

Bacteriological screening of fresh and fresh-cut fruits vended in select open air markets and supermarkets in Metro Manila, Philippines

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The recent increase in the consumption of fresh produce may be associated with risk of infection caused by microorganisms. Microbial contamination of produce may occur in different stages of the supply chain including plant cultivation, harvest, transportation, and processing. In developing countries, outbreaks linked to fresh produce consumption are not well-documented and limited data are available regarding the microbiological quality and safety of crops. In this study, microbial contamination in different types of fruits collected from select local markets in Metro Manila, Philippines was evaluated. The scope of this study is limited to screening of microbial contamination of fruits at the end of the food supply chain, where products are ready for purchase and consumption. Traditional culture methods were used to detect and quantify thermotolerant *Escherichia coli* and *Salmonella* spp. Polymerase chain reaction was utilized to confirm the identity of *E. coli* isolates. Out of 152 samples, 63 (41.5%) were positive for *E. coli* with a mean count of 0.8 log CFU/g. The highest *E. coli* count in individual samples was observed in a peeled jackfruit sample (4.7 log CFU/g). Among fruit types, the highest mean *E. coli* count (2.8 log CFU/g) was observed in jackfruit samples. *Salmonella* was not detected in any of the samples. Its presence, however, cannot be completely ruled out due to the method utilized. The commercial source of the fruits, be it

supermarkets or open air markets, did not appear to affect the observed *E. coli* counts. The study does not intend to identify the source in which contamination occurred, however, this finding suggests that the common origin of contamination of produce might have occurred in the earlier stages of the supply chain. It was also interesting to note that slicing, peeling, and other processing methods may have had an effect on the incidence of contamination of samples by *E. coli* due to the differing mean count patterns observed between whole and pre-sliced fruits. The prevalence of *E. coli* found in markets in the study could serve as persuasive reinforcement of the need to implement stronger hygienic handling, processing standards, and produce quality monitoring in the country.

KEYWORDS

Escherichia coli, food safety, fruits, microbial contamination, *Salmonella*

INTRODUCTION

Fresh produce, which includes fruits and vegetables, is part of the regular diet of an individual. Its consumption is encouraged because of the health benefits it can provide. The United States Department of Agriculture has reported a 20% increase in overall fresh produce consumption from 1970-2000 (USDA 2003). Changes in dietary habits, increasing popularity of salad bars and year-round importation of produce to the U.S. have caused the higher per capita consumption of fresh or minimally-

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processed produce (Beuchat 2002). According to the Scientific Committee on Food, this situation is similar in European countries during the last few decades where consumers tend to buy ready-to-eat and slightly-processed produce (FAO / WHO 2008). Fresh-cut salads and fruits are also gaining popularity in urban areas in Asia. In some South East Asian countries, packs of fresh-cut fruits and vegetables are increasingly sold by cottage industry suppliers and small vendors in wet markets to meet the demand for ready-to-eat produce (Harris et al. 2003). The increase in fresh produce consumption, however, presents a challenge to human health as most of these food products that are eaten raw and with minimal processing can be vehicles for microbial pathogen.

Contamination of produce may occur in various stages of the supply chain. Several factors, including irrigation water, soil, insects, human and animal feces, fertilizers, rinse water, transport equipment and vehicles, and human handling, contribute to the contamination of crops during growth, harvest, transportation, storage, and distribution (Abadias 2006; Food SC 2002; James and Ngarmasak 2011). Some of the most common bacterial pathogens that have been isolated from fruits and vegetables are *E. coli* O157:H7, *Salmonella*, *Listeria monocytogenes*, *Clostridium botulinum*, *Shigella sonnei*, *Vibrio cholerae*, and *Bacillus cereus*. These pathogens can cause symptoms such as bloody diarrhea, nausea, vomiting, and fever and are frequently associated with gastroenteritis and other intestinal illnesses (Beuchat 1998; Lewis et al. 2012). Proper management of crop production, therefore, is necessary to prevent incidence of foodborne outbreaks and to deliver safe food to consumers.

Even as the significance of fresh produce in everyday life has received increasing attention, associated disease outbreaks, especially in developing countries, have not been properly documented. Not enough foodborne disease investigations involving fresh produce have been done. This is similar to the situation in other developing countries, such as those in Latin America, where outbreaks linked to fruits and vegetables have been agreed upon by experts as underestimated at a mere 2% (Beuchat 1998), and in Asian countries like the Philippines, where no current surveillance system is available for produce-related outbreaks (FAO / WHO 2008). Insufficient information regarding proper food and water practices, as well as poor health-seeking behavior of patients from low-income communities, contribute to the pervasive lack of information regarding disease spread or infection acquired through the intake of food contaminated with disease-causing microorganisms (DOH Philippines 2017).

The gap between the rising popularity of fruits and the information available about their safety, together with contemporary shifts of food ideologies towards healthier food choices and wellness-based outlooks, underscores the importance of improved monitoring and quality assurance of food groups such as fruits and vegetables. This is recently emphasized by the Bureau of Agriculture and Fisheries Standards (BAFS) of the Philippine Department of Agriculture, an agency tasked to ensure consumer safety of local agriculture products, including fruits in a food standardization mandate applicable to farmers, vendors, and other stakeholders in the food industry. The Department of Health has also been consistent in the implementation of strategies to reduce morbidity and mortality of foodborne diseases through a multi-sectoral Food and Waterborne Disease Prevention program (DOH Philippines 2017).

The objectives of the present study were: 1) to contribute information to local disease monitoring, prevention and control efforts, and 2) to evaluate, for the first time, the microbial

content of some common fruits sold in Philippine markets specifically those of thermotolerant *Escherichia coli* and *Salmonella* spp. Its scope is limited to the screening for the presence of and contamination of fruits by aforementioned bacteria at the end of the food supply chain, where products are ready for purchase and consumption. It is assumed that contamination at the consumption point indicates that the bacteria have been introduced through industrial, environmental, or handling means, and existing food safety measures have been inadequate. The study did not intend to identify the source or exact point within the supply chain where contamination occurred but hypothesized that observations on handling and fruit preparation at the market site can provide insights into current quality control practices that contribute to contamination.

Such kind of studies are important as they can provide information regarding produce quality and safety, offer valuable data for food quality monitoring, and can make vendors and consumers more informed in ways of properly dealing with and consuming produce. They can also aid in urging production companies to maintain proper sanitation during handling and packaging of fruits and vegetables. Quite significantly, findings can influence consumers to become more critical about the quality and safety of the produce and more discerning of the conditions of the markets they purchase these from.

MATERIALS AND METHODS

Sampling collection

A total of 152 fruit samples were collected from five open air markets and five supermarkets in Metro Manila, Philippines based on seasonal availability and convenience. These samples included: *Malus domestica* Borkh. (apple, n=16); *Vitis vinifera* L. (grapes, n=16); *Ananas comosus* (L.) Merr. (pineapple, n=29); *Citrullus lanatus* (Thunb.) Matsum. & Nakai (watermelon, n=24); *Carica papaya* L. (papaya, n=7); *Psidium guajava* L. (guava, n=18); *Artocarpus heterophyllus* Lam. (jackfruit, n=5); *Prunus avium* (L.) L. (cherry, n=2); *Fragaria ananassa* (Weston) Duchesne ex Rozier (strawberry, n=15); *Cucumis melo* L. (melon, n=17); *Mangifera indica* L. (mango, n=2); and *Citrus maxima* (Burm. f.) Merr. (pomelo, n=1). The fruits collected were classified as whole fruits and sliced or peeled (fresh-cut). Whole fruits are those that can be eaten without removing the exocarp which include apple, grapes, strawberry, cherry, and guava. Sliced or peeled fruits, on the other hand, are those that undergo processing before consumption by removing the exocarp and by cutting, which include pineapple, watermelon, melon, papaya, jackfruit, mango, and pomelo. A comparison between these classifications was made to determine if the type of fruit or processing done has an effect on bacterial contamination. The samples were collected using sterile polyethylene bags to avoid direct hand contact and were kept in an ice box during transport and processed in the laboratory within 3 h after collection.

Processing of fruit samples

Fruit samples were processed using a method modified from Pui et al. (2011). Ten grams of fruits were weighed using ethanol-sterilized forceps and scalpel and placed in a sterile Whirl-Pak® bag (Nasco, USA). Thirty milliliters of 0.1% buffered peptone water (BPW) (Becton, Dickinson and Company, MD, USA) were then added to the bag and placed on a shaking platform for 5 min with moderate shaking of less than 100 rpm, or shaken vigorously for 30 sec. The wash was collected in 50 ml sterile polypropylene tubes and separated in aliquots for conventional processing.

Detection and quantification of *E. coli*

For the detection and quantification of thermotolerant *E. coli*,

Table 1: Prevalence of *E. coli* contamination in fruit samples collected from open air markets and supermarkets in Metro Manila, Philippines.

Fruit	n	Total ^a	Samples contaminated in the indicated range ^b						Range ^c	Mean ^c	
			<2 CFU/g	log	2-3 CFU/g	log	3-4 CFU/g	log			4-5 CFU/g
pineapple	29	19 (65.5)	12 (41.4)		4 (13.8)		3 (11.5)		0 (0)	0.2-3.5	1.0
watermelon	24	8 (33.3)	3 (12.5)		4 (16.7)		0 (0)		1 (4.2)	0.3-4.3	0.7
Guava	18	9 (50.0)	7 (38.9)		1 (5.6)		1 (5.6)		0 (0)	0.2-3.2	0.8
melon	17	10 (58.8)	3 (17.6)		4 (23.5)		3 (17.6)		0 (0)	1.0-3.4	1.5
Apple	16	0 (0)	0 (0)		0 (0)		0 (0)		0 (0)	0.0	0.0
grapes	16	0 (0)	0 (0)		0 (0)		0 (0)		0 (0)	0.0	0.0
strawberry	15	5 (33.3)	5 (33.3)		0 (0)		0 (0)		0 (0)	0.1-1.3	0.3
papaya	7	6 (85.7)	2 (28.6)		3 (42.9)		1 (14.3)		0 (0)	0.2-1.4	1.7
jackfruit	5	4 (80.0)	1 (0.2)		0 (0)		1 (0.2)		2 (0.4)	1.5-4.7	2.8
mango	2	1 (50.0)	0 (0)		1 (0.5)		0 (0)		0 (0)	0.0-2.9	1.4
cherry	2	0 (0)	0 (0)		0 (0)		0 (0)		0 (0)	0.0	0.0
pomelo	1	1 (100.0)	1 (100.0)		0 (0)		0 (0)		0 (0)	0.0-0.4	0.4

^a Values are expressed as number and percentage (in parenthesis) of positive samples.

^b Values are expressed as number and percentage (in parenthesis) of samples in the indicated range per log CFU/g of contamination.

^c Values are expressed in log CFU/g.

10-fold dilution of the fruit wash was made 3 times in 9 ml 0.1% BPW. Each dilution, including one undiluted wash from each sample, was filtered through a 0.45 µm nitrocellulose membrane (Pall, USA) using a vacuum pump. The filter was carefully placed on membrane-fecal coliform (m-FC) (MB Cell, South Korea) agar surface and incubated at 44.5°C for 18-24 h. Colonies showing blue to deep-blue colonies were counted. At least 4 presumptive *E. coli* colonies from a single positive sample were confirmed by streaking for isolation on MacConkey agar plates, incubated at 35°C for 18 h.

Detection and quantification of *Salmonella* spp.

For the detection and quantification of *Salmonella* spp., 5 ml of the produce wash was obtained and an initial 1:1 dilution was made in 2X BPW, followed by 3 additional serial 10-fold dilutions in 1X BPW. The pre-enrichment cultures were incubated at 35°C for 24 h. From each dilution, 0.2 ml was removed and added to wells containing 1.8 ml Rappaport Vassiliadis (RV) (MB Cell, South Korea) broth in triplicates. The RV plates were incubated overnight at 42°C. The results were recorded as positive (+) for turbid growth, weak (W) for weak positive, and negative (-) for no growth. Most probable number (MPN) counts were obtained from these data. Positive wells were confirmed by streaking on xylose lysine deoxycholate (XLD) (Becton, Dickinson and Company, MD, USA) agar plates incubated at 35°C for 24 h. Red colonies with black centers were considered as *Salmonella* spp. isolates. These procedures are based on the RV broth method of the U.S. EPA standards (US EPA 1998).

DNA extraction

DNA extraction was performed by subculturing a single colony from the positive confirmatory plates in TSB and grown overnight at 35°C. One milliliter from each of the bacterial cultures was obtained and centrifuged at 10,000 x g for 10 min after which the pellets were washed with 1ml 1X phosphate buffered saline (PBS) and centrifuged again using the above mentioned conditions. The pellets were then added with 100 µl 1X PBS, vortexed, and boiled for 15 min using a dry heat block. The resulting DNA extracts were diluted (1:50) and used as templates in the PCR reactions.

PCR assay specific for *E. coli* detection

A polymerase chain reaction (PCR) assay was performed to confirm the identity of the isolates of *E. coli* from the culture method.

Primers ECN1254F (5'GCAAGGTGCACGGGAATATT-3') and ECN1328R (5'CAGGTGATCGGACGCGT-3') were used to amplify the *uidA* gene, encoding for the β-glucuronidase enzyme of *E. coli* (Takashi et al. 2009). The reaction mix contained 1X Promega GoTaq® Green Master Mix (Promega Corporation, WI, USA), 0.5µM of each primer and 2µl DNA template. Nuclease-free water was added until a 20µl reaction volume was reached. The cycling conditions were 95°C for 2 min, followed by 35 cycles of 95°C for 30 sec, 63°C for 1 min, and 72°C for 1 min, and a final extension at 72°C for 5 min. Each run included a positive control (*E. coli* ATCC 15597) and a no template control. All PCR products were subjected to agarose gel electrophoresis and viewed under UV light.

Statistical analyses using IBM® SPSS® Statistics version 19

The microbial counts were normalized by converting the values to log 10 scale. Data were tested for normality and nonparametric tests were chosen for statistical analyses, comparing the mean counts by Mann-Whitney U test at 95% confidence level ($\alpha = 0.05$) using IBM Statistical Packages for the Social Sciences (SPSS) Version 19.

RESULTS

Using standard culture methods, 41.5% of the 152 fresh and fresh-cut fruits collected from different open air markets and supermarkets were found to be contaminated with thermotolerant *E. coli*, with an average count of 0.8 log CFU/g. The lowest *E. coli* count observed was 0.1 log CFU/g and the highest was 4.7 log CFU/g. On the other hand, none of the samples was found to harbor *Salmonella* spp. The identity of the *E. coli* isolates was confirmed by amplifying the 75-bp fragment of the *uidA* gene which encodes for the β-glucuronidase enzyme found in *E. coli* (Takahashi et al. 2009).

Of the different types of fruits collected, peeled jackfruit had the highest *E. coli* load with an average of 2.8 log CFU/g, followed

Table 2: Mean log counts of *E. coli* based on market and processing types.

	Open Air Markets			Supermarkets		
	<i>E. coli</i> ^a	Range ^b	Mean ^b	<i>E. coli</i> ^a	Range ^b	Mean ^b
Whole fruits	10/48	0.1-3.2	0.3	4/19	0.5-2.8	0.3
Sliced/Peeled	40/60	0.2-4.7	1.3	9/25	1.4-3.4	0.9

^a Values are expressed as number of positive samples over the total number of samples collected

^b Values are expressed in log CFU/g

by sliced mango and melon (Table 1). The highest counts for *E. coli* in individual samples were observed in a peeled jackfruit (4.7 log CFU/g) obtained from an open air market. All fruit samples with *E. coli* counts of 4.0 log CFU/g and above were likewise obtained from open air markets. No bacterial contaminant was found in apples, grapes, and cherries (Table 1).

Of 67 whole fruits, 14 or 20.9% were found to have *E. coli* with counts ranging from 0.1-3.2 log CFU/g, while 49 out of 85 sliced or peeled fruits or 57.6% were positive for *E. coli* with counts ranging from 0.2-4.7 log CFU/g. Results also showed that *E. coli* counts in sliced or peeled fruits (1.2 log CFU/g) are significantly higher than counts in whole fruits (0.3 log CFU/g) ($p=0.00$).

E. coli counts were also compared based on the markets sampled, classified as either open air markets or supermarkets. This comparison was done to determine if potential differences due to non-uniform sources, transportation, and handling of produce might have affected the incidence of bacterial contamination. It was found that 50/108 (46.3%) fruits from open air markets and 13/44 (29.6%) fruits obtained from supermarkets were positive for *E. coli* with counts ranging from 0.1-4.7 log CFU/g and 0.5-3.4 log CFU/g, respectively. Statistical analysis showed that *E. coli* counts on fruits from open air markets (0.9 log CFU/g) were not significantly different ($p=0.13$) from the counts on fruits from supermarkets (0.6 log CFU/g).

A comparison between two variables, namely market type and processing type to determine whether differences exist showed that fruits that were sliced or peeled and sold in open air markets have the highest incidence (66.7%) of *E. coli* contamination. Incidence of *E. coli* in whole fruits from open air markets (20.8%) was not significantly different from that in fruits from supermarkets (21.1%) (Table 2).

Statistical analyses revealed that there are no significant differences in *E. coli* content of sliced or peeled fruits whether they are purchased from open air markets or supermarkets ($p=0.08$). The same is true for whole fruits obtained from both market types ($p=0.99$).

DISCUSSION

The present study is the first report on the microbiological quality and safety of Philippine fruits purchased at local markets with emphasis on the prevalence of thermotolerant *E. coli* and *Salmonella*. It was found that 41.5% were contaminated with *E. coli* and none of the fruits were found to contain *Salmonella*. Quite significantly, *E. coli* detected, based on the methods used, can survive even at temperatures as high as 44°C. A major limitation of the study, however, is that the presence of *Salmonella* cannot be completely ruled out, for counts may have been below detectable levels. Moreover, while *Salmonella* spp. usually appear as red colonies with black centers on XLD agar, some species may form red colonies without a black center and resemble *Shigella*, and other species of *Salmonella* that fail to decarboxylate lysine would not be detected on this medium (Versalovic et al. 2011). While culture techniques remain the standard for detection of foodborne pathogens, major advance-

ments in molecular and immunological technologies are helping to shift diagnosis to more culture-independent frameworks. These methods are recommended as diagnostic adjuvants in similar future studies as they can help address the problems of time before results are obtained and of sensitivity of typical culture methods (Bell et al. 2016). Finally, results on the detection of *Salmonella* spp. may have been affected since an older technique for analysis of *Salmonella* was used. Cultured microbes were obtained through fruit wash but the US FDA now recommends sterile blending of comminuted or cut fruit with universal pre-enrichment broth prior to culture and assessment (Andrews et al. 2018).

Previous studies have shown the presence of *E. coli* in fruits from Spain, Switzerland, and Canada but mostly in lower prevalence rates (Abadias et al. 2006) (Althaus et al. 2012) (Arthur et al. 2007). One study in Egypt (Uyttendaele et al. 2014), however, reported a high prevalence rate of *E. coli* in strawberries collected from farms (72.2%) and retail outlets (66.7%). As indicators of fecal contamination, the presence of *E. coli* in the fruit samples shows that firmer hygienic methods should be employed in the processing and handling of fresh produce. Although *E. coli* may not be pathogenic, some strains may possess virulence characteristics that can cause gastroenteritis and other intestinal diseases (Sousa 2006).

From the data obtained, 29 out of 63 (46.0%) fruits were observed to have *E. coli* counts of more than or equal to 2 log CFU/g. The commission regulation on microbiological criteria for foodstuffs created by the European Commission in 2005 (EC Commission 2005) states that for every five fruits sampled from a vendor, no more than two should have *E. coli* levels between 2 to 3 log CFU/g. Based on the data obtained from the present study, much of the sliced or peeled fruits including pineapple, watermelon, melon, papaya, and jackfruit, did not pass this criterion. It is quite difficult, however, to evaluate the results using local produce safety measures. Currently, there are no clear standards since the implementing rules and regulations of the recently published Food Safety Act of the Philippines are still being formulated.

The results of this study can be useful in the advancement of policies for food safety as well as in quality monitoring of fresh produce in the country. According to the Global Agricultural Information Network report by the USDA Foreign Agricultural Service in 2015, food regulations in the Philippines are patterned after CODEX Alimentarius Commission guidelines as well as regulations established by the FDA of the United States and similar regulatory bodies in other countries since no well-established national microbiological standards for food are currently available.

Berries and melons are among the level 2 priorities in terms of microbiological safety of fresh produce around the world (FAO/WHO 2008). This study reports significant levels of contamination not only in these fruits but also in other fruits as well including jackfruit, papaya and pineapple. It is also notable that no contamination was observed in apples, grapes, and cherries. These fruits are of the fleshy types which have thin outer coverings or exocarp, making them ready-to-eat. The absence of *E. coli* and *Salmonella* in these fruits may be due to

their smooth and waxy exocarp that prevents the attachment of microorganisms on the surface of the fruit (Barth et al. 2009). Different characteristics of fruits cause differences in their potential for microbial contamination. For instance, when heavier fruits are dropped during harvest and transport, there is a higher likelihood for the occurrence of cracks or bruises, which may be sites for entry and proliferation of microorganisms (Sapers 2009). Bruised and cut surfaces in fruits may also release fluids that contain nutrients for the enhancement of microbial growth, or in some cases, these may contain antimicrobials that prevent the growth of microorganisms (Beuchat 2002).

Fruit processing is another factor associated with fresh produce quality. Fruits that have undergone peeling and cutting were observed to have significantly higher *E. coli* load compared to whole fruits which implies that bacterial contamination likely happened during the processing stages. Peeling and cutting can destroy plant cells, thereby releasing exudates with substantial amounts of nutrients suitable for the proliferation of microorganisms. Moreover, washing the produce after cutting can add to the risk for bacterial attachment and internalization (Mercanoglu Taban and Halkman 2011). Removal of bacterial pathogens by washing and use of sanitizers may not be achieved fully because they can form biofilms enabling them to adhere on the surfaces of the produce and to survive dehydration and treatment by sanitizers (Beuchat 2002). Further proliferation can also happen when high temperature is used for storage and unhygienic handling and distribution are employed (James and Ngarmsak 2011).

The source of the fruits may also be associated with their quality and safety (Vital et al. 2014). The contamination observed may have come from a number of sources. In some open air markets, fruit stalls were located near meat, poultry, and fish stalls surrounded by flies that could have acted as vectors that carried microorganisms to nearby stalls. Handling is also unsanitary as the same knife used to slice fruits in pre-packing is used several times without washing. However, it is debatable whether other sources within the supply and distribution chain of fresh produce account for contamination as there were no significant differences in mean *E. coli* counts in fruits obtained either from open air markets or supermarkets. The lack of difference in microbial load of fruits between market types implies that the higher price paid in supermarkets does not guarantee the safety of the produce.

CONCLUSIONS

As there is a dearth of published information regarding the microbiological safety of fresh produce especially in developing nations, studies of this kind become important as basis for establishing clear regulations for fresh produce safety in the Philippines. The present study highlighted the high prevalence rate of thermotolerant *E. coli* in Philippine fruits as well as the high microbial load in fruits that have undergone peeling or slicing compared to whole fruits that are ready-to-eat, with emphasis on the point where the products are ready for purchase and consumption. Although *Salmonella* was not detected in the samples, the presence of this organism cannot be completely ruled out due to the method used. Results of studies in handling and fruit preparation at the markets can provide insight into current quality control practices. It is clear that strong implementation of hygienic handling and processing of fruits is necessary to provide consumers with safe food. Lastly, the similarity in the microbial load in fruits collected from open air markets and supermarkets suggests that other sources within the supply chain may have contributed to the contamination.

Additional studies must therefore be done in order to determine specific sources of contamination and to establish clear points of control. It is also recommended that a more comprehensive report be provided for future studies on a broader scope of foodborne pathogens including not only fecal coliforms and bacteria but also viruses, protozoa and fungi.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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CONTRIBUTIONS OF INDIVIDUAL AUTHORS

ACMTAM collected and processed samples, drafted the manuscript and provided substantial contributions to the overall execution of the project. PGV designed and headed the project, provided guidance on the analysis and interpretation of results, and gave critical revisions of the final manuscript. WLR also provided guidance on the analysis and interpretation of results and directed revisions for important intellectual content. MAZD helped collect and process samples and provided guidance in the analysis and interpretation of data.

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