Original article

Detection of coliform bacteria in irrigation water and on vegetable surfaces in the Kathmandu Valley of Nepal

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Abstract

Introduction: Consumption of vegetables irrigated using polluted water is widespread in the Kathmandu Valley of Nepal. However, studies on the microbial analysis of water and vegetable samples from this region are limited. In this study, irrigation water and vegetable samples from farmers' fields in the Kathmandu Valley were examined for the presence of *Escherichia coli* and total coliforms.

Methods: Irrigation water (n = 8) and vegetable (n = 27) samples were collected from November to December 2015. The presence of *E. coli* and total coliforms in water and on vegetable surfaces was determined by the most probable number (MPN) method using Colilert reagent. In addition, information about vegetable washing and consumption was obtained through a survey to discuss changes in their microbial concentrations before selling and/or consumption.

Results: *E. coli* was detected in 75% (6/8) of the water samples, with concentrations ranging from 8.8×10^3 to 5.2×10^7 MPN/100 ml, whereas total coliforms were detected in all the 8 water samples, with concentrations ranging from 9.7×10^2 to 7.9×10^7 MPN/100 ml. *E. coli* was similarly detected in 7% (2/27) of the vegetable samples, with concentrations ranging from 0.4 to10.2 MPN/cm^2, whereas total coliforms were detected in 59% (16/27) of the vegetable samples, with concentrations ranging from 0.4 to 448 MPN/cm^2. Well, river, and tap were the predominant sources of water for washing vegetables before selling and/or consumption.

Conclusions: Unlike water samples, vegetable samples contained low microbial contamination; however, the level of contaminants was expected to increase because of washing with polluted water.

Keywords: *E. coli*, irrigation water, Kathmandu, total coliforms, vegetable contamination

Introduction

Because of inadequate sewage networks and treatment plants, nearly 75% of wastewater generated in the Kathmandu Valley of Nepal is drained into waterways without any treatment or after partial treatment, causing water resource pollution.[1] Irrigating vegetables by pumping water from polluted rivers and/or untreated sewage is common in the valley.[2,3] Contamination of vegetables can occur if polluted water comes in contact with the edible portion of the vegetable, which may lead to health risks in consumers.[4] To assess health risks, data on the concentration of pathogens in irrigation water and on vegetables are important. Several studies have reported the detection of waterborne pathogens in irrigation water, including well, river, and sewage water[2,3,5] and/or in some vegetables.[6,7] However, measurements of contaminants on vegetables are rare and the pervasiveness of pathogen contaminants on various types of vegetables in the valley is still unknown.

Given this background, the objective of this study was to determine the occurrence of *E. coli* and total coliforms in irrigation water and on vegetable samples from farmers' fields in the Kathmandu Valley. We performed a recovery test of *E. coli* from vegetable surfaces to examine recovery ratios, and we determined concentrations of *E. coli* and total coliform in river water, groundwater, and sewage samples as well as on vegetable samples. In addition, the effect of washing on vegetable contamination

was discussed based on the results from farmers' and consumers' surveys.

Methods

Recovery test of *E. coli* from vegetable surface

Preparation of E. coli suspension for inoculation

E. coli strain K12 (NRBC3301) was used to prepare an *E. coli* suspension of known concentration [approximately 10^5 colony-forming units (CFU)/ml] for inoculation to vegetable surfaces. In brief, E. coli K12 colonies were mixed with 9 ml of LB broth base (Invitrogen, California, USA) in a tube and subjected to shaking at 37°C for 3–4 h. Then, the *E. coli* suspension was centrifuged at 7000 × *g* for 5 min at 4°C. After centrifugation, the supernatant was discarded, and 10 ml of phosphate-buffered saline [PBS (−)] was added to the pellet. The expected concentration of the *E. coli* suspension in the tube was approximately 10^8 CFU/ml. Ten microliters of the *E. coli* suspension was added to another tube containing 10 ml PBS (−) to dilute the suspension to approximately 10^5 CFU/ml.

Inoculation of E. coli suspension

Carrot, cabbage, tomato, and spinach (n = 4 for tomato, n = 5 for others) were bought from a supermarket in Japan. Ten microliters of the *E. coli* suspension was inoculated to 25 cm^2 of the vegetable surfaces. Spot inoculation was performed.

Recovery of E. coli from vegetable surfaces

BM Fukitool A kit (GSI Creos, Tokyo, Japan) containing a wiping cotton and 10 ml of PBS (−) as an elution buffer was used to recover *E. coli* from vegetable surfaces. About 10 min after inoculation, vegetable surfaces were wiped with cotton over a 25 cm^2 area, and the cotton was then mixed in the elution buffer by vortexing. The wiping and vortexing was repeated 3 times. The process was repeated for non-inoculated vegetable samples as well to determine the concentration of indigenous *E. coli.*

Detection of E. coli

E. coli in the vegetable extract was determined by a culture method using Chromocult Coliform agar (Merck Millipore, Darmstadt, Germany). In short, 1 ml of the vegetable extract solution (10^0, 10^1 and 10^2 dilutions each) was added to a petri dish (2 replications each). Then, 10–15 ml of Chromocult Coliform agar was poured into the petri dish and incubated at 37°C for 24 h. After incubation, blue colonies were counted as *E. coli.* The process was repeated for the vegetable extract solution (no dilution) obtained from non-inoculated vegetable samples as well as for blank samples (1 replication) containing only agar solution with no vegetable extract solution. The same method was used to determine the concentration of the *E. coli* suspension used for inoculation using the 10^2, 10^3, and 10^4 dilutions of the suspension each.

Calculation of recovery ratios

Recovered *E. coli* number (CFU) in the vegetable extract (10 ml) was divided by actual *E. coli* number (CFU) in the inoculum (10 µl) to calculate recovery ratios. First, the dilution ratio to be used for calculating the total number of *E. coli* inoculated and/or recovered was determined based on the number of *E. coli* colonies (CFU/ml) counted in the petri dish. Dilution ratios that resulted in more than 300 *E. coli* colonies were discarded, whereas the dilution ratio that resulted the highest number of *E. coli* colonies that was less than 300 was selected for further calculation. To obtain total *E. coli* concentration (CFU/ml) in the vegetable extract and/or the inoculum, the selected dilution ratio was multiplied by the corresponding number of *E. coli* (CFU/ml) counted in the petri dish. Then, the total *E. coli* concentrations in the vegetable extract and inoculum were multiplied by 10 ml and 0.1 ml, respectively, to obtain the total numbers of *E. coli* recovered and inoculated.

Detection of *E. coli* and total coliforms in irrigation water and on vegetable samples in Nepal

Collection of water and vegetable samples

Twenty seven vegetable samples [spinach (n = 9), cabbage (n = 6), carrot (n = 6), and tomato (n = 6)] grown in 8 farmers' fields and 8 irrigation water samples used by these farmers [river water (n = 4), groundwater (n = 2), and sewage (n = 2)] were collected between November 30 and December 2, 2015, from Bhaktapur, Madhyapur Thimi, and Kirtipur municipalities of the Kathmandu Valley and brought to the laboratory of the Institute of Medicine, Tribhuvan University, for detection of *E. coli* and total coliforms.

Detection of E. coli and total coliforms in water samples

Total coliforms and *E. coli* were determined by the most probable number (MPN) method using Colilert reagent (IDEXX Laboratories, USA). This method has been successfully used by previous researchers.[2,8] In brief, 1 vial of Colilert reagent was added to 100 ml of diluted water sample (dilutions: 10^2, 10^4, and 10^6 each) in an autoclaved glass bottle. After mixing, the sample was poured into a Quanti-Tray, sealed, and incubated at 37°C for 24 h. After incubation, wells that were yellow were considered to contain total coliforms and wells that turned fluorescent blue when exposed to ultraviolet light were considered to contain *E. coli.* The concentrations of *E. coli* and total coliforms were calculated based on the number of blue and yellow wells, respectively, using IDEXX MPN Generator version 1.4 software. The limit of detection (LOD) was 2.0 $\log_{10}$ MPN/100 ml.

Detection of E. coli and total coliforms in vegetable samples

First, *E. coli* and total coliforms present over 25 cm^2 of vegetable surface were extracted into 10 ml elution buffer by regular wiping and vortexing using BM Fukitool A kit according to the manufacturer's protocol as described above. One milliliter of the eluate was then diluted to 100 ml and concentrations of *E. coli* and total coliforms were determined using the MPN method as described above for the water samples. The LOD was 0.4 MPN/cm^2. Back calculation using recovery ratios was not performed.

Farmers' and consumers' household surveys

Information about vegetable consumption, irrigation water sources, and vegetable washing prior to selling and/or consumption were obtained using a structured survey answered by farmers' households (n = 224) in April 2015 and consumers' households (n = 1136) in January–April 2015 in the Kathmandu Valley. Ethical approval and informed consent were obtained from the Nepal Health Research Council and interviewees, respectively, prior to distribution of the survey.

Results

Recovery ratio of *E. coli* from vegetable surfaces

Table 1 shows recovery ratios of *E. coli* from vegetable surfaces. The mean recovery ratios were found to be the highest for spinach (83 ± 23%, n = 5), followed by cabbage (58 ± 21%, n = 5), tomato (42 ± 17%, n = 4), and carrot (30 ± 11%, n = 5).

Table 1. Recovery ratio of *E.coli* from vegetable surfaces determined using BM Fukitool A kit

Vegetables	Number of samples	% recovery (mean ± sd)
Cabbage	5	58 ± 21
Carrot	5	30 ± 11
Spinach	5	83 ± 23
Tomato	4	42 ± 17

sd: standard deviation

Prevalence of *E. coli* and total coliforms in irrigation water and vegetable samples

Total coliforms were detected in all 8 water samples, whereas *E. coli* was detected in 6 (75%) samples. As shown in Table 2, total coliform concentrations were the highest for sewage (7.80–7.90 $\log_{10}$ MPN/100 ml), followed by rivers (4.76–7.15 $\log_{10}$ MPN/100 ml) and ground water (2.99–3.37 $\log_{10}$ MPN/100 ml). *E. coli* concentrations were similarly high for sewage (7.53–7.72 $\log_{10}$ MPN/100 ml) and rivers (3.95–5.75 $\log_{10}$ MPN/100 ml); however, *E. coli* was not detected in groundwater.

In contrast, out of 27 vegetable samples, total coliforms and *E. coli* were detected in 16 (59%) and 2 (7%) samples, respectively, with average concentrations ranging from 0.4 to 145 MPN/cm^2 for total coliforms and 0.4 to 10 MPN/cm^2 for *E. coli* (Table 3). Contamination levels of total coliforms were the highest for spinach, followed by cabbage, carrot, and tomato.

Table 2. Prevalence of *E. coli* and total coliforms in irrigation water samples

Irrigation water	Location	Total coliforms concentration ($\log_{10}$ MPN/100 ml)	*E. coli* concentration ($\log_{10}$ MPN/100 ml)
Groundwater	Salyansthan, Kirtipur	3.37	<2.0*
	Manohara, Thimi	2.99	<2.0*
River water	Balkhu River, Salyansthan, Kirtipur	4.76	3.95
	Manohara River, Manohara, Thimi	6.19	5.75
	Kalacha River, Kalacha, Bhaktapur	7.15	5.38
	Hanumante River, Jagati, Bhaktapur	5.38	5.30
Sewage	Chisa Tole, Kirtipur	7.80	7.53
	Sunga, Thimi	7.90	7.72

*: not detected, MPN: most probable number

Table 3. Prevalence of *E.coli* and total coliforms in vegetable samples

		Cabbage	Carrot	Spinach	Tomato	Total
Detection ratio, % (no. of positive samples/no. of tested samples)	Total coliforms	50 (3/6)	100 (6/6)	56 (5/9)	33 (2/6)	59 (16/27)
	E. coli	0 (0/6)	0 (0/6)	11 (1/9)	17 (1/9)	7 (2/27)
Average concentration of detected samples (MPN/cm2)						
	Total coliforms	60.5	21	145	0.4	
	E. coli	<0.4†	<0.4†	10	0.4	

†: not detected, MPN: most probable number

Results of survey

Results of surveys answered by farmers and consumers in the valley showed that washing of vegetables is a common activity by farmers prior to selling and by consumers prior to consumption. Survey results from the farmers indicated that 46%, 15%, and 37% used well, river, and tap, respectively, as their water source for washing vegetables before selling. Similarly, 61%, 26%, and 12% of the consumers used tap, well, and tanker, respectively, as their water source for washing vegetables before consumption. Wells and rivers were the dominant sources of irrigation water. Vegetables were the predominant crops in the valley in terms of area cropped and irrigation use. Vegetables such as spinach, carrot, cauliflower, radish, tomato, and cucumber were frequently produced and consumed in the valley.

Discussion

Use of the BM Fukitool A kit showed good recovery of *E. coli* from vegetable surfaces (mean recovery: 30–83%), and recovery ratios were comparatively higher for leafy vegetables, such as spinach and cabbage, than tomato and carrot (root vegetables) (Table 1). This may be because of differences in size, shape, and surface texture of these vegetables. The large leaf size and rough texture of leafy vegetables may have reduced losses during the recovery test. On the other hand, the small round shape of tomatoes and small conical shape of carrots, both with smooth surfaces, may have increased losses during the recovery test. The recovery ratios obtained in this study were also comparable with those obtained in a previous study that had adopted different recovery techniques, where recovery ratios of poliovirus from vegetables such as spinach, cabbage, lettuce, and celery varied from 36% to 74%.[9]

As shown in Table 2, 7 out of 8 water samples contained levels of bacteria that exceeded the levels allowable according to the water quality criteria and standards proposed for the Bagmati River and its tributaries, wherein total coliforms should be less than 3 $\log_{10}$ MPN/100 ml for use in agriculture.[10] Only 1 groundwater sample satisfied the standards. The World Health Organization (WHO) standards for *E. coli* with respect to wastewater use for root (≤3 $\log_{10}$ counts/100 ml) and leafy (≤4 $\log_{10}$ counts/100 ml) vegetables[11] similarly exceeded in 6 and 5 of the samples, respectively. *E. coli* was not detected in any of the groundwater samples (Table 2). Very high concentrations of *E. coli* and total coliforms in river samples, almost similar to those in sewage samples, suggested considerable contamination of river water from sewage. Poor quality of river water in the Kathmandu Valley has also been reported by previous researchers.[2,3,5]

Despite high contamination of irrigation water samples, vegetable samples from the farmers' fields showed low concentrations and detection ratios of *E. coli* and total coliforms (Table 3). This may be because of irrigation methods. Irrigation in the valley is commonly performed by pumping water from irrigation sources and pouring itinto a ditch between 2 rows of vegetables. This may have reduced direct contact of polluted water with vegetable surfaces, resulting in less contamination. Produce contamination by human pathogens is high if the produce is in direct contact with contaminated water, whereas fresh produce contamination via root uptake of human pathogens is not well understood.[4] Of 8 farmers, 1 farmer had manually irrigated spinach by pouring sewage water from a bucket, resulting in direct contact. High concentrations of *E. coli* and total coliforms were obtained from spinach grown in this farmer's field, which suggested a strong relationship between irrigation method and vegetable contamination. Total coliforms were similarly detected in all 6 carrot samples (Table 3), which may be because of its underground growth where it is in direct contact with contaminated water. Polluted water can contaminate soils, heightening produce contamination.[12]

In addition to well and river water, microbial contamination has also been reported in tap water samples in the Kathmandu Valley.[13,14,15] As mentioned before, these water sources are commonly used for

washing vegetables before selling and/or consumption in the valley. Polluted water can contaminate vegetables as a result of washing.Thus, there are health risks associated with the consumption of vegetables washed with polluted water. The risks may be even higher for vegetables that are consumed raw. The survey results showed that carrot, radish, and cucumber are frequently consumed raw and that cabbage, tomato, and spinach are sometimes consumed raw in the valley. Further studies will focus on microbial health risks in humans from consumption of vegetables irrigated and/or washed with contaminated water.

Conclusion

In this study, the presence of *E. coli* and total coliforms was measured for 8 irrigation water samples (well, river, and sewage) and 27 vegetable samples (cabbage, carrot, spinach, and tomato) from 8 farmers' fields in Kirtipur, Madhyapur Thimi, and Bhaktapur municipalities in the Kathmandu Valley of Nepal during November/December 2015. Inoculation and recovery test of *E. coli* by BM Fukitool A kit on/from vegetable surfaces was also performed, and the highest and lowest recovery ratios were obtained for spinach (83 ± 23%, n = 5) and carrot (30 ± 11%, n = 5), respectively. All water samples, except 1 from well water, had total coliform concentrations that were higher than the guideline value (3 $\log_{10}$ MPN/100 ml) in Nepal. Unlike water samples, detection ratio and concentrations of *E. coli* and total coliforms in the vegetable samples were low. However, farmers' and consumers' household survey results showed that vegetable contamination in the valley was expected to increase as a result of washing vegetables using contaminated water prior to selling and/or consumption.

Conflict of interest: None declared.

Acknowledgements

This study was supported by Science and Technology Research Partnership for Sustainable Development (SATREPS) program under Japan Science and Technology Agency (JST) and Japan International Cooperation Agency (JICA), entitled 'Hydro-microbiological Approach for Water Security in Kathmandu Valley, Nepal'.

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