Original article

Comparative analysis of *Escherichia coli* contamination on fresh produce at the market: human handling is a significant parameter of contamination

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Abstract. This study explores the effect of human handling on *E. coli* contamination of fresh produce (FP) in the overlooked market stage where customers touch FP. Study includes an observational and a comparative part with 3 experimental sample groups as; (i) Control group (CG), (ii) Touched FP group (TG) and (iii) FP touched with gloves group (GG). In the comparative part, generic *E. coli* and Shiga toxin producer *E. coli* (STEC) were screened, quantified and analyzed for antibiotic susceptibilities. The average score of sellers' knowledge level was low (39%). *E. coli* counts from banana, lettuce, carrot and tomato were found to be significantly higher (p=0.037, p=0.046, p=0.046 and p=0.034 respectively) in TG compared to CG which indicates human handling as a significant factor of bacterial contamination in the market. *E. coli* counts were significantly lower for banana, lettuce and tomato in GG (p=0.037, p=0.001, p=0.034 respectively) which is probative for the dissemination at the market through human handling and is also indicating this as a useful/practical prevention tool. Shiga toxin-producing *E. coli* (STEC-O157:H7) was isolated only from one lettuce sample in TG. The highest resistance rate was observed for ampicillin (44%), followed by cephalothin (40%), tetracycline (24%) and amoxicillin-clavulanic (24%). Here, we demonstrate that human handling of FP in the market stage is a significant contributing factor in *E. coli* contamination, which may include STEC. Furthermore, our findings suggest that this contamination is preventable by using practical materials such as gloves.

Keywords: Fruit, vegetable, fresh produce, *E. coli*, market, contamination

1. Introduction

Accumulating evidence on the health benefits of incorporating fruits and vegetables into a balanced diet is attracting consumers to raw, fresh produce (fruits and vegetables; FP) in the marketplace (1). On the other hand, the safety concerns arising from the market period and preparation methods of such products are well-documented (2). Indeed, a striking 600 million cases of foodborne diseases and 420,000 foodborne illness-attributed deaths have been reported in 2010, by the World Health Organization (3).

Three main hazard types associated with FP that can pose a threat for consumers are; physical, chemical and biological hazards (2). Physical and chemical hazards may include excess contamination by mate-

rials such as dirt or with different chemicals which could be pesticides or other agricultural products respectively. Biological hazards, however, can cause relatively higher morbidity and mortality, since biological contamination of FP can lead to food borne infections (4). Indeed, between 1996 and 2016, 88 different types of FP were reported to cause outbreaks in the United States (US) (5).

The bacterial contamination of FP takes place at different stages of the "farm to consumer" process (6). Ongoing research draws attention to certain pathogens such as *Escherichia coli,* which is associated with higher mortality and morbidity rates involving different strains (7,8). *E. coli,* especially *E. coli* O157:H7 (Shiga toxin producer *E. coli*; STEC), is one of the leading causes of foodborne infections throughout the world and in the last few years, outbreaks of STEC have been increasingly linked to consumption of fresh vegetables (4). Therefore, this pathogen is one of the biggest challenges for the microbiological safety of FP.

In terms of control and prevention, there is a growing interest in finding an effective method to get rid of bacterial contamination of FP during different stages prior to human consumption. In addition, the mechanisms behind the pathogenic dissemination is still not fully understood. In this regard, many studies have been performed to increase the understanding of pathogenic dissemination pathways during the "farm to consumer" process. Notably, post-harvest stage is often indicated as an important stage for bacterial contamination especially due to the human contact involved (9). However, dissemination which may occur at the market stage where customers randomly and repetitively touch, select and buy FP is often overlooked and remains poorly studied. Therefore, there is a need to describe the pathogenic dynamics which may have a relation to human handling in the market stage, which would allow development and testing of various preventative strategies to contribute to the safety of FP in the market stage.

This study aims to; (i) assess the knowledge and attitude of retail sellers regarding to FP safety, (ii) quantitively describe the *E. coli* (including STEC) contamination because of human handling at the market and characterize the antibiotic susceptibilities of the isolates and (iii) investigate the potential preventative role (and probative role for the dissemination at the market through human handling) of sterile gloves to be used by customers during the market stage.

2. Materials and Methods

2.1 Study Design

The present study was designed to consist of two parts as an observational and comparative/experimental study (Figure 1). Famagusta is a city located in Cyprus at 35.2857° N, 33.8411°E coordinates. FP produced in the neighboring villages is sold by the retail sellers mostly being producers directly in this city at the central open-air street market.

In the observational part, a questionnaire prepared by the researchers conducting this study was used to evaluate the knowledge and attitude of retail sellers in the market towards FP hygiene.

In the experimental part, six FP were chosen. Namely; tomato, lettuce, banana, carrot, pepper and apple. Enumeration and phenotypic characterization of bacteriological samples which were obtained from FP were investigated and their antibiotic susceptibility profiles were characterized. Also, a comparative experiment was carried out to understand the role of human handling during market stage and to assess the possible effect of gloves both to prove the effect of human handling on contamination and to test the possible preventive effect of gloves for *E. coli* transmission at the market stage.

2.2 Questionnaire

In order to investigate the knowledge and attitude about FP hygiene, questionnaires were distributed. The questionnaire consists of 14 different questions of which 4 questions were demographical, and 10 questions were asked to assess the knowledge levels and attitudes about FP hygiene. The questions which were about the assessment of sellers' knowledge were designed by considering the risk factors which were defined in US Food and Drug Administration (FDA) guidelines (8).

In order to determine if the questionnaire is easy to understand, eliminate any ambiguity and to evaluate

the questionnaire's lingual reliability, a pilot study was performed. In this regard, 20 people speaking both Turkish and English fluently were asked to complete the questionnaire in both languages. In total, 5% difference between answered questions were considered as an acceptable level. Being below the age of 18 was an exclusion criterion of this study.

2.3 FP Samples

For the experimental part of the study, six FP with 3 replicate groups were selected. The criteria for FP selection were; consisting of high nutrients, being most commonly preferred and having a high risk of bacterial contamination. Selected FP were cleaned using ethanol-sterile gauze before the experiment and were put into three separate boxes.

In order to test every condition appropriately, instructions were given to the voluntary customers in advance. Three different conditions were established for each of the FP: (i) Control Group (CG), (ii) Touched FP Group (TG) and (iii) FP touched after wearing gloves Group (GG). Explanations for these sample groups are given in Figure 1.

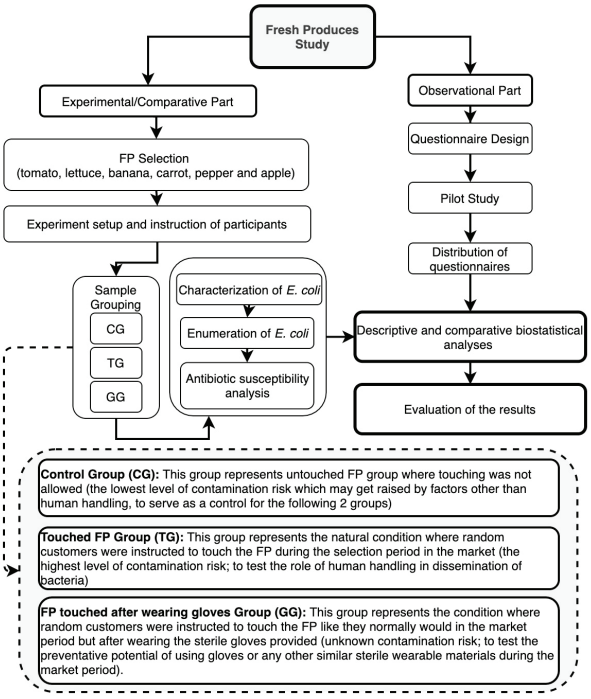

Figure. 1 Flowchart summarizing the study design for the present study. Explanations for the sample groups are also given.

2.4 Phenotypic characterization and quantification of bacteriological samples

At the end of the experiment, the FP were gathered and brought to the laboratory in sterile polythene zip bags. The samples were processed by obtaining 40g pieces from each FP sampled from their outer layers. Diluted samples (1:10, in Peptone water) were stomached and incubated at 37°C for 24 h. Then, samples were processed for enumeration of total generic *E. coli* and STEC For generic *E. coli* counting, colonies were inoculated in Eosin Methylene Blue Agar (EMB, HI media; M022-500G), and incubated for 24 hours at 37°C. Then, green metallic sheen colonies which represent *E. coli* phenotypes were counted. For the confirmation, colonies were transferred into Triple Sugar Iron (TSI) agar and standard IMVIC tests which are Indole, Methyl red, Voges-Proskauer, Citrate tests respectively, was applied. Finally, the samples resembling *E. coli* phenotype were selected. STEC were screened using the reference method (10). Counting of the colonies was done manually and calculated as log CFU/g.

2.5 Antibiotic susceptibility testing of collected isolates

Antibiotic resistance pattern of *E. coli* isolates was evaluated by performing an antibiotic susceptibility experiment with 6 different antibiotics. Namely (abbreviations and amounts are in parentheses): ampicillin (AM; 30 µg), amoxicillin-clavulanic acid (AMC; 20/10 µg), gentamycin (CN; 10 µg), imipenem (IPM; 10 µg), cephalothin (CEF; 30 µg), ceftriaxone (CRO; 30 µg), nalidixic acid (NAL; 30 µg), ciprofloxacin (CIP; 5 µg), chloramphenicol (CLR; 30 µg), tetracycline (TET; 30 µg) and trimethoprim-sulfamethoxazole (SXT; 23.75/1.25 µg). Colonies which were obtained from over-night grown bacterial culture in EMB were transferred into sterile saline solution and the turbidity was set to 0.5 McFarland standards. From each isolate, liquid was inoculated into Mueller Hinton Agar (MHA, Merck; 1.05437.0500) plate by using sterile swabs. Once the plate got dried, the antibiotic discs were applied into the inoculated plates by using an antibiotic dispenser. The plates were incubated over-night at 37°C and evaluated according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (11).

2.6 Statistical analysis

Data collected from the questionnaires were evaluated by calculating descriptive statistics as percentages for the (i) application of correct FP washing methods, (ii) for reporting knowledge levels out of 100, and (iii) attitudes towards FP safety. Pearson's chi-square tests were conducted to detect possible associations between application of correct washing methods, knowledge and attitude towards FP safety and demographic variables. Age, gender and educational level were considered as being comparison variables. Ages were categorized as 18-29, 30-49 and 50+. Educational levels were categorized as high school graduates, undergraduates and graduates. Mean and standard deviations were calculated to describe *E. coli* counts on FP. Mann-Whitney U test (for non-normal distributed counts), and independent-samples t-test (for normally distributed counts) were applied in order to compare *E. coli* counts between CG, GG and TG for each FP. A p-value less than 0.05 was considered to show a significant result.

3. Results and Discussion

3.1 Knowledge and attitudes of the sellers about safe FP handling

In the present study, knowledge and attitudes regarding the methodology of the proper washing methods was investigated for six different FP with a questionnaire. Our evaluations are based on the guideline released by FDA to minimize microbial food safety hazards for FP (8). In our study population, the average knowledge level of sellers on proper washing methods was found to be 39% in average for six types of FP (Table 1a). Specifically, this frequency was lowest for washing of carrots where 94.7% of the participants picked the incorrect washing methods when asked for how they wash carrots. According to the FDA guidelines, the proper washing method of carrots is scrubbing with a brush (8). However, using a brush for washing fruits can be time-consuming which could be the reason of the poor knowledge of sellers for the case of carrots. According to the FDA guidelines, cleaning of lettuce by the sellers should be done by using vinegar (8). However, 90.6% of sellers claimed that they do not use vinegar when they wash lettuce. Interestingly, application of proper washing method for lettuce showed a significant difference between genders of which, 95.5% of men sellers stated that they do not wash lettuce in any way while 84.5% of women participants stated that they wash lettuce by applying proper washing method (p=0.031). Also, the number of people younger than 30 years old who were applying proper washing method for lettuce was significantly higher compared to other age groups (p<0.031). Low level of knowledge of proper lettuce washing methods is especially important since consuming contaminated lettuce is strongly related with severe food borne illness and outbreaks which could be easily prevented by using vinegar. Besides, only 18.9% of the sellers stated that they clean bananas in a proper way which is washing under the running tap water. Also, based on answers, many participants wash apple, pepper and tomato properly with high percentages as 63.5%, 66.5%, and 70.1% respectively.

Our participants were also asked about food handling knowledge and the results were demonstrated in Table 1b. A big portion of sellers (76.8%) who were younger than 30 years old agreed with the statement; "I believe foodborne illness caused by bacteria on fresh FP could be problem" (p=0.017). Similarly, the number of graduate level educated participants who agreed with the statements of "Foodborne illness can occur if fresh FP are handled unsafely" and "proper storage of FP can keep them safe to eat." was significantly higher than the number of people who have lower and higher education level (p=0.002 and p=0.006 respectively). In the concept of food safety, proper hand washing and packing are other parameters which were reported to have positive effects on keeping produce clean (8). In this regard, habits and attitudes of respondents were asked in the questionnaire. In our study, 56.5% of the participants claimed that, they wash their hands before handling the FP (Table 1c). Number of men who agreed with the statements of "bagging each FP separately" and "washing whole FP can help keep them safe to eat" were significantly higher than the number of women (p=0.005 and p=0.038 respectively). As a limitation, we did not investigate

Table 1 Proper FP washing knowledge and attitudes of participants.

	Question	Percentage (%) †	Associations of percentages with demographics (p values) ‡		
			Age	Gender	Education level
a. Percentage of respondents who claimed to apply the washing method which described by FDA guidelines for washing FP.	Apple	63.5	p=0.176	p=0.786	p=0.728
	Tomato	70.1	p=0.145	p=0.307	p=0.884
	Banana	18.9	p=0.080	p=0.726	p=0.146
	Pepper	66.5	p=0.051	p=0.742	p=0.997
	Lettuce	9.4	p=0.031*	p<0.001*	p=0.534
	Carrot	5.3	p=0.428	p=0.711	p=0.092
	Average	***39***	-	-	-
b. Knowledge of respondents towards FP safety	Foodborne illness can occur if fresh FP are handled unsafely.	75.4	p=0.266	p=0.205	p=0.006*
	Washing whole FP can help keep them safe to eat.	78	p=0.109	p=0.355	p=0.046*
	Foodborne illness caused by bacteria on fresh FP could be problem.	76.8	p=0.017*	p=0.907	p=0.068
	How fresh FP are handled is important to keep them safe to eat.	74.6	p=0.056	p=0.726	p=0.007
	Proper storage of FP can keep them safe to eat.	78.6	p=0.113	p=0.708	p=0.002‡
	Average	***76.8***	-	-	-
c. Attitudes of respondents towards FP safety	Bagging each FP separately	34.9	p=0.033*	p=0.005*	p=0.046*
	Washing their hands every time before handling FP.	56.5	p=0.630	p=0.038*	p=0.995
	Washing FP because they want to remove germs and bacteria.	52.9	p=0.887	p=0.129	p=0.497

† Percentage of answers given to different questions regarding the FP knowledge/attitudes. ‡ Pearson's chi-square significance scores of associations between percentages and demographics. * Statistically significant association.

participants' hand washing or packing practices. Nevertheless, low levels of hand washing and packing knowledge prior to handling may indicate a need for education campaigns about the proper hand washing and packing methodologies since these factors strongly counteract contamination by microorganisms (8).

The poorest recorded outcome was on the awareness of respondents on the importance of putting each FP in a separate bags (34.9%). A correlation has been detected between better attitudes (about the statement in the previous sentence) with decreasing age (p=0.033), being male (p=0.005), and increasing level of education (p=0.046) (Table 1c). On the other hand, most of the respondents (78.6%) were knowledgeable about proper storage which is an important parameter to keep FP safe (Table 1b).

3.2 Testing E. coli contamination of FP in different sample groups

3.2.1. E. coli contamination of the FP at the market originate from human handling

In this study, the effect of human handling on dissemination of *E. coli* during FP selection stage in the market was investigated. In order to investigate this effect, the amount of *E. coli* was quantified, and comparison was made between FP in CG and TG. Precisely, *E. coli* counts were assessed in TG to reflect the usual situation of bacterial contamination which occurs in the market during the selection stage of FP in daily life.

The results of microbiological analysis for FP in CG and TG were presented in Table 2. Overall, generic *E. coli* counts varied between FP in CG and TG. In the beginning of the experiment for testing the human handling effect, FP in CG were cleaned with alcohol. However, it should be noted that, the cleaning did not completely eliminate the bacteria present on the surface of some FP. In this regard, *E. coli* was detected in two out of six FP in CG (Table 2). More precisely, after performing a cleaning step, carrot and lettuce still contained *E. coli* log 2.33 and 2.20 CFU/g (mean), respectively. The reason for this could be that, these two FP have a higher potential to harbor dense bacteria on their surface since lettuce is a leafy vegetable and carrot has close contact with soil (12). These features could eventually protect the adheration of bacteria to these FP more than others.

In this study, every FP in TG were reported to harbor *E. coli* and STEC was isolated only from lettuce samples in TG. Among FP in TG, *E. coli* was quantified in a mean range of log 2.10 CFU/g to log 4.33 CFU/g. The lowest numbers of bacteria were identified on banana, apple and pepper, where the mean counts were lower than log 3.60 CFU/g. However, for lettuce and carrot, the highest mean bacterial numbers were observed as log 4.33 CFU/g and log 4.02 CFU/g, respectively. Importantly, banana (p=0.037), lettuce (p=0.046), carrot (p=0.046) and tomato (p=0.034) samples in TG displayed significantly higher bacterial counts in contrast to GG. Also, STEC numbers counted from lettuce in TG were less then log 1 CFU/g. High bacterial counts may be related to the FP's morphology. For instance, the high amount of *E. coli* and presence of STEC found on lettuce and carrot is likely promoted by the large and rough surface of leaf structure (13). Also, when compared to other FP, lettuce was previously shown to harbor more *E. coli* and STEC counts (14).

E. coli, especially STEC carriage of FP can pose a health threat by causing food born illnesses. Additionally, presence of *E. coli* is considered to be an indicator

Table 2 *E. coli* counts for six different FP in CG, GG and TG.

Type of FP	*E. coli* counts in [log CFU/g] for different group of FP											
	CG				GG				TG			
	R1	R2	R3	Mean±SD	R1	R2	R3	Mean±SD	R1	R2	R3	Mean±SD
Banana*†	ND	ND	ND	ND	ND	ND	ND	ND	3.477	3.3	3.48	3.42 ± 0.06
Apple	ND	ND	ND	ND	ND	ND	ND	ND	3.602	ND	3.6	2.40 ± 1.2
Lettuce††**	3.5	ND	3.5	2.33 ± 1.17	3.6	3.8	3.7	3.70 ± 0.06	4.362	4.41	4.23§	4.33 ± 0.05
Carrot***	3.3	3.3	ND	2.20 ±1.10	3.6	ND	3.8	2.47 ± 1.23	3.903	4	4.15	4.02 ± 0.07
Tomato‡**	ND	ND	ND	ND	ND	ND	ND	ND	3.845	3.48	3.48	3.60 ± 0.12
Pepper	ND	ND	ND	ND	ND	ND	ND	ND	3.301	3	ND	2.10 ± 1.05

Symbols which represent significant decrease in bacterial counts between TG and CG; *p=0.037, **p=0.046, ***p=0.046 ****p=0.034. Symbols which represent significant decrease in bacterial counts between TG and GG; †p=0.037, ††p=0.001, ‡p=0.034. §: Symbol representing the sample with the presence of STEC with colony count of <1 log CFU/g. Abbreviations: ND: Not detectable, R1: Replica 1, R2: Replica 2, R3: Replica 3, SD: Standard Deviation. Statistical analysis has been performed by using Mann Whitney U test and Independent Samples t-test.

of hygienic conditions including personal hygiene (15). In this regard, it is important to keep the surveillance of the *E. coli* and STEC contamination on FP in each stage of the "farm to consumer" process. Besides, the concentration of *E. coli* after harvesting stage may rise up to log 3 CFU/g (12,14). Our results showed significantly higher bacterial counts among all of the FP from CG to TG together with the presence of STEC in lettuce sample. This signifies the role of human handling on transmission of bacteria because these bacteria including STEC detected in lettuce is thought to be transmitted through hand contact. Considering together, it may be discussed that human handling in market stage should be considered as an important factor in terms of bacterial dissemination as in previous steps of the "farm to consumer" process.

3.2.2. Evaluating gloves as a probative method to test the impact of human handling and as a preventative method against E. coli contamination

In the present study, the last sample group was GG where the aim was to test the sterile gloves as a preventative tool for prevention and to test GG as a probative tool for the comparisons between TG and CG. In general, FP in CG and GG remained *E. coli* free (except lettuce and carrot) while every FP in TG contained *E. coli* in the range of log 2.10 CFU/g to log 4.33 CFU/g (Table 2). STEC were not detected in any of GG samples.

When GG and TG are compared, there was a significant difference between *E. coli* counts for banana, lettuce and tomato. Analysis of *E. coli* counts on FP in TG revealed that the highest amount of *E. coli* was present on the surface of lettuce (mean 4.33 ± 0.05). *E. coli* counts from lettuce in GG, however, was lower by 1.2 times, relative to TG together with the absence of STEC.

The second highest *E. coli* count was detected on carrots with a mean of 4.02 ±0.12 CFU/g and log 2.47 ± 1.23 CFU/g in TG and GG, respectively (1.6 times lower). Previously, low temperature storage (4°C) was tested as a preventive method against bacterial contamination of carrots where the reduction of *E. coli* counts was much lower (8 times) compared to our study (16). Mean *E. coli* counts were as log 3.42 ± 0.06 CFU/g in banana and log 3.60 ±0.12 in tomato in TG. Similarly, *E. coli* was quantified as means of log 2.10 ±1.05CFU/g and log 2.40 ±1.2CFU/g for pepper and apple, respectively. In the GG group bacterial counts were significantly lower in contrast to TG for banana, lettuce, and tomato (p=0.037, p=0.001 and p=0.034).

The limit of *E. coli* counts for the "minimally processed FP" has been defined to be less than log 3 CFU/g in the document of "European Union Commission Regulations" for microbiological criteria for foodstuffs (17). By using gloves, the bacterial count of every FP which was used in our study was reduced and reached below those limits, except lettuce. STEC detected in lettuce sample in TG was not observed in GG. Containing higher number of *E. coli* could be accounted for by the denser bacteria on lettuce surface since it is a leafy vegetable with a more nested surface.

Taken together, in every FP group, reductions and elimination of STEC were detected in GG, even able to keep four out of six FP bacteria uncountable. Therefore, the findings of the present study imply that wearing sterile gloves could have a preventative effect on dissemination of *E. coli* and STEC between FP. On the other hand, these results are correlating with the findings obtained from the comparisons between TG and CG and therefore proves that human handling has a significant effect on *E. coli* contamination at the market stage. This strategy has the potential to be developed into more practical disposable sterile tools to replace gloves.

3.3. Antibiotic susceptibilities of E. coli isolates

Antibiotic susceptibilities of *E. coli* isolates which were obtained from FP were also examined. The results of in *vitro* susceptibility testing of all of the *E. coli* isolates from different sample groups are shown in Table 3. Moderately high resistance was observed against three antibiotics; AM, CEF and TET (44%, 40% and 24% respectively). In this regard, having resistance against AM is common between *E. coli* isolates from FP. To compare, a recent study reported AM resistance to be 13% among *E. coli* isolated from FP (18) which is lower than our results. The reason for this could be the sampling step where our isolates are collected at the market to observe human handling effect. Besides high resistance to AM, resistance rate to

Table 3 Antibiotic resistance rates of *E. coli* isolates from sample groups of FP.

Sample groups	FP types	No. (%) of resistant *E. coli* isolates to antibiotics: AM	AMC	CN	IPM	CEF	CRO	NAL	CIP	CLR	TET	SXT
CG	Banana	-	-	-	-	-	-	-	-	-	-	-
	Apple	-	-	-	-	-	-	-	-	-	-	-
	Lettuce (n=2)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Carrot (n=2)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Tomato	-	-	-	-	-	-	-	-	-	-	-
	Pepper	-	-	-	-	-	-	-	-	-	-	-
GG	Banana	-	-	-	-	-	-	-	-	-	-	-
	Apple	-	-	-	-	-	-	-	-	-	-	-
	Lettuce (n=3)	1 (33.3)	1 (33.3)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Carrot (n=2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Tomato	-	-	-	-	-	-	-	-	-	-	-
	Pepper	-	-	-	-	-	-	-	-	-	-	-
TG	Banana (n=3)	2 (66.6)	1 (33.3)	0 (0)	0 (0)	2 (66.6)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)	1 (33.3)
	Apple (n=2)	1 (50)	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	1 (50)
	Lettuce (n=3) †	2 (66.6)	1 (33.3)	0 (0)	0 (0)	2 (66.6)	0 (0)	1 (33.3)	0 (0)	0 (0)	2 (66.6)	2 (66.6)
	Carrot (n=3)	1 (33.3)	0 (0)	0 (0)	0 (0)	2 (66.6)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.6)	2 (66.6)
	Tomato (n=3)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Pepper (n=2)	1 (50)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Total (n=25)	11 (44)	3 (12)	0 (0)	0 (0)	10 (40)	0 (0)	1 (4)	0 (0)	0 (0)	6 (24)	6 (24)

†Symbol representing the sample with the presence of STEC which was resistant to AM, NAL, CEF, SXT and TET.

CEF (40%) among our isolates is concerning as there is a growing concern on the environmental spread of cephalosporin resistant *E. coli* strains globally (19). The only STEC isolated in this study was resistant to AM, NAL, CEF, SXT and TET. High resistance rates of our isolates to AM and CEF, could attributed to the frequent and improper usage of these antibiotics in the country. Taken together, resistant isolates on the FP is possibly sourced from human handling. Also, resistant isolates limits the antibiotic options for possible food borne infections and contributes to the transmission of resistance genes (20).

4. CONCLUSION

FP are essential ingredients of a healthy diet, and the demand for FP is increasing due to nutritional benefits. Although often shadowed by their health benefits, the consumption of FP has also been associated with health risks for consumers including infections caused by bacteria such as *E. coli* or STEC. In this context, it is important to improve the understanding the dynamics of FP pathogens, dissemination mechanisms, risks to the consumer. Such understanding should ultimately lead to development of strategies to eliminate or control of pathogenic contaminants. Here, we demonstrate that human handling in the market stage have significant effects on *E. coli* and STEC contamination of FP by making comparisons between 3 sample groups. Also, we provide evidence that *E. coli* contamination on FP can be decreased significantly by using sterile gloves before selecting the FP in the market stage which is a concept that has potential to be developed into more practical and industrialized similar materials. Additionally, our data regarding

knowledge and attitudes of the general population on FP handling highlights a possible link between people's understanding of bacterial contamination on FP. Antimicrobial resistance of *E. coli* on FP is another concerning result of the present study. Governments and public should be aware of the risks for the dissemination of pathogens through human handling of FP at the markets. Therefore, continuous audit of sellers' hygiene practices and surveillance of contaminants are recommended.

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