

Article

Microbial quality evaluation of fresh vegetables from distinct markets in urban areas of Bangladesh

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Abstract: This study was carried out from January to May 2018 to isolate, identify, and determine microbial loads (*Staphylococcus aureus*; *Salmonella*; *Escherichia coli*; *Campylobacter*) in fresh-marketed vegetables sold at several marketplaces in the districts of Netrokona, Kishoregonj, and Jamalpur. In this study, 90 fresh vegetable samples (carrot, cucumber, coriander leaf, green chili, and tomato) were collected. The spread plate dilution method was utilized for this work, and the organisms were cultivated on selected culture media. Microbes that were expected were discovered utilizing culture and staining procedures. *S. aureus*, *Salmonella*, *E. coli*, and *Campylobacter* were recovered from all tomatoes (100%), cucumber (100%) and green chili (100%). In carrot samples, the number (%) of *S. aureus*, *E. coli* and *Campylobacter* was 100% except for *Salmonella* (88.88%). However, 94.44% *S. aureus*, 77.77% *Salmonella*, 100% *E. coli*, and 100% *Campylobacter* were identified in the coriander leaf sample. In conclusion, in salad vegetable samples, high bacterial load and the presence of these organisms, particularly *S. aureus*, *Salmonella*, *E. coli*, and *Campylobacter*, could serve as an indicator of the need to raise awareness about the potential health hazards caused by improper handling of these vegetables.

Keywords: microbial quality; fresh vegetables; markets; Bangladesh

1. Introduction

Vegetables are regarded as a healthy nutritional source of minerals, micronutrients, vitamins, and fiber, all of which are essential for human health and well-being. Well-balanced, vegetable-rich diets are especially beneficial for preventing vitamin C deficiency, while vitamin A deficiency has been linked to a lower risk of numerous diseases (Fung *et al.*, 2011). Salad veggies that are commonly consumed raw include tomato, cucumber, carrot, green chile, lemon, and coriander leaf. Their traditional use in salad preparation is well known around the world. In recent years, a surge in health consciousness has resulted in the consumption of minimally processed foods (Warriner *et al.*, 2009). Salad veggies have thus become popular since they meet a current need and do not require any complex preparations (Carmo *et al.*, 2004). When vegetables come in contact with soil, dust or water, as well as during harvest and after harvest, vegetables are contaminated by microorganisms. As a result, they contain some bacteria, including plant and human pathogens (Dunn *et al.*, 1995; Carmo *et al.*, 2004).

Unrelated factors such as resident microflora in the soil, application of nonresident microflora via animal dung, sewage, or irrigation water, and transportation and handling by specific retailers all contribute to differences in microbial profiles of distinct vegetables (Ray and Bhunia, 2007; Ofor *et al.*, 2009). Contamination is exacerbated by the use of untreated wastewater and manure as fertilizers in the production of fruits and vegetables (Amoah *et al.*, 2009).

Food safety is harmed by microbial deterioration and pathogen contamination. The Centers for Disease Control (CDC) reported that 76 million cases of food-borne disease occur each year in the USA, with the majority of cases being caused by bacteria (Linscott, 2011). The intake of infected salad vegetables has been connected to several gastroenteritis outbreaks. Salad veggies have been reported to include *S. aureus*, *E. coli*, *Enterobacter* spp., *Klebsiella* spp., *Providencia* spp., and *Pseudomonas aeruginosa* ($>10^{10}$ CFU/g) (Moayed *et al.*, 2013). Pingulkar *et al.* (2001) investigated the microbiological quality of fresh leafy vegetables, salad components, and ready-to-eat salads, reported faecal coliforms, *Listeria* spp., and *Yersinia* spp. (10^6 - 10^8 CFU/g).

Microbiological quality is often classified into four categories based on standard plate counts, indicator organism levels, and the number or presence of pathogens. These are satisfactory, marginal, unsatisfactory, and potentially harmful. Good microbiological quality is indicated by satisfactory results. Marginal results are borderline because they are within acceptable microbiological quality levels but may signal potential hygiene issues in the handling of salad vegetables. Unsatisfactory microbiological limits indicate poor hygiene or food-handling procedures. Levels in this range are potentially harmful, and prompt action should be taken to prevent food-borne illness. Actions should be taken to withdraw the veggies and fruits still available for sale or distribution (Itohan *et al.*, 2011). An investigation of food production or handling practices should be investigated to determine the source/cause of the problem so that remedial actions can be started.

Although limited research has been done in Bangladesh to detect microbial contamination of vegetables (Nipa *et al.*, 2011; Rahman and Noor, 2012), a comprehensive investigation is needed to determine the wide range of bacteria linked to vegetable contamination. This study aimed to assess microbial loads of fresh vegetables from various Bangladeshi urban areas.

2. Materials and Methods

2.1. Area and design of the study

Samples were taken from local marketplaces in the Bangladeshi districts of Netrokona, Kishoregonj, and Jamalpur (Table 1). For this study, 90 fresh vegetable samples (carrot, cucumber, coriander leaf, green chili, and tomato) were taken from several marketplaces in the Netrokona, Kishoregonj, and Jamalpur districts. Plate count agar (PCA), Mannitol salt agar (MSA), Xylose lysine deoxycholate agar (XLDA), MacConkey Agar (MCA) and Blood base agar no. 2 were used to determine the total viable count (TVC), total *Staphylococcus aureus* count (TStAC), total *Salmonella* count (TSC), total *Escherichia coli* count (TEC) and total *Campylobacter* count (TCC) respectively.

2.2. Collection and transportation of samples

Fresh vegetable samples (500 g each) were collected from numerous markets in the Netrokona, Kishoregonj, and Jamalpur districts. Tomato, carrot, cucumber, coriander leaf, and green chili samples were collected separately and stored in sterile polythene bags in a cool chain environment. To avoid touch or cross-contamination, precautions were taken. The samples were carefully carried to the bacteriology lab of the Department of Microbiology and Hygiene, Bangladesh Agricultural University, Mymensingh, Bangladesh, maintaining a cool chain in the ice box.

2.3. Sample preparation for detection of *S. aureus*, *Salmonella* and *E. coli*

Traditional culture-based methods were used to detect *Salmonella*, *E. coli*, and *S. aureus*, according to the International Organization for Standardization protocol (ISO, 1995). In order to homogenize a total of 10 g of sample, 90 ml of 0.1% peptone water was used. To assess TVC, TStAC, TSC, and TEC a 10-fold serial dilution was performed and inoculated on bacterial media (PCA, MSA, XLDA and MCA). Cultures were incubated at 37°C for 24 h before colony analysis and observation of colony features for *S. aureus*, *Salmonella* and *E. coli* detection.

Table 1. Number of fresh vegetable samples collected from different markets of Netrokona, Kishoregonj and Jamalpur districts.

SL No.	Locations	Types of sample	No. of sample collected
1	Netrokona	Tomato	6
		Carrot	6
		Cucumber	6
		Coriander leaf	6
		Green chili	6
Sub-Total			30
2	Kishoregonj	Tomato	6
		Carrot	6
		Cucumber	6
		Coriander leaf	6
		Green chili	6
Sub-Total			30
3	Jamalpur	Tomato	6
		Carrot	6
		Cucumber	6
		Coriander leaf	6
		Green chili	6
Sub-Total			30
Total			90

2.4. Sample preparation for detection of *Campylobacter* species

Campylobacter spp. were isolated using the filtration method (0.45 µm filter) reported by Neogi *et al.* (2020). The collected samples (10 g) were allowed to be prepared into fresh vegetable homogenates and 100 µl of homogenates before being dispersed on filter papers placed on the surface of Blood base agar no. 2. After that, the plates were allowed to stand for 30 min at room temperature before being incubated at 37°C for 48 h under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂). After 48 h, the incubated media were tested for bacterial growth. Colonies from the agar plate were then stained using Gram's method and examined under a microscope for the Gram-negative curve. Gram-negative curves from the agar media were subsequently sub-cultured to obtain single pure colonies. Pure colonies were maintained in glycerol stock and stored at -20°C for future work.

2.5. Preparation of culture media and processing of salad vegetables

The following agars were employed in this study: Blood base agar no. 2 (Oxoid, Germany), MacConkey agar (MC) (Hi-media, India), Xylose lysine deoxycholate agar (XLD) (Hi-media, India), and Mannitol salt agar (MS agar) (Hi-media, India). These materials were all made according to the manufacturer's specifications. Following the criteria of the International Organization for Standardization, each fresh vegetable sample was macerated in a sterile diluent (ISO, 1995). Each fresh vegetable sample (10 g) was taken aseptically with sterile forceps and placed in sterile containers containing 90 mL of 0.1% peptone water. In a sterile zipper bag, a homogenized solution was made and a 10-fold dilution (1:10) of sample was obtained. Subsequently, serial dilutions (up to 10⁻⁸ dilution) of each of the samples were prepared using 0.1% peptone water. Afterwards, the samples were tested for the bacterial load as well as microbiological assessment and analysis.

2.6. Enumeration of total viable count (TVC)

From each ten-fold dilution, 0.1 mL was transferred and dispersed on PCA using a sterile glass spreader for total bacterial count determination. After that, the plates were incubated at 37°C for 24 h. Plates with 30-300 colonies were counted after incubation. To calculate the total viable count, the average number of colonies in each dilution was multiplied by the dilution factor. The total viable count was estimated using ISO guidelines (1995). The overall bacterial count was represented as the number of organisms or colony-forming units per gram of vegetable sample (CFU/g).

2.7. Enumeration of total *Staphylococcus aureus* count (TStac)

For the total *Staphylococcus aureus* count (TStac), 0.1 ml of each ten-fold dilution was transferred and disseminated on Mannitol salt agar. The plates were then incubated at 37°C for 24 h. The overall bacterial count

was represented as the number of organisms or colony-forming units per gram of vegetable sample (CFU/g).

2.8. Enumeration of total *Salmonella* count (TSC)

The sampling, dilution, and streaking procedures used to get the total *Salmonella* count were the same as those used to determine the total *Staphylococcus aureus* count. Only for the *Salmonella* count, xylose lysine deoxycholate agar (XLDA) was utilized. TSC was calculated similarly to the total viable count and was expressed as the number of organisms or colony-forming units per gram of vegetable sample (CFU/g).

2.9. Enumeration of total *E. coli* count (TEC)

Total *E. coli* count procedure was similar to total *Salmonella* count procedures in terms of sampling, dilution, and streaking. MacConkey agar (MC Agar) was only used to count *E. coli*. TEC was calculated in the same way as TVC.

2.10. Enumeration of total *Campylobacter* count (TCpC)

To calculate the total *Campylobacter* count (TCpC), 0.1 ml of each ten-fold dilution was transferred and distributed on selected blood base agar containing 5% sheep blood. A sterilized glass spreader was used to disseminate the diluted samples as rapidly as possible on a 0.45mm filter placed on blood agar base no. 2. After that, the plates were incubated in a 42°C incubator for 24-48 hours. Plates containing 30-300 colonies were counted after incubation. The total viable count was calculated by multiplying the average number of colonies in each dilution by the dilution factor. According to ISO (1995), the total viable count was determined.

2.11. Data analysis

The results from the fresh vegetable sample analysis of TVC, TSC, TEC, TStC, and TCpC were tabulated in Microsoft Excel and then transferred to SPSS v. 20. (Statistical Package for Social Sciences). The statistical significance of the data was determined using the ANOVA test, with p-values of 0.05 considered significant.

3. Results

3.1. Determination of TVC, and bacterial load of *S. aureus*, *Salmonella*, *E. coli* and *Campylobacter* (log CFU/g) from the vegetable samples

The total amount of TVC, *S. aureus*, *Salmonella*, *E. coli* and *Campylobacter* (log CFU/g) identified in fresh vegetable samples from Bangladesh's districts of Netrokona, Kishoregonj, and Jamalpur is shown in Table 2. Total viable count (TVC) of the vegetables sample ranged from 5.89 log CFU/g to 9.43 log CFU/g. The highest concentration was found in tomato (9.43 log CFU/g) from Jamalpur, carrot (9.43 log CFU/g) from Netrokona, green chili (9.19 log CFU/g) from Netrokona, cucumber (9.16 log CFU/g) from Kishoregonj and Jamalpur, coriander leaf (8.98 log CFU/g) and. On the other hand, comparatively lower concentration was recorded in carrot (5.89 log CFU/g) from Jamalpur, coriander leaf (8.44 log CFU/g) from Kishoregonj, and green chili (8.47 log CFU/g) from Kishoregonj. No significant variation ($p > 0.05$) of the data according to the location was observed.

The highest concentration of *S. aureus* was found in tomato (7.53 log CFU/g) at Netrokona, carrot (7.89 log CFU/g) at Kishoregonj, cucumber (6.84 log CFU/g), coriander leaf (7.64 log CFU/g), and green chili (8.33 log CFU/g) at Jamalpur district of Bangladesh, while the lowest concentration was recorded in tomato (6.84 log CFU/g) at Jamalpur, carrot (5.69 log CFU/g) at Netrokona, cucumber (5.77 log CFU/g) at Kishoregonj, coriander leaf (5.55 log CFU/g), and green chili (5.69 log CFU/g) at Netrokona district of Bangladesh.

The highest concentrations of *Salmonella* were found in tomato (4.52 log CFU/g) and carrot (4.96 log CFU/g) at Jamalpur, cucumber (4.64 log CFU/g) at Kishoregonj, coriander leaf (4.29 log CFU/g) at Kishoregonj, and Green chili (4.07 log CFU/g) at Kishoregonj district of Bangladesh, and tomato (4.02 log CFU/g) from Netrokona, carrot (3.15 log CFU/g) from Kishoregonj, cucumber (4.13 log CFU/g) from Netrokona, coriander leaf (0.70 log CFU/g) from Netrokona, and green chili (3.75 log CFU/g) from Jamalpur in Bangladesh had the lowest amounts.

In terms of *E. coli*, the maximum amounts were detected in tomato (7.36 log CFU/g) in Kishoregonj, carrot (7.58 log CFU/g) in Jamalpur, cucumber (6.59 log CFU/g) in Netrokona, coriander leaf (6.91 log CFU/g), and green chili (6.61 log CFU/g) in Kishoregonj and the lowest quantities were found in tomato (7.31 log CFU/g) from Netrokona, carrot (6.91 log CFU/g) from Kishoregonj, cucumber (4.39 log CFU/g) from Kishoregonj, coriander leaf (4.07 log CFU/g) from Netrokona, and green chili (4.28 log CFU/g) from Netrokona in Bangladesh.

Table 2. Determination of TVC, *S. aureus*, *Salmonella*, *E. coli* and *Campylobacter* count (log CFU/g) from fresh vegetable samples.

Parameter	Place	Tomato	Carrot	Cucumber	Coriander leaf	Green chili
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
TVC	Netrokona	8.73±0.51	9.43±1.00	9.01±0.54	8.98±0.55	9.19±0.88
	Kishoregonj	8.57±0.42	5.89±4.59	9.16±0.48	8.44±0.07	8.47±0.50
	Jamalpur	9.43±0.79	8.89±0.56	9.16±0.48	8.46±0.07	8.68±0.62
	p value	0.088	0.141	0.1440	0.232	0.079
<i>Staphylococcus aureus</i>	Netrokona	7.53±0.49	5.69±3.29	6.74±0.47	5.55±3.14	5.69±3.29
	Kishoregonj	7.22±1.79	7.89±1.92	5.77±4.49	6.78±3.34	7.87±1.78
	Jamalpur	6.84±3.41	7.55±0.68	6.84±3.36	7.64±0.51	8.33±0.73
	p value	0.125	0.718	0.140	0.132	0.063
<i>Salmonella</i>	Netrokona	4.02±2.72	3.59±2.02	4.13±1.32	0.70±1.56	3.88±0.78
	Kishoregonj	4.11±2.72	3.15±2.48	4.64±1.01	4.29±2.20	4.07±0.66
	Jamalpur	4.52±1.20	4.96±0.87	4.51±1.26	3.19±2.58	3.75±0.75
	p value	0.075	0.108	0.110	0.212	0.059
<i>E. coli</i>	Netrokona	7.31±0.02	6.96±0.92	6.59±0.33	4.07±3.74	4.28±3.99
	Kishoregonj	7.36±0.64	6.91±0.60	4.39±3.42	6.91±0.47	6.61±0.98
	Jamalpur	7.32±1.25	7.58±1.55	5.30±2.60	6.79±0.49	5.28±2.39
	p value	0.114	0.214	0.215	0.412	0.219
<i>Campylobacter</i>	Netrokona	4.05±0.02	4.55±0.38	3.81±1.91	3.44±1.69	4.18±0.47
	Kishoregonj	3.82±0.29	3.63±1.78	3.81±1.91	2.73±2.17	4.18±0.34
	Jamalpur	4.42±0.82	4.36±0.04	3.64±1.79	4.24±0.69	4.16±0.31
	p value	0.131	0.214	0.152	0.114	0.073

In terms of *Campylobacter*, the highest concentrations were found in tomato (4.42 log CFU/g) at Jamalpur, carrot (4.55 log CFU/g) at Netrokona, cucumber (3.81 log CFU/g) at Netrokona and Kishoregonj, coriander leaf (4.24 log CFU/g) at Jamalpur, Green chili (4.18 log CFU/g) at Netrokona and Kishoregonj district of Bangladesh while the lowest concentrations were found in tomato (3.82 log CFU/g) at Kishoregonj, carrot (3.63 log CFU/g) at Kishoregonj, cucumber (3.64 log CFU/g) at Jamalpur, coriander leaf (2.73 log CFU/g) at Kishoregonj, and green chili (4.16 log CFU/g) at Kishoregonj in Bangladesh.

3.2. Prevalence of different bacteria isolated from fresh vegetable samples

The prevalence of various microorganisms found in various fresh vegetables is shown in Table 3. *Salmonella*, *E. coli*, *S. aureus*, and *Campylobacter* were also found in 6 (100%), 6 (100%), and 6 (100%) tomato samples from the Netrokona district respectively. *Salmonella*, *E. coli*, *S. aureus*, and *Campylobacter* were also found in 6 (100%), 6 (100%), and 6 (100%) tomato samples from the Kishoregonj district respectively. *Salmonella*, *E. coli*, *S. aureus*, and *Campylobacter* were also found in 6 (100%), 6 (100%), and 6 (100%) tomato samples from the Jamalpur district respectively.

Out of 6 carrot samples of Netrokona district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 6 (100%), 6 (100%), 6 (100%) and 6 (100%), respectively. Out of 6 carrot samples of Kishoregonj district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 4 (66.66%), 6 (100%), 6 (100%) and 6 (100%), respectively. Out of 6 carrot samples of Jamalpur district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 6 (100%), 6 (100%), 6 (100%) and 6 (100%), respectively.

Out of 6 cucumber samples of Netrokona district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 6 (100%), 6 (100%), 6 (100%) and 6 (100%), respectively. Out of 6 cucumber samples of Kishoregonj district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 6 (100%), 6 (100%), 6 (100%) and 6 (100%), respectively. Out of 6 cucumber samples of Jamalpur district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 6 (100%), 6 (100%), 6 (100%) and 6 (100%), respectively.

Out of 6 coriander leaf samples of Netrokona district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 4 (66.66%), 6 (100%), 6 (100%) and 6 (100%), respectively. Out of 6 coriander leaf samples of Kishoregonj district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 5 (83.33%), 6 (100%), 5 (83.33%) and 6 (100%), respectively. Out of 6 coriander leaf samples of Jamalpur district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 6 (83.33%), 6 (100%),

6 (100%) and 6 (100%), respectively.

Out of 6 green chili samples of Netrokona district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 4 (100%), 6 (100%), 6 (100%) and 6 (100%), respectively. Out of 6 green chili samples of Kishoregonj district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 6 (100%), 6 (100%), 6 (100%) and 6 (100%), respectively. Out of 6 green chili samples of Jamalpur district, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 6 (100%), 6 (100%), 6 (100%) and 6 (100%), respectively.

Table 3. Prevalence of different bacteria isolated from fresh vegetable samples of various places of Netrokona, Kishoregonj and Jamalpur districts.

Types of sample	Place	No. of sample	Number (%) of bacteria			
			<i>S. aureus</i>	<i>Salmonella</i>	<i>E. coli</i>	<i>Campylobacter</i>
Tomato	Netrokona	6	6 (100)	6 (100)	6 (100)	6 (100)
	Kishoregonj	6	6 (100)	6 (100)	6 (100)	6 (100)
	Jamalpur	6	6 (100)	6 (100)	6 (100)	6 (100)
	Total	18	18 (100)	18 (100)	18 (100)	18 (100)
Carrot	Netrokona	6	6 (100)	6 (100)	6 (100)	6 (100)
	Kishoregonj	6	6 (100)	4 (66.66)	6 (100)	6 (100)
	Jamalpur	6	6 (100)	6 (100)	6 (100)	6 (100)
	Total	18	18 (100)	16 (88.88)	18 (100)	18 (100)
Cucumber	Netrokona	6	6 (100)	6 (100)	6 (100)	6 (100)
	Kishoregonj	6	6 (100)	6 (100)	6 (100)	6 (100)
	Jamalpur	6	6 (100)	6 (100)	6 (100)	6 (100)
	Total	18	18 (100)	18 (100)	18 (100)	18 (100)
Coriander leaf	Netrokona	6	6 (100)	4 (66.66)	6 (100)	6 (100)
	Kishoregonj	6	5 (83.33)	5 (83.33)	6 (100)	6 (100)
	Jamalpur	6	6 (100)	5 (83.33)	6 (100)	6 (100)
	Total	18	17 (94.44)	14 (77.77)	18 (100)	18 (100)
Green chili	Netrokona	6	6 (100)	6 (100)	6 (100)	6 (100)
	Kishoregonj	6	6 (100)	6 (100)	6 (100)	6 (100)
	Jamalpur	6	6 (100)	6 (100)	6 (100)	6 (100)
	Total	18	18 (100)	18 (100)	18 (100)	18 (100)

For a total of 18 tomato samples, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 18 (100%), 18 (100%), 18 (100%) and 18 (100%), respectively. For a total of 18 carrot samples, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 16 (88.88%), 18 (100%), 18 (100%) and 18 (100%), respectively. For a total of 18 cucumber samples, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 18 (100%), 18 (100%), 18 (100%) and 18 (100%), respectively. For a total of 18 coriander leaf samples, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 14 (77.77%), 18 (100%), 17 (94.44%) and 18 (100%), respectively. For a total of 18 green chili samples, the number (%) of *Salmonella*, *E. coli*, *S. aureus* and *Campylobacter* was found 18 (100%), 18 (100%), 18 (100%) and 18 (100%), respectively.

4. Discussion

In recent decades, the consumption of ready-to-eat fresh veggies has expanded dramatically. There are currently no statistics on the bacterial burden and prevalence of food-borne diseases in ready-to-eat salad veggies, fresh-cut fruit, or sprouts on the market (Althaus *et al.*, 2012). Fresh produce-related food-borne disease outbreaks are growing increasingly pervasive. High-impact outbreaks, like the one linked to spinach tainted with *E. coli* O157:H7, resulted in nearly 200 cases of food borne illness and >300 market losses across North America (Warriner *et al.*, 2009). Over the last few decades, there has been a lot of research into the interaction of human pathogens with plants and how to improve the microbiological safety of fresh produce. As a result, the current study was chosen to determine the microbial loads in salad vegetables sold in six markets in the districts of Netrokona, Kishoregonj, and Jamalpur, as well as isolate and identify bacteria found on salad vegetables. The bacterial load has a major impact on the microbiological quality of vegetables. A high total viable count (TVC) denotes a dangerous situation and, as a result, the possibility of contamination. Various places had different

TVCs for vegetables. The disparity in hygienic conditions maintained by the vegetable dealers in those locations caused this discrepancy. When the source was in a good hygienic condition, the variance in bacterial contamination was nearly identical, but it differed when the source was in poor hygienic condition. Similar findings were found by Uzeh *et al.* (2009) and Rajvanshi (2010). However, Itohan *et al.* (2011) disagree with this conclusion.

With 6 to 7 log CFU/g, the TVC of the samples from all tests was not within the UK Public Health Laboratory Services (PHLS, 2000). Abdullahi and Kareem (2010) reported high TVCs from various salad ingredients. In the current study, mean values of the TVC of mixed fresh salad vegetable samples are very similar to those of Nipa *et al.* (2011). High TVC suggests poor handling, improper processing, or a general lack of hygiene, implying that the operators in this study most likely used inadequate hygiene standards. This study's TVC values are over the permissible range. When selling vegetables, supermarkets and local markets should take the required precautions in terms of good handling and hygienic practices. This is especially important given that the salad veggies were kept at room temperature for a long time during the sales process. The TVC mean value of carrot and cucumber samples reported in this investigation agrees closely with the findings of Adjrah *et al.* (2013). This could be attributed to differences in the hygienic, handling, and preservation conditions of vegetables. Doores (1983) advised paying particular attention to maintaining a microbiologically stable environment in order to achieve high-quality raw vegetables and processed products.

Salmonella spp. are found all over the world. The most often implicated sources of *Salmonella* outbreaks include poultry and other animal items, eggs, and dairy products (D'Aoust, 2000). Fresh product, on the other hand, has recently been linked to large epidemics (Fung *et al.*, 2011). *Salmonella* spp. was found in the RTE veggies salad samples examined, according to the findings. *Salmonella* spp. was found in concentrations ranging from 4.75 to 5.27 log CFU/g, which is unsatisfactory. *Salmonella* spp. in any proportion poses a substantial risk to consumers, according to PHLS (2000). Adjrah *et al.* (2013), in related studies in Lome, did not detect *Salmonella* spp. in any of the salad samples analyzed, which is slightly different from the findings of our study. Other studies on salad and salad vegetables conducted in Uganda (Mugampoza *et al.*, 2013) and Iran (Moayed *et al.*, 2013) found no *Salmonella* spp. in any of the samples examined.

E. coli is the most common fecal indicator discovered in salad samples. According to the results, *E. coli* levels ranged from 5.74 log CFU/g to 8.16 log CFU/g, crossing the permissible threshold of 2 log CFU/g. This implies that *E. coli* was present in high concentrations in the fresh vegetable samples examined. Consumers are extremely vulnerable to *E. coli* illness. During this investigation, it was discovered that most local market and super shop operators did not adhere to hygienic conditions and serving utensils dedicated to serving only salad; instead, the majority of the salad given to consumers was handled and held at room temperature. This shows that, while most operators may be well-intentioned and concerned with consumer safety and well-being.

Different selective and differential agar media were employed in this study to isolate *E. coli* from samples. The colony properties of *E. coli* seen in EMB agar were identical to those observed by Sharada *et al.* (1999). *E. coli* were Gram-negative small rods that might be single or paired and were motile. Several researchers have described similar *E. coli* culture staining and motility features (Thomas *et al.*, 2005). The detected bacteria were reconfirmed using various sugar fermentation and other biochemical assays, which were found to be consistent with the findings of other researchers (Thomas *et al.*, 2005).

S. aureus is a pathogen that has been linked to food handling. *S. aureus* in individual fresh-cut vegetables shows poor hygiene procedures, and levels more than 4 log CFU/g are potentially harmful. The count of *S. aureus* in this investigation ranged from 6.33 log CFU/g to 9.28 log CFU/g. All of the samples tested for identification of *S. aureus* from different local markets had above the permitted range of *S. aureus*, indicating that the test RTE veggies salads are harmful to health. The presence of *S. aureus* at such high levels in most marketplaces indicates poor handling procedures before and/or after salad production. The nose, throat, skin, and hair of healthy humans and animals, as well as the feathers of birds, are major sources of *S. aureus* food contamination (Garvani, 1987). Food handlers are the primary carriers of *S. aureus* into food. Poor food handling might occur due to contaminated hands or other means such as coughing or sneezing. Staphylococci are likely to present in any and all foods that are handled directly by people or are of animal origin unless heat procedures are used (Loeto *et al.*, 2007). As a result, substantial amounts of *S. aureus* were found in samples from most markets. Most operators' time and temperature abuse of the goods, as well as a lack of basic handling standards during sales, are suggested. Many people on the BAU campus have worked with vegetable salad samples in order to discover food-borne pathogens in salad veggies. In 45 samples of vegetable salads collected on and near the BAU campus, Jasim (2015) discovered *E. coli* in 21 samples and *S. aureus* in 24 samples.

There was a lot of *Campylobacter* in the fresh vegetable samples that were analyzed in this study. Mellou *et al.* (2010) and Danis *et al.* (2009) reported that fresh vegetables and fruits could be considered as risk factors for *Campylobacter* infection.

5. Conclusions

In conclusion, high bacterial load and food-borne pathogens in the salad vegetable samples, particularly *S. aureus*, *Salmonella*, *E. coli*, and *Campylobacter*, may indicate the need to raise awareness about the potential health risks associated with improper handling of these vegetables. As a result, regulatory agencies must ensure that microbiological standards for handling and distribution of fresh vegetable samples are set and followed by farmers and marketers.

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Data availability

The data presented in this study are contained in this manuscript.

Conflict of interest

None to declare.

Author's contribution

Conceptualization: S. M. Lutful Kabir; methodology: Ohiduzzaman, S.M. Akramul Islam; data analysis: Mohammad Arif, Md. Jahidul Islam Saddam; writing—original draft preparation: Ohiduzzaman, Jasim Uddain; writing—review and editing: Muhammad Tofazzal Hossain, S. M. Lutful Kabir. All authors have read and approved the final manuscript.

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