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Fecal Contamination on Produce from Wholesale and Retail Food Markets in Dhaka, Bangladesh

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Abstract. Fresh produce items can become contaminated with enteric pathogens along the supply chain at the preharvest (e.g., irrigation water, soil, fertilizer) or postharvest (e.g., vendor handling or consumer handling) stages. This study assesses the concentrations of fecal indicator bacteria *Escherichia coli*, enterococci (ENT), and Bacteriodales on surfaces of carrots, eggplants, red amaranth leaves, and tomatoes obtained from both a wholesale market (recently harvested) and neighborhood retail markets in Dhaka, Bangladesh. We detected *E. coli* in 100% of carrot and red amaranth rinses, 92% of eggplant rinses, and 46% of tomato rinses. Using a molecular microbial source tracking assay, we found that 32% of produce samples were positive for ruminant fecal contamination. Fecal indicator bacteria were more likely to be detected on produce collected in retail markets compared with that in the wholesale market; retail market produce were 1.25 times more likely to have *E. coli* detected ($P = 0.03$) and 1.24 times more likely to have ENT detected ($P = 0.03$) as compared with wholesale market produce. Bacteriodales was detected in higher concentrations in retail market produce samples compared with wholesale market produce samples (0.40 $\log_{10}$ gene copies per 100 cm^2 higher, $P = 0.03$). Our results suggest that ruminant and general fecal contamination of produce in markets in Dhaka is common, and suggest that unsanitary conditions in markets are an important source of produce fecal contamination postharvest.

INTRODUCTION

Ingestion of contaminated produce, when eaten raw or cooked insufficiently to kill pathogens, poses a substantial risk to human health.[1] The diarrheal disease burden attributed to foodborne illness is difficult to assess, but it is estimated that 32% of gastroenteritis cases are transmitted by food in developed countries.[2] In the United States alone, around 48 million diseases each year are estimated to be caused by foodborne transmission, resulting in 3,037 deaths.[2] More than 25 million disability-adjusted life years (DALYs) were attributed to foodborne pathogens globally in 2010.[3] Although surveillance systems in low-income countries struggle to capture high quality data, it is estimated that Africa and Southeast Asia have the highest per capita foodborne disease burden in the world.[3,4]

High concentrations of fecal contamination have been found on produce in low- and middle-income countries. For example, a study in Tanzania assessing contamination on produce collected from markets and households found geometric means of 2,500 colony-forming units (CFU) of *Escherichia coli* and 6,300 CFU of enterococci (ENT) per item.[5] Several studies have found leafy vegetables, such as lettuce and spinach, to be more contaminated than other types of produce per unit mass. For example, in Mexico City, spinach and lettuce had the highest concentrations of fecal coliforms in comparison to radish, celery, and parsley; spinach and lettuce had geometric means of 2,400 and 3,600 most probable number (MPN) fecal coliforms per 100 g wet weight, respectively.[6] Similarly, leafy vegetables were found to be more contaminated than nonleafy vegetables in Pakistan,[7] and in Ghana, lettuce had higher contamination than cabbage and onions.[8] Although measuring fecal bacterial contamination per gram is relevant for food consumption, the units of normalization could be influencing the conclusion that leafy vegetables are more contaminated than other produce, because leafy items have larger surface areas per 100 g than many nonleafy vegetables.[9]

Food can become contaminated through preharvest mechanisms, including irrigation and fertilizer application, and through postharvest mechanisms, such as handling of produce during transport and in markets by vendors and customers. Some studies have found associations between irrigation water quality and fecal contamination of produce.[10–12] Wastewater irrigation can contaminate both the surface and the flesh of the produce.[6,13] Contaminated soil and manure used as fertilizer can also cause crop contamination.[13,14] A study in Pakistan assessing postharvest contamination of produce found that produce items in markets were more contaminated with fecal coliforms than preharvested produce items.[7] Another study in Ghana found that increased storage time in the markets was associated with increased contamination on lettuce leaves.[12] In addition, food markets in low-income countries can have poor drainage and sanitation infrastructure.[7,13] There has been limited work to identify the source of fecal contamination on produce (e.g., from human or animal hosts). Identifying the mechanisms of contamination in produce markets, the final distribution stage before direct consumer contact, could lead to the development of interventions to reduce health risks associated with handling and consuming contaminated produce.

This study explored the extent of fecal contamination on produce items in local markets in Dhaka, Bangladesh. Carrots, eggplants, red amaranth (*Amaranthus gangeticus*, a leafy green or purple vegetable), and tomatoes were collected from the largest wholesale market in the city as well as from four neighborhood food retail markets. We compared concentrations of fecal indicator bacteria found in rinses of produce items between a wholesale market (selling produce transported directly from farms) versus neighborhood retail markets. In addition, we used a ruminant-specific molecular source tracking assay to assess the prevalence of ruminant

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fecal contamination on produce, which could occur from manure used as fertilizer or contact with animal feces in markets.

METHODS

Study area and market selection. One wholesale and four neighborhood retail markets in Dhaka, Bangladesh, were included in the study. We selected the main wholesale market in Dhaka, *Kawran Bazar*, as it is a common place for vendors to purchase produce from growers to sell at smaller distribution markets. We selected neighborhood retail markets in the Mirpur community where members of the research team had previous experience. We completed a census of all known food retail markets in the Mirpur community. In Mirpur, we visited 10 markets known by our study team and selected four markets with the greatest number of vendors (based on visual estimation).

Market observation and produce sample collection. Data collection took place in December 2012. Trained Bangladeshi field enumerators conducted visual observations of each study market, collecting information on the types of water sources and drainage systems present, as well as on the presence of animals in the market. Field enumerators also estimated the number of vendors and customers present in the market. Data were recorded on handheld computers using The Survey System (TSS) software (Creative Research Systems, Petaluma, CA).

At each market, enumerators collected the following four types of produce items (selected as a representative variety of commonly eaten produce): carrots, eggplants, red amaranth leaves, and tomatoes (Figure 1). For each produce type, enumerators selected one vendor from each of the four retail markets and four vendors from the wholesale market (for a total of eight vendors per produce type and 32 vendors in all). From each vendor, the enumerator identified three pieces of the same type of produce from the top of the pile on display (for a total of 24 items per produce type). The enumerator asked the vendor to pick up each item and place it in an individual Whirl-Pak bag (Nasco, Fork Atkinson, WI) that had been prefilled with 250 mL sterile (autoclaved at 121°C for 15 minutes), 1/4-strength Ringer's solution (Oxoid Ltd., Hampshire, United Kingdom).[7,10] The enumerator then vigorously shook the sample bag for 15 seconds and subsequently massaged the item for 15 seconds while holding the outside of the sample bag.[5] The enumerator removed the produce item by manipulating it from the outside of the sample bag and then sealed and placed the bag in a cooler with ice until delivery to the laboratory for processing. All rinse samples were processed within 6 hours of collection.

Information was collected on the produce items using a handheld computer and TSS software. Enumerators made the following observations: location of produce items on display (i.e., ground versus table), visible wetness, visible dirt on items, and number of flies present near produce stand at the time of survey (flies were recorded on a scale of none, 1–5, 6–10, and greater than 10). Enumerators asked the vendor questions regarding produce handling practices, including any methods used to clean the produce items while in their possession.

Microbial processing of produce rinses. The produce rinses were processed for determining the MPN of *E. coli* (EC) and ENT enumeration using IDEXX defined-substrate assays with Quanti-tray 2000s (IDEXX, Westbrook, ME). Colilert-18 media was used for culturing EC and Enterolert was used for ENT. The rinse samples were processed following the manufacturer's instructions, except that the Colilert-18 trays were incubated at 44.5°C for 18–22 hours, allowing for enumeration of fecal coliforms in addition to EC.[15,16]

To enumerate ENT, 1 mL of the produce rinse was processed. One hundred milliliter of sample was required for the IDEXX assay, so 99 mL of sterile water was added to the sample volume to result in a total volume of 100 mL. To enumerate EC, 50 mL of the produce rinse was added to 50 mL of sterile water and processed. Half of the produce rinses were also processed at 10 mL for EC enumeration to increase the upper detection limit of the assay. Trays with zero positive wells were recorded as 0.5 MPN per volume assayed.[17,18] Trays with all positive wells were recorded as 2,420 MPN per volume assayed. For rinse samples with two volumes processed with Colilert-18, if both trays had positive wells within the range of quantification, then an average concentration was calculated. If only one of the rinse sample volumes resulted in a concentration within the range of quantification, then that concentration was recorded. The lower limits of quantification for the ENT and EC methods, reported as MPN per 100 cm^2 based on the mean surface areas for each produce type, are presented in Supplemental Table 1.

The concentrations of the fecal indicators detected in the rinse sample were reported as MPN per 100 cm^2 surface area of the item. The concentration of the fecal indicator bacteria in the rinse volume assayed was converted to report the amount of fecal indicator bacteria in the entire rinse volume (250 mL).

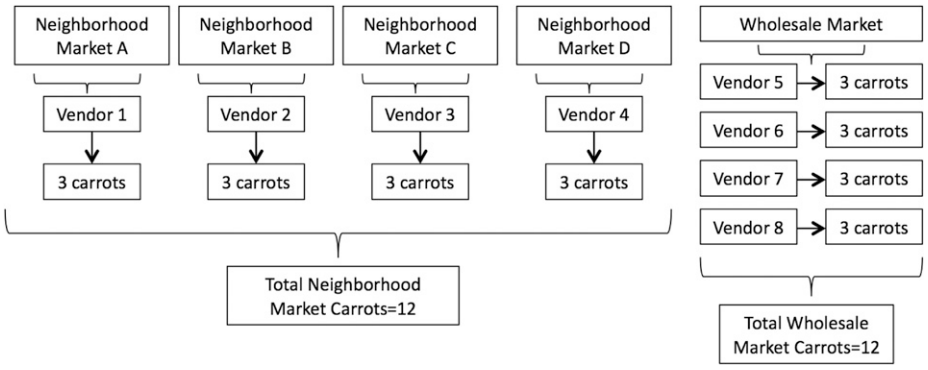

FIGURE 1. Markets sample scheme for carrots. Same sample scheme followed for all produce types (carrot, eggplant, red amaranth, and tomato).

This value was then divided by the approximate surface area of the item and multiplied by 100, resulting in a final value represented as MPN per 100 cm^2 of surface of the item. Surface areas of the produce items were approximated by using external measurements, similar to previously published methods.[19] For carrots, eggplants, and tomatoes, surface area approximations were made based on length and circumference measurements of individual items, assuming cylindrical shapes. The surface area of red amaranth leaf clusters was approximated by taking the square of the main stem length measurement.

Five method blanks were processed with the IDEXX assays over the course of the study. A method blank consisted of processing sterile water in place of a produce rinse sample in the laboratory. Two field blanks were also processed; a field blank consisted of processing an unused produce rinse bag stored in the field cooler.

DNA extraction and analysis from produce rinses. Three quarters of produce rinses were processed for detection of molecular markers of ruminant and general fecal contamination. All produce items collected from the neighborhood retail markets were processed for molecular analysis (N = 12 per produce type), whereas half of the produce rinses from the wholesale market were processed for molecular analysis (N = 6 per produce type). Fifty milliliter of each produce rinse was filtered through a 47-mm diameter, 0.4-μm pore size polycarbonate membrane filter (Isopore, Millipore, Billerica, MA). To stabilize the nucleic acids, 0.5 mL of RNAlater was added to the filter, allowed to sit for 3–5 minutes, and then vacuumed through the filter to remove the residual solution. Laboratory technicians ran three method blanks of the membrane filtration procedure using autoclaved (121°C for 15 minutes) distilled water as rinse water. Laboratory technicians also archived the filter from one field blank. The filters were placed in 2-mL tubes with glass beads (GeneRite, North Brunswick, NJ) and stored at –80°C until transported to the laboratory at Stanford University, where they were stored at –80°C until DNA extraction.

DNA was extracted from the filters using the DNA-EZ extraction kit (GeneRite, North Brunswick, NJ). DNA extraction was performed in sets of 10–20 filters at a time, and a process blank was created with each extraction set. Extraction process blanks were treated in the same way as the produce rinse samples, except that the extraction blank bead tube had no filter. GenBac3[20] and BacR[21] microbial source tracking qPCR assays were performed on the produce rinse DNA extracts. The BacR assay was previously found to be sensitive and specific for detecting ruminant fecal contamination in Dhaka, Bangladesh, and GenBac3 (a molecular target for bacteria of the order Bacteriodales) was a general indicator of fecal contamination in Dhaka.[18] The master mix used for the qPCR assays was ABI Universal (Applied Biosystems, Carlsbad, CA).[18] Previously published cycling parameters and primer and probe concentrations of the assays were used for the GenBac3 assay.[20] For the BacR assay, modified cycling parameters were used.[18] Each qPCR plate included a standard curve run in triplicate with concentrations ranging from 20 copies per qPCR reaction to 2×10^5 copies per reaction (alternatively, 10 copies per μL of DNA extract to 1×10^5 copies per μL of DNA extract). Two microliter of DNA extract was added per reaction (total reaction volume was 25 μL). The GenBac3 standard was genomic DNA from *Bacteroides thetaiotaomicron* and the BacR standard was a synthetic plasmid (IDT, Coralville, IA). Triplicate no-template negative controls were included with each plate. Produce rinse DNA extracts were processed in duplicate reactions.

The lower limit of quantification was 20 copies per reaction (the lowest standard that reliably amplified). For the GenBac3 assay, rinse samples with the molecular target either not detected or detected below the lower limit of quantification have concentrations reported as 10 copies per reaction (i.e., 1/2 the lower limit of quantification, based on the standard curve). Results of the BacR assay are reported as target detected (or not detected) and as concentrations if within the range of quantification. Rinse samples with BacR targets detected within the range of quantification or below the lower limit of quantification are considered positive. Concentrations of GenBac3 and BacR were reported as copies per surface area, which was calculated considering that 100 μL of DNA extract was prepared from 50 mL of rinse sample processed out of the total 250 mL in the rinse bag. The lower limits of quantification for the molecular target assays, reported as copies per 100 cm^2 based on the mean surface areas for each produce type, are presented in Supplemental Table 1.

Inhibition tests. To assess inhibition, a modified "spike and dilute" method was used.[18,22] One produce rinse sample per produce type was processed for inhibition. The sample DNA extracts were tested undiluted and at a 1:10 dilution, with the undiluted extract spiked with 2×10^4 copies of standard and the 1:10 dilution of the extract spiked with 2×10^3 copies of standard. If the difference in mean C_T values between the undiluted and diluted sample reactions was greater than 2, then the sample was considered uninhibited.[18]

Data analysis. All general indicator concentrations (EC, ENT, and GenBac3) were $\log_{10}$ transformed. Pearson's correlation was used to describe and characterize contamination on produce items. Clustering of produce contamination at the vendor level was accounted for in modeling using robust standard errors.[23] Poisson regression models were used to estimate the prevalence ratio of the presence of EC, ENT, and BacR at retail markets compared with the wholesale market (dependent variables indicated whether the target was detected or not). Linear regression models were used to estimate the difference in fecal indicator concentrations between retail markers and the wholesale market; dependent variables were $\log_{10}$ transformed concentrations of the general fecal indicators, EC, ENT, and GenBac3, reported per 100 cm^2 of surface area of the produce item. Produce type was controlled for in the regression models. P values less than 0.05 were considered statistically significant, and P values between 0.05 and 0.10 were considered marginally significant.

RESULTS

Market characteristics. The wholesale market had approximately 2,500 vendors, and one retail market had a similar number of vendors. The other three retail markets had between 400 and 600 vendors. All markets had at least one piped water source from the municipal water service provider, Dhaka Water Supply and Sewerage Authority. Three of the four retail markets had open concrete-lined ditches for drainage, in which enumerators observed water, trash, and food waste. The remaining retail market and the wholesale market had fully covered concrete ditches. All markets had chickens present at

the time of observation, and a subset of markets had cows ($N = 2$), goats ($N = 1$), sheep ($N = 1$), and ducks ($N = 2$). Similar quantities of flies were observed at produce stands in both the wholesale and retail markets. Overall, 10% of vendor stands had no flies, 70% had 1–10 flies, and 20% had > 10 flies at the time of the enumerator's observation.

Produce characteristics. Vendor-reported handling practices varied for the different produce items (Table 1). Some tomatoes and red amaranth leaves were reported by the vendor to have been wiped with a rag before rinse sample collection (38% of tomatoes and 13% of red amaranth leaves). Some eggplants (38%) and red amaranth (75%) leaves were reported by vendors to have been submerged in a bucket of water for cleaning before rinse sample collection. The majority of the study eggplants were visibly wet (75%) and the majority of the study tomatoes were dry (70%). All study carrots were visibly dry and all study red amaranth leaves were visibly wet.

Blanks and inhibition tests. All method and field blanks were negative for EC and ENT contamination. However, all membrane filtration blanks (both method and field) had the GenBac3 target detected below the range of quantification of the assay. As almost all produce rinse samples (96%) had the GenBac3 target detected within the range of quantification, the low concentrations of background GenBac3 in the method and field blanks unlikely impacted the results. The field and method blanks were negative for BacR. All extraction blanks and no-template controls were negative for GenBac3 and BacR molecular targets. All samples tested for inhibition were uninhibited; therefore, no additional efforts to correct for inhibition were used.

Fecal contamination on produce. Evidence of fecal contamination was found on the majority of produce (Figure 2), with detection of EC on 100% of carrots and red amaranth leaves, 92% of eggplants, and 46% of tomatoes. Concentrations of EC (MPN per 100 cm^2) ranged from a $\log_{10}$-mean of 2.7 on carrots, 2.3 on eggplants, 1.9 on red amaranth leaves, and 0.87 on tomatoes. ENT were detected on 100% of eggplants and red amaranth leaves, 75% of carrots, and 50% of tomatoes. The concentration of ENT varied by produce type from 2.5 to 3.4 $\log_{10}$-mean MPN per 100 cm^2 (Figure 2). GenBac3, a molecular marker for general fecal contamination, was detected on 100% of each vegetable, except for tomatoes (94%). When detected, concentrations of GenBac3 varied between 4.0 and 4.5 $\log_{10}$-mean target copies per 100 cm^2 for the different produce types (Table 2). There was a positive correlation between EC and ENT concentrations (Pearson's r correlation = 0.48, $P < 0.001$), between EC and GenBac3 concentrations (Pearson's r correlation = 0.20, $P = 0.10$), and between ENT and GenBac3 concentrations (Pearson's r correlation = 0.31, $P = 0.01$).

Presence of more than 10 flies at the vendor stand was not associated with increased contamination on produce as measured by the fecal indicators (linear regression, $P > 0.1$). Visible wetness was associated with concentrations of fecal contamination on eggplants. Wet eggplants were more contaminated with EC (1.5 $\log_{10}$ MPN EC per 100 cm^2 higher on average, linear regression, $P = 0.03$) and GenBac3 target copies (0.9 $\log_{10}$ copies per 100 cm^2 higher on average, linear regression, $P = 0.03$) as compared with dry eggplants. There were no significant associations between the detection of fecal indicators and a binary indicator of whether tomatoes were wet (Poisson regression, $P > 0.1$). Similar analyses were not undertaken for red amaranth and carrots because all red amaranth leaves were characterized as visibly wet and all carrots were characterized as dry.

The ruminant marker (BacR) was detected in a total of 23 produce rinses (32%) (Table 3). The BacR target was detected in rinses from 22% of carrots, 28% of eggplants, 39% of red amaranth leaves, and 39% of tomatoes. Only rinses obtained from red amaranth leaves (22%) had BacR detected within the range of quantification of the assay at concentrations between 9,300 and 37,000 copies per 100 cm^2. Six of 23 rinses positive for the BacR target (26%) were negative for ENT and/or EC.

Comparison of neighborhood retail and wholesale market produce. Retail market produce rinse samples were 1.25 times more likely to have EC present than wholesale market produce rinses (Poisson regression, binary outcome of indicator detected or not, $P = 0.03$) (Table 4). Similarly, retail market produce rinses were 1.24 times more likely to have ENT detected compared with wholesale market produce rinses (Poisson regression, $P = 0.03$). With the exception of ENT on amaranth leaves, retail market produce rinses had higher concentrations of all general fecal indicators as compared with wholesale market produce rinses (Table 2). For continuous outcome models of EC and ENT concentrations using multivariate linear regression, market type was marginally statistically significant (Table 4). *Escherichia coli* mean concentrations were 0.52 $\log_{10}$ MPN per 100 cm^2 higher ($P = 0.07$) and ENT were 0.36 $\log_{10}$ MPN per 100 cm^2 higher ($P = 0.06$) in retail versus wholesale market produce rinses. In addition, retail markets had statistically significant higher concentrations of the general fecal marker GenBac3 ($P = 0.03$) than the wholesale market, with mean concentrations 0.40 $\log_{10}$ GenBac3 copies per 100 cm^2 higher (equivalent to a 2.5-fold increase in contamination) (Table 4).

The ruminant fecal marker (BacR) was detected in both retail and wholesale market rinse samples. Market type was not significantly associated with BacR target detection in the produce rinses (Poisson regression, $P = 0.90$, $N = 72$) (Table 4). In addition, there was no association between the detection of the BacR target in a produce rinse and the produce item

TABLE 1

Characteristics related to vendor handling practices and visual appearance for carrot, eggplant, red amaranth, and tomato samples

	Carrot ($N = 24$)	Eggplant ($N = 24$)	Red Amaranth ($N = 24$)	Tomato ($N = 24$)
Displayed on table vs. ground	9 (38%)	12 (50%)	12 (50%)	12 (50%)
Appeared visibly wet	0 (0%)	18 (75%)	24 (100%)	7 (29%)
Appeared unclean/dirty	12 (50%)	15 (63%)	24 (100%)	15 (63%)
Wiped with rag	0 (0%)	0 (0%)	3 (13%)	9 (38%)
Submerged in bucket of water	0 (0%)	9 (38%)	18 (75%)	0 (0%)
Sprayed with water	0 (0%)	0 (0%)	0 (0%)	3 (13%)

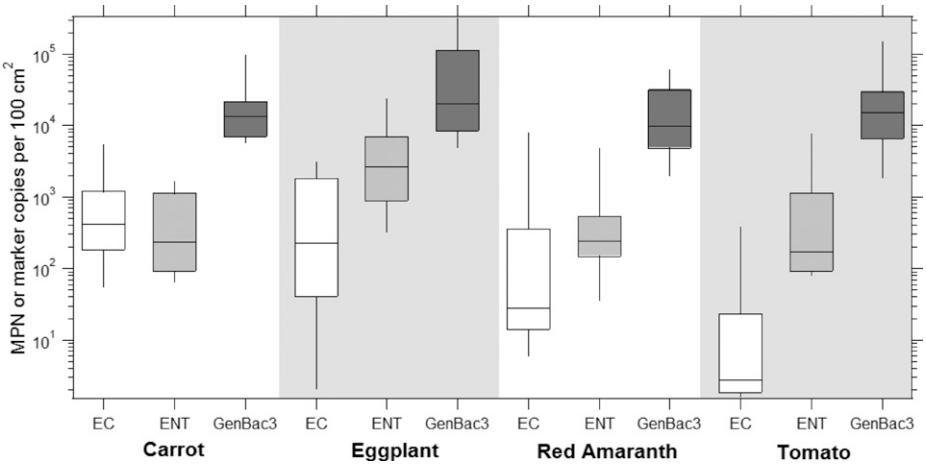

FIGURE 2. Concentrations of *Escherichia coli* (EC), enterococci (ENT), and GenBac3 in carrot, eggplant, red amaranth, and tomato rinse samples (for each produce type, $N = 24$ for EC and ENT, $N = 18$ for GenBac3). Concentrations are reported as the most probable number (MPN) per 100 cm^2 for EC and ENT, and as target copies per 100 cm^2 for GenBac3. Box plots show the range of 25th to 75th percentile of samples as the bottom and top edges of the box, with the center line of the box displaying the median concentration. The top and bottom lines show the 10th and 90th percentile range.

coming from a market where ruminants were observed to be present (Chi-square test, $P = 0.89$).

DISCUSSION

General and ruminant fecal contamination on produce was widespread in the markets we sampled in Dhaka. Produce items from neighborhood retail markets were generally more contaminated than similar items from wholesale markets. This relationship was identified across three different general indicators of fecal contamination (EC, ENT, and GenBac3), suggesting that transport to retail markets and/or neighborhood retail market conditions contribute to fecal contamination on produce postharvest. There are several possible mechanisms for produce contamination in markets, particularly related to vendor and consumer handling practices. Vendors often rinse or spray produce with stored water collected from community water sources (Table 1), which may be contaminated and lead to fecal contamination of produce.[12,24] Moist surfaces are stable environments for bacteria, and bacterial pathogens may persist longer on wet produce.[24,25] More efficient transfer of bacteria and viruses between hands and surfaces can occur when surfaces are moist.[26–28] Previous work suggests that fecal contamination on hands is high in these communities as compared with developed countries,[29] and unwashed hands could potentially transmit contamination to produce. More generally, hand sorting of produce has been identified as a critical control point for contamination, and pathogen transfer from hands during the sorting process of tomatoes was a potential contributor to a *Shigella flexneri* outbreak in the United States.[30] In addition, surfaces on which produce items are displayed (e.g., tables or mats) may themselves be contaminated.[14]

Our study found indicators of fecal contamination on all produce types. A smaller fraction of tomatoes had fecal contamination detected compared with other produce types as measured by EC and ENT (potentially because of their smooth surface), which is consistent with previous work.[7] As a point of reference, several studies assessing EC concentrations on lettuce leaves in developed countries (i.e., Norway, Spain, Canada, and Belgium) found that only a fraction of lettuce samples had EC detected (5–22%); however, caution must be used when making comparison across studies as detection methods vary.[31] The detection of multiple types of fecal markers (EC, ENT, and GenBac3) in rinses of produce items from Dhaka markets suggests potential for fecal pathogen presence and risk of enteric disease if produce are eaten raw or undercooked.

Highly contaminated produce may also serve as a vehicle for introducing pathogens into the household environment.[1] Prior research in Tanzania, for example, found that the act of preparing food increased ENT concentrations on female caregiver hands.[32] Another study in Tanzania found that recent handling of food items was positively associated with enteric pathogen detection on female caregiver hands.[33] In Mexico, hand contamination has also been linked to produce contamination;

TABLE 2

Log_{10}-mean concentrations and standard deviations (SDs) of the log_{10} transformed concentrations of *Escherichia coli* (EC), enterococci (ENT), and GenBac3 per 100 cm^2

Produce type	Mean surface area, cm^2 (SD)	Log_{10}-mean MPN EC per 100 cm^2 (SD)			Log_{10}-mean MPN ENT per 100 cm^2 (SD)			Log_{10}-mean GenBac3 copies per 100 cm^2 (SD)		
		Overall	Retail	Wholesale	Overall	Retail	Wholesale	Overall	Retail	Wholesale
Carrot	190 (39)	2.68 (0.62)	2.76 (0.64)	2.60 (0.62)	2.53 (0.77)	2.90 (0.87)	2.16 (0.43)	4.19 (0.42)	4.08 (0.32)	4.41 (0.53)
Eggplant	405 (68)	2.27 (1.07)	2.52 (0.93)	2.03 (1.18)	3.38 (0.66)	3.58 (0.69)	3.17 (0.59)	4.47 (0.68)	4.63 (0.77)	4.14 (0.26)
Red Amaranth	990 (380)	1.85 (1.05)	2.01 (0.55)	1.69 (1.40)	2.48 (0.74)	2.32 (0.51)	2.63 (0.92)	4.04 (0.55)	4.26 (0.53)	3.60 (0.27)
Tomato*	72 (26)	0.87 (0.88)	1.42 (0.95)	0.32 (0.19)	2.50 (0.68)	2.82 (0.74)	2.19 (0.45)	4.16 (0.63)	4.41 (0.59)	3.66 (0.37)

Log_{10}-means are reported overall and stratified by market type (retail vs. wholesale markets). Means and SDs of estimated item surface area by produce type.

* Fifty-four percent of tomato rinse samples did not have EC detected and 50% did not have ENT detected; these samples have nonzero values assigned for analysis purposes.

TABLE 3

Number (%) of produce rinse samples with the BacR ruminant-specific target detected by produce type

	Overall	Retail	Wholesale
Carrot (N = 18)	4 (22%)	3 (25%)	1 (17%)
Eggplant (N = 18)	5 (28%)	1 (8%)	4 (67%)
Red Amaranth (N = 18)	7 (39%)	7 (58%)	0 (0%)
Tomato (N = 18)	7 (39%)	4 (33%)	3 (50%)

TABLE 4

Association between market type (retail vs. wholesale) and the following fecal indicators: *Escherichia coli,* enterococci (ENT), ruminant-specific fecal marker (BacR), and general fecal marker (GenBac3)

		PR	SE	*P* value
Binary outcomes	*E. coli*	1.25	0.13	0.03
	ENT	1.24	0.12	0.03
	Ruminant marker	1.07	0.54	0.9
		β	SE	*P* value
Continuous outcomes	*E. coli*	0.52	0.27	0.07
	ENT	0.36	0.2	0.06
	General marker	0.4	0.17	0.03

Binary outcome models (indicator detected = 1 and indicator nondetected = 0) estimated prevalence ratios (PR) using Poisson regression of detectable indicators in produce rinses in retail markets compared with wholesale markets. Continuous outcome models used multivariate linear regression to estimate associations between indicator concentrations (MPN/copies per 100 cm^2) and market type. Standard errors (SE) of β coefficients are reported. All models include robust standard errors to account for clustering at the vendor level, and control for produce type (coefficients not shown).

concentrations of a general molecular fecal target (AllBac) on hands of agricultural farmworkers were positively correlated with concentrations of the target on produce they were handling.[10] Notably, caregiver handwashing with soap before and during food preparation has been associated with less frequent diarrhea among their children.[34] However, washing produce and handwashing may not prevent pathogens from being transferred from produce into the home environment. Taken together with previous evidence, our work suggests that attention should be given to produce as a potential pathway of fecal contamination into the home.

We found evidence of ruminant fecal contamination on produce. Exposure to ruminant feces may pose substantial health risks to humans, as ruminant feces can contain zoonotic pathogens such as *Campylobacter*, *Giardia*, *Cryptosporidium*, *Salmonella*, and EC O157:H7.[14,35,36] We do not know how the produce in our study became contaminated with ruminant feces. In each of the study markets, animals were observed in close proximity to produce stands. In addition, farmers in Bangladesh often use cow manure as fertilizer to grow produce.[37] Future work to elucidate mechanisms for the transmission of animal feces to produce could help inform development of management strategies to prevent fecal contamination of produce items.

There are several limitations to our study. Surface area measurements were approximated with imperfect assumptions regarding produce shape. Comparisons between produce types should be interpreted with caution because of potentially different detection limits and recovery efficiencies. We have no reason to suspect systematic error within produce types; therefore, we believe the relative comparisons between market types and wetness conditions are appropriate for our data. Market type was only marginally statistically significant (*P* values between 0.05 and 0.1) in the continuous outcome regression models for EC and ENT concentrations. We had limited statistical power for these comparisons because of the small sample size and accounting for clustering at the vendor level using robust standard errors. However, market type was statistically significant in the binary outcome models for detection of *E. coli* and ENT, so we believe the combined results provide support for the conclusion that contamination on produce is related to market type. The recovery efficiency of the sampling method is unknown; however, systematic bias of recovery efficiency between produce from wholesale and retail markets is unlikely. The culture-based fecal indicators used in this study are commonly used to measure contamination on fresh produce[38]; however, some EC and ENT could be of environmental, not fecal, origin.[39–45] By using a rinse method, we were only able to detect contamination on the surface of the produce items; thus, we did not capture internalized contamination of the produce. We found low concentrations of GenBac3 (below the lower limit of quantification) in membrane filtration blanks, which may have occurred because of difficulty of processing samples in an active field laboratory with limited space to work. Analyses were conducted considering samples (N = 2) with GenBac3 targets detected below the lower limit of quantification as nondetects, thus minimizing the potential impact on results of the low concentrations found in the blanks. Another limitation of the indicators is that they may behave differentially on produce compared with some pathogens, such as soil transmitted helminths.[8] Additional study of the association between fecal indicator organisms and pathogens on produce would improve interpretation of indicator measurements.[31]

The results of our study suggest that local market conditions contribute to produce contamination. Improving sanitary conditions and produce handling practices by vendors could be explored as methods to prevent the introduction of fecal contamination on produce in food markets.[12,46] For instance, improving drainage and management of human and animal waste at markets, using treated (e.g., chlorinated) water for rinsing produce, and keeping produce dry could reduce contamination.[24,47,48] Future work could evaluate the feasibility and effectiveness of interventions such as installing handwashing stations in markets for use by vendors and consumers, or providing liquid disinfectants such as chlorinated water or acetic acid for cleaning produce.[48–50] Informing buyers of proper food preparation methods, such as cooking at a temperature of 70°C and washing, soaking, and scrubbing produce items in chlorinated water to remove microbiological contamination[46,47,50–52] could be another strategy to be explored to reduce contamination before consumption.

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