

Received:
8 November 2018
Revised:
8 January 2019
Accepted:
30 January 2019

Cite as: Sunzid Ahmed,
Md. Abubakkar Siddique,
Matiur Rahman,
Md. Latiful Bari,
Shahnila Ferdousi. A study on
the prevalence of heavy
metals, pesticides, and
microbial contaminants and
antibiotics resistance
pathogens in raw salad
vegetables sold in Dhaka,
Bangladesh.
Heliyon 5 (2019) e01205.
doi: [10.1016/j.heliyon.2019.
e01205](https://doi.org/10.1016/j.heliyon.2019.e01205)

A study on the prevalence of heavy metals, pesticides, and microbial contaminants and antibiotics resistance pathogens in raw salad vegetables sold in Dhaka, Bangladesh



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Abstract

The presence of undesirable heavy metals, pesticide residues, and microbial contaminants in fresh produces is a worldwide public health concern. This study was undertaken to evaluate the residual pesticides (Diazinon, Malathion, Cypermethrin, Dimethoate, Quinalphos, and Chloropyrifos), heavy metal contamination (Pb, Cd, and Cr), and microbiological quality and safety of 4 common raw salad vegetables (RSVs) samples from different local markets in Dhaka. Results showed the presence of heavy metals residues were within the acceptable limits of local and international standards. None of the above-mentioned pesticides were found in tomato and cucumber samples but presence of Dimethoate was noticed in 13 coriander samples (12.94–158.3 µg/kg) and 7 lettuce samples (9.6–74.8 µg/kg) exceeding the maximum permissible limit of

EU guideline. The microbiological analysis showed irrespective of RSV types, total aerobic bacteria was present in higher number (4.0–7.0 log CFU/g), whereas 3.36–5.57 log CFU/g coliform count was recorded. In comparison with retail markets, lower level of total aerobic, and coliform bacterial presence was observed in the samples collected from sophisticated shops, but presence of *E. coli* and *Salmonella* spp. were evident in more than 60% samples in these shops. However, 50% and 33% samples from wholesale and retail markets respectively were noticed to be contaminated with *Staphylococcus* spp. Irrespective of RSV types, isolated *E. coli* were found resistance to 2–5 different antibiotics, where *Salmonella* spp. isolated from cucumber and coriander leaves showed resistance against 4–8 different antimicrobials. Therefore, the study results demonstrated that, the presence of residual pesticides, multidrug resistant *E. coli* and *Salmonella* spp. in the RSV samples posing concern when consumed raw. The regulatory bodies are expected to monitor and ensure the overall quality standards are in place and practiced by food producers and marketers responsible for handling and distribution of RSVs.

Keywords: Agriculture, Food science, Food analysis, Food safety

1. Introduction

Fruit and vegetables are recognized as an important key component of a healthy diet. They are low fat and low energy-dense foods, relatively rich in vitamins, minerals and other bioactive compounds, as well as being a good source of fiber (Rekhy and McConchie, 2014). A high intake of fruit and vegetables in the diet is positively associated with the prevention of cardiovascular disease; cancer; diabetes; and osteoporosis (Wang et al., 2014), due to these health benefits, its consumption worldwide has increased considerably in recent years, making such produce an important economic output. However, despite these health benefits, epidemiological investigations ranked raw fruits and vegetables as the second most common source of outbreaks of foodborne illness (Callejón et al., 2015). These foodborne outbreaks are not only a burden on public health but also cause heavy economic loss to the food industry (Hussain and Dawson, 2013). A recent report by Center for Science in the Public Interest (CSPI) showed that the highest number of outbreaks was attributed to fresh produce commodity in the USA during 2002–2011 (CSPI, 2014). It is estimated that fresh produce causes the greatest number of illnesses and the largest average number of illnesses per outbreak, thus, there is pressure on producers to focus even more on hygiene to minimize exposure to food hazards. A chemical/microbiological risk exists especially if the fresh produce is grown outdoors in the field, because several groups of microorganisms can colonize or contaminate fruits and vegetables at any point of production throughout the produce

supply chain. The greatest risk is when vegetables and fruits are consumed without being washed and become worst if the biological/chemical contamination is not washed off from the fruits and vegetables by both the farmer and the consumer. In Bangladesh, post-harvest handling, poor processing practices, non-sanitized water wash, poor or no packaging, and transportation system and poor personal hygiene practices are the critical food safety issues in horticultural sectors and varies widely in the communities. Excessive use of pesticides and toxic chemicals is quite common among the farmers of Bangladesh. In addition, the middlemen who collect the vegetables from farmers also use harmful chemicals to keep fruits and vegetables 'fresh'. Furthermore, indiscriminate use of pesticides and not maintaining the waiting period before harvest leads to the accumulation of pesticide residues in fruits and vegetables, were reported by many researchers (Ali et al., 2012; Nur et al., 2015; Chowdhury et al., 2014; Hossain et al., 2013; Lozowicka et al., 2015). On the other hand, heavy metals are natural components of the earth's crust that cannot be destroyed or degraded, but can be transformed from one form to another. Accumulation of relatively high density of metal is toxic or poisonous (Harrison et al., 1981) and discharge of untreated industrial effluents in nearby pond and environment is so common in Bangladesh, and the subsequent use of these waste water in agriculture resulted in heavy metals contamination of soil and vegetation and thus enter into food chain. Consumption of these contaminated fruits and vegetable may impart major detrimental impacts on human health. Heavy metals like Pb, Cd, Cr etc. are recognized as metals of immediate concern (Hamid et al., 2017; WHO, 2004) if small extent of these metal entered into human bodies through food, drinking water and/or air (Sobukola et al., 2010). As the external morphology of vegetables cannot guarantee safety from microbial or chemical contamination, thus, heavy metals contamination ranked highest among the major contaminants of leafy vegetables. Vegetables take-up metals by absorbing them from contaminated soils, as well as from polluted environments and excessive amount of Pb and Cd in food is associated with etiology of a number of diseases (e.g. cardiovascular, kidney, neurology and orthopedic diseases). Again, foodborne illness due to contaminated vegetables increased many folds worldwide and many research studies reported that the presence of pathogenic bacteria like *E. coli* O157:H7, *Salmonella* spp., *Listeria* spp., *Staphylococcus* spp., *Yersinia enterocolitica* etc. in various fresh vegetable samples of Dhaka city markets (Islam et al., 2015; Jeddi et al., 2014; Kabir et al., 2014). In addition, when these pathogens were found resistant to multiple antibiotics, could pose significant public health risk and resistance of pathogenic bacteria could leads to the anti-infective therapy of both humans and animals (Lai et al., 2016). Bacteria are able to adapt rapidly to new environmental conditions and can acquire genes or undergo molecular changes with increasing exposure to antimicrobials in human and veterinary medicine, leading to resistance to these agents (Pruden et al., 2006). The linkages of antibiotic exposure to vegetables might be due to the indiscriminate use of antibiotics in human and food animals to eliminate

diseases, and the human and animal excreta containing resistant bacteria might discharge into the soil and/or water, thus contaminated the field soil and uptake by the vegetable while growing, or can be contaminated during handling by handlers who doesn't aware of good hygiene practices (GHP) or good agricultural practices (GAP). On the other hand, the use of antimicrobials has been deemed to be the major factor in the development of bacterial resistance to these antimicrobials, the use of biocides (including disinfectants, antiseptics, preservatives, and sterilants) might also have some contribution. In the laboratory, resistance to biocides has been linked to the appearance of resistance to antimicrobials, although such linkage has as yet not been conclusively identified in practice (McDonnell and Russell, 1999).

Thus, to reduce the foodborne pathogens incidence, cleaning and sanitation operation have become standard postharvest practice to ensure quality and safety of the vegetables. Cleaning must be done to remove adhering soil and other debris and sanitation must be done to remove spoilage and pathogenic microorganisms from the surfaces of the produce. Unfortunately, in Bangladesh, fruits and vegetables are hardly clean, and not sanitized at all before entering into the marketing channel, which contribute to unsafe and poor quality vegetables. Since, the safety of fruits and vegetables is an increasing consumer concern related to public health issues, thus, to face these constraints and to ensure preventing food contaminants, monitoring the activities from farm to fork and to produce baseline data of the principle contaminants and major pathogens that contribute illness to human, are essential. However, no such research data is yet to develop in Bangladesh and thus, studies to be undertaken to understand the magnitude and type of food contamination. Therefore, this study was designed to analyze the chemical contaminants (heavy metal and pesticide residues) and presence of pathogens and its AMR pattern in fresh salad vegetables including cucumber, lettuce, tomato and coriander leaf sold in the Dhaka city markets (Fig. 1).

2. Materials and methods

2.1. Sample collection

The types of markets included open, municipal, chain shops and wholesale shops ensuring the association of low-middle-high income family purchased RSV items. Total 12 markets were selected depending on the popularity within Dhaka metropolitan city area. Among them, three markets [Agora (Gulshan-2), Swapno (Banani), and Minabazar (Dhanmondi)] were chosen as popular chain shops. Three markets (Kawran bazar, Shyambazar, and Jatrabari) were chosen as popular wholesale markets, and the rests 6 were the popular retails market (New Market, Mohakhali, Khilkhhet, Mirpur-1, Mohammadpur Krishi Market, and Santinagar bazaar).



Fig. 1. Sample collection areas in Dhaka city.

2.2. Inclusion and exclusion criteria of vendors

Randomly selected vendors, their age and business duration was considered while vendors' selection for this study was done. The age should be between 25 and 45 years, and selling vegetables more than 5 years at the same markets. In case of multiple vendors selling same vegetable items, the vendors who was willing to participate in this study was in the inclusion criterion. In case of multiple vendors from the same market was willing to participates, different vegetable samples were taken in the study. One vegetable category from one vendor-criteria was followed in this study to cover maximum vendor's inclusion in this study.

2.3. Sample collection

Sample collection was performed by the trained and experienced “sanitary inspectors” and ‘analysts’ working at the Institute of Public Health. Sample collection was done from 01st February to 15th April, 2018 and detailed sample collection plan has been presented in Table 1. A total of 120 RSV samples were collect as such usually bought by the consumers. Lettuce (200 gm; n = 30), Cucumber (500 gm; n = 30), Tomato (500 gm; n = 30), and Coriander leaf (200 gm; n = 30) samples were collected from the vendors in the markets of Dhaka city. The samples were collected in sterile sample collection bag and kept in cool box to maintain the temperature during transporting to the laboratory within 1–2 hours of collection. The samples were checked and received with laboratory code marks and divided in to three equal parts (one for chemical analysis, another one was for microbial analysis and the last one was for storage). Finally, the samples were stored refrigerator (2–8 °C) until analysis completed.

2.4. Quality compliance laboratories (QCL)

This work was collaborated with the Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka- 1000, Bangladesh and a systematic random sampling method was used and one third of the total samples were cross checked in a second laboratory (WAFFEN Research Laboratory, - a professional microbiology laboratory at Gulshan, Dhaka, Bangladesh) to determining the quality of analysis and variances.

2.5. Chemical contaminants

2.5.1. Heavy metals analysis

A total 120 samples were analyzed to detect Lead (Pb), Chromium (Cr), and Cadmium (Cd) through AAS (NFSL validated method, EN 13805:2002). Sample

Table 1. Sample collection plan.

Types of vegetables	Chemical parameters	Microbial contaminants	No. of samples	Sampling size	Sampling time
Tomato	Heavy metal (Pb, Cr, Cd), and pesticide residues (Diazinon, Malathion, Cypermethrin, Dimethoate, Quinalphos and Chloropyrofos)	Aerobic plate count, Total coliform count, <i>E. coli</i> , <i>Salmonella</i> spp., <i>Staphylococcus</i> spp.	30	Min 500 g composite/sample	10:00–12:00 hrs + 2 hours to reach Lab
Lettuce	Same as above	Same as above	30	Min 200 g composite/sample	Same as above
Cucumber	Same as above	Same as above	30	Min 500 g composite/sample	Same as above
Coriander leaf	Same as above	Same as above	30	Min 200 gm composite/sample	Same as above

preparation for heavy metals was done as per the protocol of EN 13805:2002. The certified reference material (CRM, Signa Aldrich, UK) was used to check the analysis performance.

2.5.2. Pesticide residues analysis

A total 120 samples were analyzed. The QuEChERS, 2009 (Quick, Easy, Cheap, Effective, Rugged and Safe) method was used to analyze targeted 6 pesticide residues of organophosphorus pesticides group from different vegetables sample in an un-rinsed state as such received from markets. Analysis was done in NFSL by GC-MSD (NFSL validated method) to identify diazinon, malathion, cypermethrin, dimethoate, quinalphos and chloropyrifos pesticide residues. The certified reference material (CRM, Dr Ehrenstorfer GmbH) was used to check the analysis performance.

2.6. Microbiological analysis

The composite samples weighted (25 gm) and poured into sterile stomacher bags (Japan) and appropriate amount of sterile saline was added and stomached at 230 rpm for 90 seconds. The diluted or non-diluted stomached samples were surface plated on selective and non-selective agar plates. Briefly, 100 μ l of stomacher treated samples were surface plated on to Tryptic Soya Agar (Oxoid, England) plate for the isolation of total aerobic bacteria (TABC); Sorbitol MacConkey Agar (Oxoid, England) for total coliform count (TCC); EC agar (Nissui, Japan) for *E. coli* count; and Bismuth Sulfite Agar (Oxoid, England) for *Salmonella* spp. count. On the other hand, the presence or absence of the *E. coli* and *Salmonella* spp. microorganisms were also confirmed by placing all the samples separately into Tryptic Soya Broth (Oxoid, England) and incubated at 30 °C overnight, and then spread plated onto both selective and non-selective medium and incubated at 37 °C for 24–48 hours. The presumptive bacteria grown in selected medium were confirmed using API 20E biochemical test set. All the plate counts data were computed and recorded as log CFU/g.

2.7. Antimicrobial susceptibility testing

The standard disc diffusion technique was used (Bauer et al., 1966). The antibiogram profile of randomly selected isolates was performed against 18 different antibiotics. The following antibiotic discs from Oxoid (Basingstoke, England) was used: Gentamycin (30 μ g), Ampicillin (10 μ g), Ciprofloxacin (5 μ g), Ceftazidime (30 μ g); Chloramphenicol (30 μ g); Aztreonam (30 μ g) Streptomycin (10 μ g); Amoxicillin (10 μ g); Nitrofurantoin (300 μ g); Rifampicin (5 μ g); Erythromycin (15 μ g); Novobiocin (30 μ g); Nalidixic Acid (30 μ g); Tetracycline (30 μ g); Bacitracin (10 μ g);

Polymyxin B (300 µg); Kanamycin (30 µg) and Azythromycin (15 µg). The diameters of inhibition zones were compared with those of the Clinical Laboratory Standards Institute (CLSI, 2015).

2.8. Quality control and quality assurance

Trained observers and sufficient number of respondents, matching, stratification technique, statistical modeling method was used as needed to minimize probable confounders or potential biases. About 25% data was rechecked or cross checked at NSFL and CARS to increase reliability of the data analyzed.

2.9. Ethical considerations

The ethical and official permission was taken from Institute of Public health, and informed written consent was taken from each vendor before sample collection, and the confidentiality of collected information was maintained throughout the study. Non-discrimination of choosing vendors and respondents had the rights to refuse and withdraw from the survey.

2.10. Statistical analysis

After collection and analysis the samples for pesticide and heavy metals, the results were analyzed by using SPSS 19 and reported plate count data represented the mean values obtained from three individual trials, with each of these values being obtained from duplicated samples. Data were subjected to analysis of variance using the Microsoft Excel program (Redmond, Washington DC, USA.). Significant differences in plate count data were established by the least-significant difference at the 5% level of significance.

3. Results

3.1. Chemical quality

The consumptions of un-cooked fresh vegetables, due to many health benefits have increased many folds that simultaneously increased the food safety risk worldwide. On the other hand, consumption of pesticide contaminated vegetables pose a major threat to public health. The presence of five widely used organophosphorus (OP) pesticides (diazinon, malathion, dimethoate, quinalphos, and chloropyrofos) and one pyrethroid (cypermethrin) residues in four common RSV commodities (tomato, cucumber, coriander leaf and Lettuce) were determined. The levels of pesticide residues found in the analyzed samples and their maximum residue limits were outlined in Tables 2 and 3. It was observed that except Dimethoate in coriander leaf and lettuce samples, all other pesticides analyzed were not detected and the Dimethoate

Table 2. Comparison of pesticide residues determined in marketed salad vegetables sold at Dhaka city with EU maximum permissible limit. (EU regulation; EU pesticides)

Types of pesticides tested	EU maximum residue levels ^a (µg/kg)	Regulation (EU) No.	Entry in force	Tomato (30)	Cucumber (30)	Coriander leaf (30)	Lettuce (30)
No of positive samples against the following pesticides	NA ^b	NA	NA	0	0	13	7
Diazinon (F)	10	834/2013	31/08/2013	ND ^c	ND	ND	ND
Malathion (sum of malathion and malaoxon expressed as malathion)	20	2015/399	14/03/2015	ND	ND	ND	ND
Cypermethrin (cypermethrin including other mixtures of constituent isomers (sum of isomers)) (F)	50	2017/626	04/07/2017	ND	ND	ND	ND
Dimethoate (W)	10 (0.01 mg/kg)	396/2005 2017/1135	27/06/2017	ND	ND	Min. 12.94 Max. 158.3	Min. 9.6 Max. 74.8
Quinalphos (F)	10	2015/868	06/10/2015	ND	ND	ND	ND
<i>Chlorpyrifos</i> (F)	50	2018/686	16/05/2018	ND	ND	ND	ND

^a Groups of vegetables, FRESH or FROZEN. a: Fruiting vegetables; b: other fruiting vegetables; c: Leaf vegetables, herbs and edible flowers; to which the MRLs apply.

^b Not applicable.

^c ND = Not detected; Detection limit was 1.0 µg/kg.

residues level were found exceeded the maximum permissible limit of EU MRL regulation. Dimethoate were detected in 13 Coriander leaf samples ranging from 12.94 to 158.3 µg/kg and all the 13 Coriander leaf samples were found exceeded the EU MRL values. On the other hand, dimethoate were detected in 7 lettuce samples ranging from 9.6 to 74.8 µg/kg, of which 6 samples possess residues level 5–7

Table 3. Analysis of heavy metals (mg/kg) in cucumber, tomato, coriander leaf, and lettuce leaf samples.

Names of RSVs	Level of heavy metals contamination		
	Lead (Pb)	Cadmium (Cd)	Chromium (Cr)
Cucumber (n = 30)	≤0.066; n ₁ = 16	≤0.016; n ₁ = 3	≤0.060; n ₁ = 8
Tomato (n = 30)	≤0.025; n ₁ = 23	≤0.045; n ₁ = 3	≤0.094; n ₁ = 6
Coriander leaf (n = 30)	0.011–0.22	0.04–0.21	0.05–0.44
Lettuce leaf (n = 30)	0.018–0.30	0.14; n ₁ = 4	0.022–0.25

n = Total number of samples.

n₁ = Number of samples where heavy metals were non-detectable.

Maximum permissible limit of Pb = 0.3 mg/kg (Mohod, 2015); Cd = 0.1 mg/kg (Mohod, 2015); Cr = 2.4 mg/kg (Hamid et al., 2017).

time higher than the EU MRL values (Table 3). However, tomato, and cucumber analyzed was found free from OP group of pesticide residues.

The presence of these pesticide residues in vegetables makes the safety of these vegetables more venerable to public health. Since these vegetables usually eaten raw in salads thus have direct effect on health and the persistence of the pesticides on the vegetables is of great concern due to their bioaccumulation and toxic biological effects on human (Damalas and Eleftherohorinos, 2011). On the other hand, vegetables are known to accumulate heavy metals either from waste water or from toxic chemicals and or pesticides (Hezbollah et al., 2016). However, the presence of low level of heavy metals (Pb, Cd, and Cr) contamination was observed in all the tested RSV, and was found within the acceptable limits of local and international standard. The concentrations of Pb, Cd, and Cr found at different concentrations were shown in Table 3. The results obtained in this study are comparable with some literature values of similar studies reported previously (Hezbollah et al., 2016; Nogaim et al., 2013).

3.1.1. Lettuce

The microbiological analysis was done at the WAFFEN Research Laboratory as per the ISO methods and the results were presented in Table 4. Microbiological results showed that irrespective of market location higher number of APC was recorded ranging from 4.84 to 7.04 log CFU/g, in lettuce samples (Table 4). Although, the sophisticated shops showed lower number APC in lettuce sample, but *E. coli* and *Salmonella* spp. were evident in lettuce samples analyzed. On the other hand, wholesale market sample showed higher microbial load than that of retail and sophisticated

Table 4. Microbiological quality of lettuce collected from 12 different markets of Dhaka city.

Market type	Location	Average microbiological population (log CFU/g)				
		Aerobic bacterial count	Total coliform count	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Staphylococcus</i> spp.
Sophisticated shops (2)	Gulshan-1 Shopno	5.39 ± 0.09	3.36 ± 0.28	2.78 ± 0.18	2.34	4.30 ± 0.00
	Mena Bazar Bansree	4.84 ± 0.03	4.43 ± 0.07	2.75 ± 0.21	<1.0	3.50 ± 0.04
Wholesale market (4)	Mirpur-1	5.39 ± 0.05	3.57 ± 0.09	2.41 ± 0.09	<1.0	2.45 ± 0.21
	Kawran Bazar	5.09 ± 0.13	4.05 ± 0.21	<1.0	<1.0	<1.0
	Jatrabari bazar	7.04 ± 0.09	6.87 ± 0.17	3.42 ± 0.42	2.52 ± 0.52	4.80 ± 0.10
	Syambazar	6.95 ± 0.31	6.76 ± 0.00	3.31 ± 0.08	3.08 ± 0.38	4.47 ± 0.06
Retail market (6)	Uttora (Sector-3)	5.50 ± 0.74	4.78 ± 0.02	1.69 ± 0.12	<1.0	2.69 ± 0.12
	Tejgaon Colony Bazar	5.43 ± 0.05	3.84 ± 0.08	<1.0	<1.0	3.14 ± 0.08
	Khilkhet	5.28 ± 0.18	3.75 ± 0.04	<1.0	<1.0	2.30 ± 0.0
	Mohakhali	5.95 ± 0.04	5.96 ± 0.01	2.78 ± 0.18	<1.0	4.52 ± 0.00
	Bashundhara R/A	5.72 ± 0.05	5.30 ± 0.09	3.75 ± 0.21	<1.0	3.45 ± 0.17
	New market	5.236 ± 0.00	4.848 ± 0.04	<1.0	<1.0	3.25 ± 0.0

*The average values of three individual trial ± SD; <1.0 = Not detected.

shops. Higher number of coliform, *E. coli* and *Salmonella* spp. was evident in the wholesale market; this might be due to unhygienic market places, holding containers, and poor personal hygiene of the food handlers. On the contrary, retail market data showed that lower number of samples were contaminated with *E. coli*, and *Salmonella* spp., this might be due to the vendors personal hygiene knowledge and periodic application of sanitizers on the displayed lettuce. Therefore, irrespective of market types, improvement of hygienic conditions of the places, holding containers and personal hygiene of vendors is needed. Introduction of non-chlorine sanitizers for washing, drying and wrapping after postharvest should be applied to improve the safety and quality of lettuce.

3.1.2. Tomato

Microbiological results showed that irrespective of market location higher number of APC ranging from 4.14 to 6.95 log CFU/g, coliform count ranging from (3.36–5.57 log CFU/g), *E. coli* count ranging from (1.69–2.42 log CFU/g), and *Staphylococcus* spp. count ranging from (2.45–3.51 log CFU/g) was recorded in tomato samples (Table 5). Although, the presence of *Salmonella* spp. was not detected in sophisticated shops, but the presence of *E. coli* was evident in tomato samples analyzed. On the other hand, non significant differences of bacterial counts were observed in wholesale market and retail market samples, however, periodic presence of *Salmonella* spp. was observed in wholesale market and no *Salmonella* spp. was found in the retail market. Although the retailers purchased their tomatoes from wholesale markets and presence of *E. coli* in 25% tomatoes, and presence of *Salmonella* spp. in 50% tomato samples were found, but, 100% tomatoes samples were free from *Salmonella* spp. and 50% tomatoes was found free of *E. coli* reminds that the

Table 5. Microbiological quality of tomato collected from 12 different markets of Dhaka city.

Market types	Locations	Average microbial population (Log CFU/gm)				
		Total aerobic bacterial count	Total coliform count	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Staphylococcus</i> spp.
Sophisticated shops (2)	Agora, Gulshan-2	4.29 ± 0.09	3.36 ± 0.28	1.78 ± 0.18	<1.0	3.30 ± 0.00
	Mena Bazar, Uttara	4.14 ± 0.03	3.43 ± 0.07	1.75 ± 0.21	<1.0	2.5 ± 0.04
Wholesale markets (4)	Mirpur-1	5.39 ± 0.05	3.57 ± 0.09	2.42 ± 0.09	<1.0	2.45 ± 0.21
	Karwanbazar	5.09 ± 0.13	4.05 ± 0.21	<1.0	<1.0	<1.0
	Jatrabari Bazar	6.44 ± 0.09	5.57 ± 0.17	2.42 ± 0.42	2.52 ± 0.52	3.47 ± 0.10
	Syambazar	6.95 ± 0.31	5.66 ± 0.00	2.31 ± 0.08	2.08 ± 0.18	2.71 ± 0.06
Retail markets (6)	Uttara- 3	5.50 ± 0.74	4.78 ± 0.02	1.69 ± 0.12	<1.0	2.71 ± 0.12
	Tejgaon colony bazar	5.43 ± 0.05	3.84 ± 0.08	<1.0	<1.0	3.14 ± 0.08
	Khilkhet	5.28 ± 0.18	3.75 ± 0.04	<1.0	<1.0	2.30 ± 0.00
	Mohakhali	5.95 ± 0.04	4.19 ± 0.01	1.78 ± 0.18	<1.0	3.51 ± 0.00
	Bashundhara R/A	5.72 ± 0.05	4.30 ± 0.09	1.75 ± 0.21	<1.0	3.43 ± 0.17
	New market	5.24 ± 0.00	4.85 ± 0.04	<1.0	<1.0	3.25 ± 0.00

*The average values of three individual trial ± SD; <1.0 = not detected.

retailer might use some disinfectant/antimicrobials to eliminate pathogens from tomato samples. Nevertheless, presence of higher coliform bacteria in wholesale & retail markets were evident this might be due to unhygienic market places, holding containers, and poor personal hygiene of the food handlers. The absence of *E. coli* and *Salmonella* spp. in tomatoes of retail market, despite the higher prevalence of *E. coli* and *Salmonella* spp. in tomatoes of wholesale shops, is suspicious. Hence, a study is needed to see what disinfectant/antimicrobials are using to kill pathogens. Alternatively, introduction of non-chlorine sanitizers and market availability will reduce the use of other mysterious sanitizers. Therefore, irrespective of market types, improvement of hygienic conditions of the places, holding containers and personal hygiene of vendors is needed. Introduction of non-chlorine sanitizers for washing, drying and waxing after postharvest should be applied to improve the safety and quality of tomatoes.

3.1.3. Cucumber

The microbiological analysis was done at the National Food Safety Laboratory (NFSL) as per the ISO methods and the results were presented in Table 6. All the cucumber sample analyzed possess higher APC count ranging from 4.56 to 8.09 log CFU/g; and total coliform bacteria ranging from 2.75 to 6.45 log CFU/g. Although, presence of higher coliform bacteria in one sophisticated shop (Agora) was noticed, but no *E. coli*, nor *Salmonella* spp. or *Staphylococcus* spp. was observed in cucumber samples of this sophisticated shop. In addition, the presence of *Salmonella* spp. was not detected in any cucumber samples of

Table 6. Microbiological quality of cucumber samples collected from 12 different markets of Dhaka city.

Market type	Sample collection area	Average microbiological population (log CFU/g)				
		Aerobic plate count	Total coliform count	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Staphylococcus</i> spp.
Sophisticated market (2)	Dhanmondi (Minabazar)	6.08 ± 0.09	3.95 ± 0.7	3.70 ± 0.02	<1.0	<1.0
	Gulshan-2, (Agora)	7.00 ± 0.11	6.45 ± 0.16	<1.0	<1.0	<1.0
Wholesale market (4)	Mirpur-1 (Prince Bazar)	5.26 ± 0.12	3.90 ± 0.04	3.60 ± 0.14	<1.0	4.26 ± 0.12
	Kawranbazar	8.09 ± 0.7	5.51 ± 0.06	3.50 ± 0.17	<1.0	4.46 ± 0.16
	Jatrabari	7.91 ± 0.05	3.38 ± 0.0	2.6 ± 0.05	<1.0	<1.0
	Shyambazar	6.47 ± 0.3	3.84 ± 0.4	<1.0	<1.0	<1.0
Retail market (6)	Khilkhet	6.57 ± 0.13	2.75 ± 0.18	<1.0	<1.0	5.43 ± 0.28
	Mohammadpur Krishi	8.09 ± 0.34	2.88 ± 0.4	<1.0	<1.0	<1.0
	Mohammadpur Town	6.98 ± 0.17	4.98 ± 0.7	3.81 ± 0.10	<1.0	2.00 ± 0.09
	Mohakhali	4.56 ± 0.05	3.51 ± 0.05	0.67 ± 0.0	<1.0	4.30 ± 0.04
	Mirpur-11	6.10 ± 0.0	5.30 ± 0.05	1.53 ± 0.09	<1.0	4.32 ± 0.05
	Santinagar	6.60 ± 0.02	5.14 ± 0.10	2.47 ± 0.07	<1.0	<1.0

*The average values of three individual trial ± SD; <1.0 = Not detected.

markets in this study, but the presence of *E. coli* was evident in one (Minabazar) sophisticated shops, four wholesale markets (Syambazar), and six retail markets (Khilkhet, & Mohammadpur Krishi). On the other hand, no *Staphylococcus* spp. contamination was observed in cucumbers of sophisticated shops, but periodic presence of *Staphylococcus* spp. was noticed in wholesale and retail markets. About 50% cucumber samples of wholesale markets and 33% cucumber samples of retail market were found contaminated with *Staphylococcus* spp. Therefore, irrespective of market types, improvement of hygienic conditions of the places, holding containers and personal hygiene of vendors is needed. Introduction of non-chlorine sanitizers for washing, followed by drying and waxing after post-harvest should be applied to improve the safety and quality of cucumber samples.

3.1.4. Coriander leaves

The microbiological analysis was done at the National Food Safety Laboratory as per the ISO methods and the results were presented in Table 7. Presence of higher number of aerobic bacteria ranging from 6.45 to 8.45 log CFU/g; coliform bacterial contamination ranging from 4.60 to 7.40 log CFU/g; and *E. coli* contamination ranging from 3.85 to 6.34 log CFU/g. was evident in the coriander samples analyzed (Table 7). Although *Salmonella* spp. was not present, but higher number of *E. coli* and *Staphylococcus* spp. was observed in sophisticated shops. *Salmonella* spp. was found in one wholesale market (Syambazar) and one retail market (Santinagar). Despite market type, all the coriander leaves investigated in this study possess higher number of *E. coli* than that of tomato, cucumber and lettuce. Only 30% of coriander leaves were found contaminated with *Staphylococcus* spp. on the other hand, 18% of

Table 7. Microbiological quality of coriander leaves collected from 12 different markets of Dhaka city.

Market type	Sample collection area	Average microbiological population (log CFU/g)				
		Aerobic plate count	Total coliform count	<i>E. coli</i>	<i>Salmonella</i> spp.	<i>Staphylococcus</i> spp.
Sophisticated (2)	Dhanmondi (Minabazar)	6.78 ± 0.05	6.18 ± 0.05	5.65 ± 0.05	<1.0	4.18 ± 0.05
	Gulshan-2 (Agora)	6.92 ± 0.05	5.30 ± 0.05	4.41 ± 0.05	<1.0	<1.0
Wholesale market (4)	Mirpur-1 (Prince Bazar)	6.45 ± 0.05	4.60 ± 0.05	3.95 ± 0.05	<1.0	4.26 ± 0.05
	Kawranbazar	7.08 ± 0.05	6.48 ± 0.05	4.08 ± 0.05	<1.0	4.70 ± 0.05
	Jatrabari	7.88 ± 0.05	7.40 ± 0.05	6.34 ± 0.05	<1.0	5.20 ± 0.05
	Shymbazar	6.53 ± 0.05	6.26 ± 0.05	3.85 ± 0.05	Present	<1.0
Retail Market (6)	Mohammadpur Krishi	7.74 ± 0.05	4.90 ± 0.05	4.38 ± 0.05	<1.0	4.85 ± 0.05
	Mirpur-11	8.32 ± 0.05	6.48 ± 0.05	5.23 ± 0.05	<1.0	<1.0
	Mohammadpur town	8.45 ± 0.05	7.32 ± 0.05	6.34 ± 0.05	<1.0	4.23 ± 0.05
	Khilkhet	8.08 ± 0.05	5.88 ± 0.05	4.18 ± 0.05	<1.0	5.23 ± 0.05
	Santinagar	7.00 ± 0.05	6.41 ± 0.05	5.26 ± 0.05	Present	<1.0
	Mohakhali	6.48 ± 0.05	5.48 ± 0.05	3.99 ± 0.05	<1.0	4.15 ± 0.05

coriander leaves sample was found contaminated with *Salmonella* spp. and 100% of the coriander leaves sample was found contaminated with *E. coli*. This finding suggested that significant risk associated with these salad vegetables exist if consumed fresh. On the other hand, it has been evident that washing these vegetables with tap water may eliminate the debris, soils and other contaminants, however, unable to eliminate pathogens attached to the surfaces of these vegetables (Damalas and Eleftherohorinos, 2011). Therefore, introduction of non-chlorine sanitizers for washing, followed by drying and packaging after postharvest should be applied to improve the safety and quality of coriander leaves samples.

3.2. Antibiotic resistance pattern

The antibiogram of randomly selected 10 *E. coli* and 6 *Salmonella* spp. isolates from cucumber and coriander leaves was performed against 18 different commonly used antibiotics and the results were shown in Table 8. The results showed that the *E. coli* isolated from cucumber leaves were resistance to minimum 2 antibiotics (Novobiocin, and Bacitracin), and maximum 8 antibiotics (Amoxycillin, Ampicillin, Nitrofurantoin, Streptomycin, Rifampicin, Erythromycin, Novobiocin, Bacitracin). On the other hand, *E. coli* isolated from coriander leaves were resistant to at least 3 antibiotics (Amoxycillin, Rifampicin, Bacitracin), and maximum 5 antibiotics (Amoxycillin, Rifampicin, Erythromycin, Novobiocin, Bacitracin). On the other hand, *Salmonella* spp. isolated from cucumber and coriander leaves showed minimum resistance to 4 antibiotics (Rifampicin, Erythromycin, Novobiocin, Bacitracin), and maximum 8 antibiotics (Amoxycillin, Ampicillin, Nalidixic Acid, Tetracycline, Rifampicin, Erythromycin, Novobiocin, Bacitracin).

4. Discussion

Vegetables are the second major food group consumed after cereals and their products in Bangladesh (Timsina et al., 2016). Furthermore, vegetables consumption promotes good health because of their nutritive components (Liu, 2003). But most of the vegetable crops are lost on the farm due to pest infestation, as a result, most farmers (over 50%) in Bangladesh use pesticides and also to protect the crops quality and reduce cost of production. The widespread use of pesticides may contaminate the environment as well as foods, which may create health problem (Parveen and Nakagoshi, 2001).

In this study, total chromium was chosen for analysis because exposure of chromium may occur naturally or from industrial sources. Naturally occurring chromium exists in trivalent chromium (Cr + 3) state and industrial environment chromium can exist in hexavalent chromium (Cr + 6), state. However, Cr + 6 is relatively unstable and undergoes chemical reaction with another compound promptly. Thus, it is difficult to

Table 8. The AMR pattern of *E. coli* and *Salmonella* spp. isolated from fresh salad vegetables against commonly used 18 antibiotics.

Sl No.	Name of antibiotics	Randomly selected <i>E. coli</i> isolates										Randomly selected <i>Salmonella</i> spp. isolates						
		CuMo01	CuKh15	CuKa27	CuMi39	CuSh97	CoMo55	CoJa68	CoSh100	CoSa111	CoDh121	Ja68	Sh99	Sh100	Sh101	Sh111	Sa113	
1	Amoxicillin (10 µg)	S ^a	R ^b	R	S	R	R	R	R	R	R	R	I ^c	S	R	S	S	S
2	Ceftazidime (30 µg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
3	Piperacillin (110 µg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
4	Chloramphenicol (30 µg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
5	Ampicillin (10 µg)	S	R	S	S	S	I	R	S	S	S	S	S	R	S	S	S	S
6	Nitrofurantoin (300 µg)	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
7	Aztreonam (30 µg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
8	Nalidixic Acid (30 µg)	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S
9	Tetracycline (30 µg)	S	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S
10	Streptomycin (10 µg)	I	R	S	I	S	S	S	S	S	S	I	I	I	S	I	I	I
11	Azythromycin (15 µg)	S	S	S	S	I	S	S	S	S	S	S	S	S	S	I	R	R
12	Ciprofloxacin (5 µg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
13	Rifampicin (5 µg)	R	R	S	I	S	R	I	I	R	R	R	R	R	R	R	R	R
14	Erythromycin (15 µg)	R	R	S	I	R	R	S	S	R	S	R	R	R	R	R	R	R
15	Polymyxin B (300 µg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	I	I	I
16	Novobiocin (30 µg)	R	R	R	R	R	R	R	I	R	S	R	R	R	R	R	R	R
17	Bacitracin (10 µg)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
18	Kanamycin (30 µg)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S

Where, S = ^aSensitive; R = Resistant; I = Intermediate.

differentiate between chromium Cr + 3 and chromium Cr + 6 during analysis hence, total chromium was analyzed in this study.

The microbial quality of the salad vegetables including tomato, lettuce, cucumber and coriander leaves were evaluated in this study. In this study, all the vegetables were found contaminated with pathogenic *E. coli* and some of the samples were contaminated with *Salmonella* spp., that can cause serious public health problem. However, the microbiological data as such do not signify a risk but point to unacceptable levels of contamination in some samples. For example, *E. coli* is an indicator bacterium of faecal contamination, presence of *E. coli* in any food indicates that this food is somehow contaminated with faecal materials. The comparison of aerobic and coliform bacteria between “retail market” and “sophisticated” shops do not necessarily mean a difference is risk between the different samples. However, higher presence of these two bacteria indicates the quality, processing conditions and the risk of pathogen contamination. *E. coli*, *Salmonella* spp., *Staphylococcus* spp. etc., were predominantly may present in agriculture soils, improperly composted manures, and/or irrigation water may contribute to contaminate the RSVs during production, harvest handling, transportation and storage and even during display (Alam et al., 2015; Rahman et al., 2010).

As, fruits and vegetables are hardly clean, and not sanitized at all before entering into the marketing channel in Bangladesh that contribute unsafe and poor-quality vegetables, therefore, cleaning and sanitation practices must be introduced to improve the quality and safety of these vegetables. In addition, number of factors including 1) lack basic knowledge and awareness in safe handling practices during production and post-harvest operations; 2) inadequacy of postharvest specific infrastructure such packing houses; 3) pre-cooling, sorting and storage facilities; 4) lack of auxiliary industries for the production of packaging materials, tools and equipment; 5) deterioration of produce quality owing to rough handling, improper packaging; 6) overloading and damage during transportation; 7) lack of cold chain systems; 8) unskilled farmers coupled with poor technical extension and training facilities, and 9) poor access to market information, were found responsible for the poor quality and unsafe vegetables. Thus, introduction of non-chlorine sanitizers for washing, along with improvement of the above-mentioned factors should be taken care to improve the safety and quality of RSVs samples (Ahmed et al., 2017).

5. Conclusion

The persistent nature of the pesticides is of great concern due to their bio-accumulation nature and toxic biological effects on human and wildlife. This study results demonstrated the presence of heavy metals residues were within the

acceptable limits of local and international standards. On the other hand, no pesticide residues were found in tomato and cucumber samples but presence of dimethoate samples was noticed in coriander and lettuce samples. Furthermore, the presence of foodborne pathogens including *E. coli*, and *Salmonella* spp. was evident in almost all the salad vegetables samples. The presence of microbiological hazard can cause serious public health problem, a continuous monitoring by the regulatory authority in the presence and absence of any chemical and microbiological hazard is imperative to reduce the foodborne pathogen incidences and thereby protecting public health. In addition, initiative should be taken to introduce non-chlorine sanitizers, awareness program on cleaning and sanitization practices, and capacity building of farmers and stakeholders who directly involved in the vegetables value chain, as a long-term goal, to improve food safety and quality of fresh produce.

Declarations

Author contribution statement

Sunzid Ahmed, Matiur Rahman: Performed the experiments; Analyzed and interpreted the data.

Md. Abubakkar Siddique: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Md. Latiful Bari, Shahnila Ferdousi: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This study was partially supported by Bangladesh Medical Research Council.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

The authors would also like to thanks Dr. Sakila Parvin, Md Rezaul Karim, Md Saiful Islam & Md. Hedayet of NFSL for their technical support on microbiological

analysis and Ms Farida Yeasmin and Md Amdadul Huq of NFSL for their technical support on heavy metal analysis.

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