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Comparative study on the microbiological quality of vegetables collected from local markets and super shops of Dhaka city

Mofijur Rahman Mamum, Kamal Kanta Das*, Md. Sohel Rana, Halimatus Sadia Tanim and Farahnaaz Feroz

Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh

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Vegetables are major source of vitamins and fibers but presence of pathogens in salad vegetables can cause outbreak of diseases. Several studies have been conducted on commonly consumed vegetables so far to detect the level of microbial contamination. However, few of them compare the microbial quality of local and super shop vegetables. The present study was conducted for microbiological assessment and comparing four types of vegetables collected from two different market conditions. In this study, Green chili (*Capsicum frutescens L.*), Tomato (*Solanum lycopersicum*), Coriander (*Coriandrum sativum*) and Lady's finger (*Abelmoschus esculentus*) were analyzed to determine Total Viable Bacterial (TVB) Counts, Total Fungal (TF) Counts, Total Coliform Counts (TCC), Total Fecal Coliform (TFC) Counts and occurrence of *Staphylococcus aureus*, *Pseudomonas* spp., *Listeria* spp., *Bacillus* spp., *Salmonella* spp., and *Vibrio* spp. Among these microorganisms, *Klebsiella* spp. and *Pseudomonas* spp. were significantly present in coriander of both local market and super shop. All pathogenic bacteria and fungi were found in huge amounts in the vegetable samples of the local market. Although some pathogenic bacteria and fungi were also found in the same samples of the super shop, relatively lower than the local market's vegetables. In conclusion, contamination in collected vegetable samples were not acceptable but comparatively higher load in local market samples indicated that the hygienic condition in these markets is not sufficiently maintained.

Keywords: Food safety, Vegetables, Food contamination, Consumer's risk, Super shop.

INTRODUCTION

Vegetables are commonly consumed all over the world due to their nutritional value and their availability. Also, the people are now more careful than previous days and follow healthy lifestyle which has boosted up the trend of consumption of raw vegetables and food globally (1-3). Vegetables and food are low fat and low energy-dense foods besides containing varieties of vitamins, minerals, and other phytochemicals that help to prevent many serious diseases like cardiovascular disease; cancer; diabetes; and osteoporosis (4-6). However, along with nutritional value consumption of raw or undercooked vegetables can cause many food-borne diseases (1, 7-13). Growth of microorganisms on vegetables depends on various intrinsic and extrinsic factors such as pH, temperature, moisture, acidity, water activity, oxygen concentration, redox potential, etc. (14). Generally, vegetables contain varieties of fungi and bacteria including *Aspergillus* spp., *Escherichia coli*, *Klebsiella* spp., *Staphylococcus aureus*, *Bacillus* spp., *Pseudomonas* spp., *Listeria* spp., *Salmonella* spp., *Shigella* spp., *Vibrio* spp. etc (7, 10, 13, 15). These microorganisms cause many food-borne diseases such as food poisoning, diarrhea, dysentery, cholera, etc. (7, 10, 13). The sources of microorganisms in vegetables can be identified as air, water, the soil of cultivated land, pots in where vegetables are kept, shopkeeper's hands, hair, dresses, etc. (16-20).

Like other countries, ranges of vegetables are available in Bangladesh which are and also popular to Bangladeshi people. However, poor processing practices, post-harvest handling using non-sterile, use of poor quality water for washing and faulty packaging and transportation system make them more susceptible to microbial contamination (23). In Dhaka city, vegetables are sold in local markets as well as from super shops. People prefer local markets for purchasing vegetables for several reasons such as, low price, easily accessible locations and vegetables seem fresh, etc. However, super shops are becoming popular day by day in Dhaka city because vegetables and other food items are kept in a chiller in hygienic conditions which are properly maintained in local market (10).

Generally, local markets are located in open places whereas super shops are placed in closed building. Local markets are congested and dirty whereas super shop is not congested but much cleaner. Water, air, floor, vegetable pots are much clean in super shops than local markets. Furthermore, many studied reported that preservation systems and hygienic practice are very decent in super shops comparing to the local markets (36, 37).

Considering all these facts, this study was designed

***Corresponding Author**: *Mailing address.* Kamal Kanta Das, Lecturer, Department of Microbiology, Stamford University Bangladesh, 51, Siddeswari Road, Dhaka-1217, Bangladesh; E-mail: kkanta_36@stamforduniversity.edu.bd.*

to investigate and compare the microbiological quality of common vegetable samples from local and super markets of Dhaka city.

MATERIALS AND METHODS

Samples and Sampling Sites. Total 24 vegetable samples of four categories (Lady's finger, Coriander, Green chili and Tomato) were collected from three local markets (Karwan Bazar, Malibag, Shantinagar) and three super shops (near Bailey Road) early in the morning and transported to the laboratory as soon as possible according to the standard methods suggested by American Public Health Association (21).

Microbiological analysis of sample. Ten grams of each of the vegetable sample was homogenized in 90 ml saline and diluted up to 10^{-6} following the standard methods then the volume of 0.1 ml from each sample suspension was spread onto nutrient agar (NA) and incubated at 37°C for 24 hours for enumerating total viable bacteria (TVB). Sabouraud dextrose agar (SDA) (Oxoid Ltd., Hampshire, England) was inoculated followed by incubation at 25°C for 48 hours for isolation of fungi. On the other hand, for the isolation of coliform bacteria (*Escherichia coli*, *Klebsiella* spp.), 0.1 ml of each sample suspension was spread over MacConkey (Oxoid Ltd., Basingstoke, Hampshire, England) agar and incubated at 37°C for 24 hours (7, 10, 22). For enumerating total fecal coliform, 0.1 ml of each sample suspension was spread onto membrane fecal coliform (MFC) (Oxoid Ltd., Hampshire, England) agar and incubated at 45°C for 24 hours. 0.1 ml of each sample suspension was spread on mannitol salt agar (MSA) (Oxoid Ltd., Hampshire, England) for the estimation of *Staphylococcus aureus*, and the plates were incubated at 37°C for 24 hours. For the estimation of starch hydrolyzing bacteria (*Bacillus* spp.), 0.1 ml of each sample suspension was spread onto starch agar (SA) (Oxoid Ltd., Hampshire, England) and incubation at 37°C for 24 hours (23-24).

For the enumeration of *Pseudomonas* spp., 0.1 ml of each sample suspension was spread onto Pseudomonas agar (Oxoid Ltd., Hampshire, England) and plates were incubated at 37°C for 24 hours. For the estimation of *Listeria* spp., 0.1 ml of each sample suspension was spread onto Listeria identification agar (LA) (Oxoid Ltd., Hampshire, England) containing *Listeria* supplements and the plates were incubated at 37°C for 24 hours (23-24).

Enrichment of *Salmonella* spp., *Shigella* spp. and *Vibrio* spp. The *in vitro* cultivation of the species of *Salmonella*, *Shigella* and *Vibrio* often appears difficult or with faulty results (false-negative) due to their viable but non-culturable (VBNC) attributes (32-33). Therefore, enrichment was used prior to isolating these bacteria (7, 24). Enrichment was performed for *Salmonella* spp. and *Shigella* spp., in the selenite cysteine broth (SCB). 1 ml of homogenized sample suspension was transferred to SCB followed by incubation at 37°C for 4 hours and serially diluted up to 10^{-5}, and from 10^{-3} dilution, 0.1 ml was spread onto *Salmonella Shigella* (SS) agar (Hi media, India) followed by the incubation at 37°C for 24 hours. For the enrichment of *Vibrio* spp., 0.1 ml of the homogenized sample suspension was transferred to 10ml alkaline peptone water (APW) and incubated at 37°C for 4 hours and serial dilutions were made up to 10^{-5} and from 10^{-3} dilution, 0.1 ml was spread onto TCBS (Oxoid Ltd., Hampshire, England) agar followed by the incubation at 37°C for 24 hours (23, 24). Finally, representative isolates were confirmed by the number of biochemical tests, like the triple sugar iron (TSI), motility indole urease (MIU), methyl-red (MR), Voges-Proskauer (VP), citrate utilization, catalase, and oxidase tests (23-24)

RESULTS

Incidence of bacteria and fungi in vegetable samples. In our investigation, contamination was found in most of the samples (Lady Finger, Coriander, Green Chili, and Tomato), the total viable bacteria count (TVBC) was observed within a range of 10^5 to 10^7 cfu/g in all samples collected from local markets and super shops. The highest TVBC is observed in Lady Finger (3.2×10^7 cfu/g) and Coriander (2.6×10^6 cfu/g) also showed the maximum total fungal count, both samples are collected from local shops (Table 1). In the case of local shop samples, most of them were contaminated by all pathogenic bacteria. Where

Table 1. Microbial proliferation of vegetables collected from local markets.

Sample	TVB	TF	*Escherichia coli*	*Klebsiella* spp.	TFC	*Staphylococcus* spp.	*Bacillus* spp.	*Pseudomonas* spp.	*Listeria* spp.	*Salmonella* spp.	*Vibrio* spp.
Lady's Finger	3.2×10^7	1.5×10^6	2.6×10^3	8.2×10^2	4.0×10^2	1.1×10^3	2.6×10^2	0	1.8×10^2	8.5×10^2	0
Coriander	2.9×10^5	2.6×10^6	3.0×10^3	9.0×10^2	8.0×10^2	1.9×10^3	1.4×10^2	2.1×10^2	2.4×10^2	0	0
Green Chili	2.9×10^7	1.6×10^5	4.8×10^3	2.5×10^2	0	6.8×10^3	4.8×10^2	0	1.3×10^2	2.8×10^2	0
Tomato	1.9×10^7	1.1×10^5	3.2×10^3	1.4×10^2	0	6.4×10^3	4.0×10^2	2.8×10^2	1.7×10^2	0	0

Note: TVB-Total Viable Bacteria, TF-Total Fungus, TFC-Total Fecal Coliform.

Table 2. Microbial proliferation of vegetables collected from super shops.

Sample	TBV	TF	*Escherichia coli*	*Klebsiella* spp.	TFC	*Staphylococcus* spp.	*Bacillus* spp.	*Pseudomonas* spp.	*Listeria* spp.	*Salmonella* spp.	*Vibrio* spp.
Lady Finger	2.0×10^6	1.0×10^4	2.5×10^2	0	0	2.0×10^4	0	6.5×10^2	0	0	0
Coriander	3.5×10^6	1.0×10^4	3.0×10^2	8.0×10^2	0	4.0×10^2	0	7.6×10^3	0	0	0
Green Chili	7.5×10^6	0	0	0	0	1.7×10^2	0	8.0×10^3	0	0	0
Tomato	1.2×10^6	0	0	0	0	2.0×10^2	0	8.0×10^3	0	0	0

Note: TVB-Total Viable Bacteria, TF-Total Fungus, TFC-Total Fecal Coliform.

Table 3. Confirmative biochemical tests for the isolates.

Assumed Organism	TSI				Indole test	MR test	VP test	Citrate test	Catalase test	Oxidase test
	slant	butt	gas	H_2S						
Escherichia coli	Y	Y	+	-	-	-	-	+	+	-
Klebsiella spp.	Y	Y	+	-	-	-	+	+	+	-
Pseudomonas spp.	R	R	-	-	-	-	-	+	-	-
Staphylococcus spp.	Y	R	+	+	-	+	-	+	+	-
Bacillus spp.	Y	Y	-	-	-	-	+	+	+	-
Listeria spp.	Y	Y	-	-	-	+	+	-	+	-
Salmonella spp.	R	Y	-	+	-	+	-	-	+	-

Note: TSI-Triple Sugar Iron Test, Y-Yellow (Acid), R-Red (Alkaline), MR-Methyl red, VP-Voges-Proskauer.

Klebsiella spp., *Staphylococcus* spp., *Bacillus* spp., and *Listeria* spp. found in all samples within a range of 10^2 to 10^3 cfu/g. Fecal coliform, *Pseudomonas* spp. and *Salmonella* spp. were found to be present in only two samples and *Vibrio* spp. could not be isolated in any sample. On the other hand, super shop vegetables harbored only *Escherichia* coli, *Staphylococcus* spp., and *Pseudomonas* spp. within a range of 10^2 to 10^3 cfu/g. However, fecal coliform, *Bacillu*s spp. *Listeria* spp., *Salmonella* spp., and *Vibrio* spp. are totally absent in all samples collected from super shops.

Highest total coliform (4.8×10^3 cfu/g) was found in Green Chili of local market whereas lowest 3.0×10^2 cfu/g count was found in coriander of super shop. Similarly, highest count (8.0×10^2 cfu/g) of fecal coliform was found in coriander of local market whereas absent in super shop samples. The presence of total coliform in the food sample indicates the presence of fecal materials which is definite by the presence of *E. coli*.

Comparison of vegetables quality collected from local markets and super shops. Microbiological quality of vegetable sold in local markets and super shops were found to be different in our research. Many pathogenic bacteria and fungi were found in most of the samples collected from local markets and super shops. Total viable bacterial count was greater in both samples of local markets than super shops samples. In the case of fecal coliform count, local market samples showed unacceptable results whereas super shop samples showed no count. Similarly total coliform, *S. aureus, Bacillus* spp., *Pseudomonas* spp., *Listeria* spp., *Salmonella* spp., *Shigella* spp. and *vibrio* spp. showed huge count in local market vegetables whereas the same samples of super shops showed very limited count and some of these microorganisms showed no growth. Since almost all microbial counts were found greater in samples (Lady Finger, Coriander Green Chili and Tomato) collected from local markets than the similar samples from super shops.

Antibiogram of the bacterial isolates. Six presumptively identified *Staphylococcus* spp. from blood agar plates were selected for the determination of antibiotic sensitivity pattern against six different types of antibiotics. The results are shown in Table 4. Upon antibiogram profiling, it was clearly evident that *Staphylococcus* spp. were mostly resistant against Cotrimoxazole (84%) and highly sensitive to Vancomycin (100%) and Ciprorofloxacin (100%). Mixed sensitivity was observed against Amoxicillin (50%), Chloramphenicol (67%) and Erythromycin (50%).

DISCUSSION

The present investigation of vegetables (Lady Finger, Coriander Green Chili and Tomato) quality was different in both local markets and super shops in Dhaka city. The most contaminated vegetable was coriander from both local markets and super shops. Moreover, in every vegetable sample, almost all pathogenic microorganisms such as *Escherichia coli, Klebsiella* spp., fecal coliform, fungi, *Staphylococcus aureus, Bacillus* spp., *Pseudomonas* spp., *Listeria* spp., *Salmonella* spp., *Shigella* spp., *and Vibrio* spp. were found in undesirable amounts (38). The presence of *Staphylococcus* spp. in both categories of shops may be introduced from food handlers. The presence of other pathogenic bacteria like *Listeria* spp. and *Salmonella* spp. in the vegetables of local markets is of public health concern.

Previous study by Rahman *et al*. and Ahmed *et al*. found similar results and these microorganisms could cause various food-borne diseases such as food poisoning, diarrhea, dysentery, cholera as well as typhoid & pneumonia (24, 26). Raw vegetables were not safe to eat without processing or cooking, so, before eating as salad or food, these vegetables must be washed properly with clean water or other recommended washing methods (24, 27).

Moreover, bacteria isolated in this study may be part of the natural flora of the vegetables or contaminants from the pre and post-harvesting stage like air, water, cultivated soil, pots or places where vegetables kept, shopkeeper's as well as customer's hands, hair, dresses, etc. (28-31). Since local markets were placed in an open place which were congested and dirty, so vegetables could easily get contaminated from air, water and surround environments (34, 35). On the other hand, super shops were placed in closed building which was comparatively clean likewise supermarket salesman's were more carefully maintained almost all hygienic rules before handling vegetables to prevent microbial contaminations (36,37). So, government needs to give more attention in inspection on the local markets and organize some free session to literate vegetables handler about hygienic handling practice.

CONCLUSION

The presence of pathogenic bacteria contamination in fresh vegetables is not desirable, in any condition which may cause food-borne illness in human. That is why, GHP, GAP, and HACCP need to be introduced to avoid contamination. On the other hand, Government should take some necessary steps in the agriculture sector to reduce the health risk factors by foodborne illness. In conclusion, we found that four samples of vegetables (Green Chili, Tomato, Lady Finger and Coriander) under study were more or less contaminated but a comparatively higher load in local market samples indicated that the hygienic condition of these markets was poor. Our study thus reported not only a complete scenario on the microbial profile of the common vegetables from local and super shops but also compared their results.

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