l, respectively. A Similar finding was reported by Mohammad *et al.* (48) in which the bacteria from post-chlorinated water samples were more resistant to chlorine disinfection compared to pre-chlorinated water samples. Furthermore, a recent study reported that a lethal dose of chlorine rapidly inactivates fecal coliforms like *E. coli,* but leaves a little effect on chlorine-resistant *Enterococcus faecalis* unaffected (38).

Resistant to different concentrations of chlorine might be due to different factors, like natural transformation of antibiotic-resistant genes and mobile genetic elements. Even the naked DNA from chlorine-killed bacteria may transform to chlorine-injured, cultivable species at a rate of more than 550-fold in comparison to the untreated one (38). A review suggests that antimicrobial genes concurrently occur with other genes that promote resistance to various harmful chemicals like disinfectants, a phenomenon also called co-selection (49).

CONCLUSIONS

Drinking water samples in Kathmandu Valley contained a high number of coliforms, with untreated water showing greater contamination than treated water. The presence of *E. coli* and *Klebsiella* spp. even in treated water indicates fecal contamination in the drinking water distribution system, and potential public health risks. Resistance of coliforms to antibiotics did not significantly differ between treated and untreated water, suggesting widespread environmental dissemination of antibiotic-resistant bacteria. Chlorine resistance at high chlorine concentration was observed in coliforms, which might indicate the presence of chlorine-tolerant strains in the drinking water distribution system. No significant correlation between chlorine treatment and antibiotic resistance was observed. However long-term study would be needed to find out the causes of antibiotic resistance in bacteria.

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CONFLICTS OF INTEREST

The authors have declared no conflict of interest.

REFERENCES

1. Park K. 2000. Park's textbook of preventive and social medicine. 16th ed. Banarsidas Bhanot, Jabalpur, p. 495.
2. Curtis T. 2003. Bacterial pathogen removal in wastewater treatment plants. In: Mara D, Horan N, editors. Handbook of water and wastewater microbiology. Academic Press, London, pp. 477–490.
3. Korajkic A, McMinn BR, Harwood VJ. 2018. Relationships between microbial indicators and pathogens in recreational water settings. International Journal of Environmental Research and Public Health, 15:2842.
4. Singh RK, Iqbal S, Seth PC. 2001. Bacteriological pollution in a stretch of river Narmada at Hoshangabad, Madhya Pradesh. Pollution Research, 20:211–213.
5. World Health Organization. 1993. Guidelines for drinking-water quality. Vol. II. WHO, Geneva.
6. Maal-Bared R, Bartlett KH, Bowie WR, Hall ER. 2013. Phenotypic antibiotic resistance of Escherichia coli and E. coli O157 isolated from water, sediment and biofilms in an agricultural watershed in British Columbia. Science of the Total Environment, 443:315–323.
7. Pereira A, Santos A, Tacão M, Alves A, Henriques I, Correia A. 2013. Genetic diversity and antimicrobial resistance of Escherichia coli from the Tagus estuary, Portugal. Science of the Total Environment, 461:65–71.
8. Collee JG, Miles RS, Watt B. 1996. Tests for the identification of bacteria. In: Collee JG, Marmion BP, Fraser AG, Simmons A, editors. Mackie & McCartney practical medical microbiology. 14th ed. Churchill Livingstone, New York, pp. 131–151.
9. Khan S, Beattie TK, Knapp CW. 2016. Relationship between antibiotic and disinfectant resistance profiles in bacteria harvested from tap water. Chemosphere, 152:132–141.
10. McCurry DL, Krasner SW, von Gunten U, Mitch WA. 2015. Determinants of disinfectant pretreatment efficacy for nitrosamine control in chloraminated drinking water. Water Research, 84:161–170.

11. Nwachcuku N, Gerba CP. 2004. Emerging waterborne pathogens: can we kill them all? Current Opinion in Biotechnology, 15:175–180.
12. Liu SS, Qu HM, Yang D, Hu H, Liu WL, Qiu ZG, et al. 2018. Chlorine disinfection increases both intracellular and extracellular antibiotic resistance genes in a full-scale wastewater treatment plant. Water Research, 136:131–136.
13. Shi P, Jia S, Zhang XX, Zhang T, Cheng S, Li A. 2013. Metagenomic insights into chlorination effects on microbial antibiotic resistance in drinking water. Water Research, 47:111–121.
14. Xi C, Zhang Y, Marrs CF, Ye W, Simon C, Foxman B, et al. 2009. Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Applied and Environmental Microbiology, 75:5714–5718.
15. Zhang S, Wang Y, Lu J, Yu Z, Bond PL, Guo J. 2021. Chlorine disinfection facilitates natural transformation through ROS-mediated oxidative stress. ISME Journal, 15:2969–2985.
16. Datta N. 1969. Drug resistance and R-factors in the bowel bacteria of London patients before and after admission to hospital. British Medical Journal, 2:407–411.
17. Farrar WE Jr, Eidson M, Guerry P, Falkow S, Drusin LM, Roberts RB. 1972. Interbacterial transfer of R-factor in the human intestine. Journal of Infectious Diseases, 126:27–33.
18. Jin M, Liu L, Wang DN, Yang D, Liu WL, Yin J, et al. 2020. Chlorine disinfection promotes the exchange of antibiotic resistance genes across bacterial genera by natural transformation. ISME Journal, 14:1847–1856.
19. Helbling DE, VanBriesen JM. 2007. Free chlorine demand and cell survival of microbial suspensions. Water Research, 41:4424–4434.
20. Adefisoye MA, Olaniran AO. 2022. Does chlorination promote antimicrobial resistance in waterborne pathogens? Antibiotics, 11:564.
21. Geldreich EE. 1996. Microbial quality of water supply in distribution systems. CRC Press, Boca Raton, FL.
22. LeChevallier MW. 1990. Coliform regrowth in drinking water: a review. Journal of the American Water Works Association, 82:74–86.
23. Kamal MA, Khalaf MA, Ahmed Z, Jakee JE. 2019. Evaluation of the efficacy of commonly used disinfectants against chlorine-resistant strains from drinking water used in Egyptian cattle farms. Veterinary World, 12(12):2025–2035.
24. Carey DE, McNamara PJ. 2015. The impact of triclosan on the spread of antibiotic resistance in the environment. Frontiers in Microbiology, 5:780.
25. Templeton MR, Oddy F, Leung WK, Rogers M. 2013. Chlorine and UV disinfection of ampicillin- and trimethoprim-resistant Escherichia coli. Journal of Environmental Engineering, 139(2):231–237.
26. American Public Health Association. 2017. Standard methods for the examination of water and wastewater. 23rd ed. APHA, Washington DC.
27. Cheesbrough M. 2012. District laboratory practice in tropical countries. 2nd ed. Cambridge University Press, India.
28. Clinical and Laboratory Standards Institute. 2021. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100. 31st ed. CLSI, USA.
29. Al-Berfkani MI, Zubair AI, Bayazed H. 2014. Assessment of chlorine-resistant bacteria and their susceptibility to antibiotics from water distribution systems in Duhok Province. Journal of Applied Biology and Biotechnology, 2(6):10–13.
30. Koju N, Prasai TP, Joshi T, Shrestha S, Raut P. 2015. Drinking water quality of Kathmandu Valley. Nepal Journal of Science and Technology, 15(1).
31. Diwakar J, Yami KD, Prasai T. 2008. Assessment of drinking water of Bhaktapur municipality area in pre-monsoon season. Scientific World, 6:94–98.
32. Jayana BL, Prasai T, Singh A, Yami KD. 2010. Assessment of drinking water quality of Madhyapur-Thimi and study of antibiotic sensitivity of bacterial isolates. Nepal Journal of Science and Technology, 10:167–172.
33. Shakya P, Joshi TP, Joshi DR, Bhatta DR. 2013. Evaluation of physicochemical and microbiological parameters of drinking water supplied from distribution systems of Kathmandu Municipality. Nepal Journal of Science and Technology, 13(2):179–184.
34. Bishanka S, Joshi TP, Joshi DR, Bhatta DR. 2012. Assessment of microbial quality of chlorinated tap water and susceptibility of Gram-negative bacterial isolates towards chlorine. Nepal Journal of Science and Technology, 13(1):173–178.
35. Prasai T, Lekhak B, Joshi DR, Baral MP. 2007. Microbiological analysis of drinking water of Kathmandu Valley. Scientific World, 5:112–114.
36. Karimi KJ, Ngumba E, Ahmad A, Duse AG, Olago D, Ndwigah SN, et al. 2023. Contamination of groundwater with sulfamethoxazole and antibiotic-resistant Escherichia coli in informal settlements in Kisumu, Kenya. PLOS Water, 2(4):e0000076.
37. Sarker S, Mahmud S, Sultana R, Biswas R, Sarkar PP, Munayem MA, et al. 2019. Quality assessment of surface and drinking water of Nakla Paurosova, Sherpur, Bangladesh. Advances in Microbiology, 9(8):703–727.
38. Nitol S, Kabir MM, Sultana F, Sultana N, Hossain MS, Saadat AHM. 2016. Quality analysis of potable water at Dakshin Khan in Dhaka City, Bangladesh. Journal of Biodiversity and Environmental Sciences, 8:1–10.
39. Ferro P, Morales E, Ticona E, Ferró-Gonzales P, Oblitas A, Ferró-Gonzáles AL. 2024. Water quality and phenotypic antimicrobial resistance of Escherichia coli isolated from water for human consumption under a One Health approach. Heliyon, 10(1):e23961.
40. Prasai T, Lekhak B, Joshi DR, Baral MP. 2010. Microbiological analysis of drinking water of Kathmandu Valley. Scientific World, 5(5):212–214.
41. Daly M, Powell J, O'Connell NH, Murphy L, Dunne CP. 2023. Antimicrobial resistance is prevalent in Enterobacterales isolated from public and private drinking water supplies in Ireland. Microorganisms, 11(5):1224.
42. Larson A, Hartinger SM, Riveros M, Salmon-Mulanovich G, Hattendorf J, Verastegui H, et al. 2019. Antibiotic-resistant Escherichia coli in drinking water from rural Andean households in Peru. American Journal of Tropical Medicine and Hygiene, 100(6):1363–1370.
43. Zhao J, Dang H. 2011. Identification of a globally distributed streptomycin-resistance plasmid in a coastal bay of China. Letters in Applied Microbiology, 52:1–8.
44. Poonia S, Singh TS, Tsering DC. 2014. Antibiotic susceptibility profile of bacteria isolated from natural water sources in East Sikkim. Indian Journal of Community Medicine, 39:156–160.
45. Khan S, Beattie TK, Knapp CW. 2016. Relationship between antibiotic and disinfectant resistance profiles in bacteria harvested from tap water. Chemosphere, 152:132–141.
46. Franz E, et al. 2015. Pathogenic Escherichia coli producing extended-spectrum beta-lactamases isolated from surface water and wastewater. Scientific Reports, 5:14372.
47. Najmuldeen H, Rashid H. 2021. Assessment of chlorine-resistant Enterobacter cloacae isolated from water storage tanks in Sulaimaniyah City, Iraq. Passer Journal of Basic and Applied Sciences, 3(1):87–94.
48. Al-Berfkani MI, Zubair AI, Bayazed H. 2014. Assessment of chlorine-resistant bacteria and their susceptibility to antibiotics from water distribution systems. Journal of Applied Biology and Biotechnology, 2(6):10–13.
49. Reyes-Fernández EZ, Schuldiner S. 2020. Acidification of cytoplasm in Escherichia coli provides a strategy to cope with stress and facilitates development of antibiotic resistance. Scientific Reports, 10:9954.