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Prevalence and concentration of *Escherichia coli* and *Salmonella* species in fresh vegetables collected from different types of markets in Phnom Penh

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Freshly-eaten vegetables have become more popular in Cambodian diet. However, these vegetables are also one of the main sources of infection from pathogenic microorganisms if they become contaminated. Outbreaks of foodborne diseases caused by fresh fruits and vegetables have been increasingly reported, raising concerns regarding their safety. Therefore, it is very important to conduct an inquiry into the contamination of fresh vegetables by *Escherichia coli* and *Salmonella* spp. This study investigates the presence and concentration of *E. coli,* total coliforms, and *Salmonella* in samples of lettuce, saw-leaf herb, and cucumber, collected from different types of market in Phnom Penh. The spread-plate technique was used to detect the number of bacteria in each sample. The results indicate that the incidence of bacterial contamination in fresh vegetables, particularly lettuce and saw-leaf herbs, was higher in the wholesale markets than the other three types of markets. The concentration of *E. coli* in each of the samples ranged between 2×10^3 and 7×10^5 CFUs/g from the retail markets. The concentration of total coliforms was also found to be quite high. *Salmonella* was present in almost all leafy vegetable samples but in very few in cucumber samples. Bacteria reduction from the samples by thorough washing under flowing tap water was effective in most cases.
Keywords: fresh vegetables, *Escherichia coli*, total coliforms, *Salmonella*, pathogen removal

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Introduction

There are many kinds of vegetables people prefer eating raw believing they can obtain more vitamins, dietary fiber and protein from their diet (Slavin & Lloyd, 2012; Chau et al., 2014). These freshly eaten vegetables include lettuce, cucumber, water spinach, carrot, tomato, saw-leaf herb. All have become more popular recently in the Cambodian diet. However, these vegetables are also considered a major source of infection by pathogenic microorganisms via agents such as fungi, bacteria, viruses, and parasites (WHO, 2008). Reports of foodborne disease outbreaks related to fresh fruits and vegetables have been increasing in number, raising concerns about the safety of these products (WHO, 2008; Berger et al., 2010; Dolye & Erickson, 2008; Heaton & Jones, 2008; Lynch *et* al., 2009).

Bacterial pathogens are considered to be the most typical agent causing foodborne diseases, followed by viruses (Painter et al., 2013). Food-borne pathogens have a serious impact on public health outcomes. For example, in the United States alone, about 14 million incidents of food-related illness occurred in one year (Mead et al., 1999). Between 1996 and 2008, most foodborne disease outbreaks were linked to leafy green vegetables. *Escherichia coli* O157: H7, a Shiga toxin-producing serotype of the bacterial species *E. coli* and causing hemorrhagic diarrhea and kidney failure, and *Salmonella,* a bacterial species causing Salmonellosis and/or typhoid fever,

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were involved in up to 72% of these cases (Gravani, 2009). Contamination of vegetables, especially by *E. coli* and *Salmonella* has also been reported in many countries including the United States, Mexico, Spain, Brazil, India, Sri Lanka, Philippines, Vietnam, Thailand and Cambodia (Johnston et al., 2005; Quiroz-Santiago et al., 2009; Ruiz et al., 1987; Santos et al., 2010; Tambekar & Mundhada, 2006; Silva et al., 2013; Vital et al., 2014; Chau et al., 2014; Ananchaipattana et al., 2012; Chhay et al., 2017; Kheang, 2016; Hay, 2018).

Among the developed countries, a log reduction value (LRV) of between 1 and 1.5 for *Escherichia coli* and a very low incidence of *Salmonella* (< 1%) was detected in green leafy vegetables in the United States (Johnston *et al.*, 2005). *Salmonella* was found to be present in approximately 5.8% of vegetable samples in Mexico (Quiroz-Santiago et al., 2009), while *Salmonella* and *E. coli* were found to be present in 7.5% and 86.1% of vegetable samples, respectively collected from different market types in Granada, Spain (Ruiz *et al.,* 1987). In 2010, a microbiological analysis showed a high presence by heat-tolerant *E. coli* species in 32% of vegetable samples collected in Brazil (Santos et al., 2010). There are also many reports of bacterial contamination in vegetables sampled in developing countries (Tambekar & Mundhada, 2006; Silva et al., 2013; Vital et al., 2014; Chau *et al.*, 2014; Ananchaipattana et al., 2012; Chhay et al., 2017; Kheang, 2016; Hay, 2018). For example, the presence *E. coli* and *Salmonella* was detected in 38.3% and 5.8% of various types of salad vegetables sold in Amravati city of India, respectively (Tambekar & Mundhada, 2006); in Sri Lanka, the presence of *Salmonella* in leafy vegetables was more common (6% of samples), than *E. coli* (2% of samples) (De Silva et al., 2013); microbiological tests of the quality of fresh produce from open-air

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markets and supermarkets in the Philippines revealed that 16.7% and 24.7% of 300 samples tested positive for thermo-tolerant *E. coli* and *Salmonella*, respectively (Vital et al., 2014); a study in Vietnam reported that 100% and 17.6% of fresh vegetable samples collected from traditional markets in Hue city were contaminated with *E. coli* and *Salmonella,* respectively (Chau *et al.*, 2014); the presence of *E. coli* was detected in 34% of vegetables collected from open-air markets and supermarkets in Thailand (Ananchaipattana et al., 2012); and surprisingly, the presence of *E. coli* and *Salmonella* species in lettuce leaves from supermarkets in Phnom Penh city (40% and 20%, respectively) was higher than from open-air markets (14% and 10%, respectively) (Chhay et al., 2017).

Vegetables may become contaminated with bacteria, as well as other parasites, during the planting, cultivation, transport, post-harvest processing or preparation of freshly-eaten vegetables (Johnston *et al.*, 2005; Enabulele & Uraih, 2009). Contamination can arise from organic fertilizers, irrigation, soil-borne pathogens, or post-harvest handling (Enabulele & Uraih, 2009; Gosh et al., 2004; Kłapeć & Borecka, 2012; Pachepsky et al., 2011; Ingham et al., 2004; Vital et al., 2014). Many Cambodian farmers still use traditional methods to both produce crops for sale and many risks of microbial contamination may are introduced through some of these methods. Interestingly, there is little mention of microbial contamination of foods in Cambodian, compared to many studies on chemical contamination from pesticides or herbicides (Phoeurk, 2007; Neufeld et al., 2010). Therefore, the importance of this study, investigating the presence and concentration of *Escherichia coli,* total coliforms, and *Salmonella* in freshly-eaten vegetables in Cambodia cannot be

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understated. This research will raise awareness of the probable contamination thus of these vegetables with pathogenic bacteria and help promote the need for practices that reduce adverse health impacts.

The overall objective of this study is to determine the presence and concentration of *E. coli,* total coliforms, and *Salmonella* in cucumber (*Cucumis sativus*), saw-leaf herb (*Eryngium foetidum*) and lettuce (*Lactuca sativa*) sampled from four different types of markets in Phnom Penh. Specifically this involves (i) determining the prevalence and concentration of *E. coli,* total coliforms and *Salmonella* contamination in cucumber, saw-leaf herb and lettuce collected from different market types in Phnom Penh; (ii) compare the *Escherichia coli,* total coliform and *Salmonella* prevalence and concentration from wholesale markets, roadside markets, mobile vegetable stalls, and supermarkets; and (iii) compare the change in *Escherichia coli,* total coliform, and *Salmonella* concentrations before and after thorough washing under flowing water.

Methodology

The research is based on a cross-sectional study of four types of markets in Phnom Penh, including (i) three wholesale markets (Doeum Kor, Stung Meanchey and Takhmao); (ii) a total of nine road stalls comprising three stalls surrounding each of the wholesale markets; (iii) nine mobile vegetable carts in Phnom Penh; and (iv) nine supermarkets in Phnom Penh (Figure 1). These markets are broadly representative of where most freshly-eaten vegetable purchases are made in the city that accounts for approximately 10% of the total population of Cambodia. Samples of cucumber, lettuce and saw-leaf herb were collected over a period of three months between July and

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September in 2017. A total of nine samples of each vegetable were taken from each type of market. This resulted in a total of 36 samples of each vegetable as shown in Table 1. After the sample was purchased, it was transferred to a sterile plastic bag and transported under cool conditions to a laboratory at the Bioengineering Department of the Royal University of Phnom Penh. Each sample was analyzed on the same day as collection. *E. coli* and *Salmonella* bacteria were cultured and a qualitative and quantitative analysis of their presence conducted.

Table 1: The number of water samples collected for this study.

Type of vegetable	Samples used in this study		
	E. coli	total coliforms	*Salmonella*
Lettuce	36	36	36
Saw-leaf herb	36	36	36
Cucumber	36	36	36

Sample Preparation

All equipment (knife, tray, glass spreaders, etc.) used for the culturing of bacteria was disinfected with 95% ethanol and a short period of flaming while the glass tubes, falcon tubes and 500ml Erlenmeyer flasks were autoclaved for sterilization. Each vegetable sample was divided in two with one subsample washed under flowing tap water for at least two minutes, while the other was left unwashed (De Silva, *et al*., 2013). Both subsamples were then were minced and 25 g of each subsample was transferred into 225 ml of Tryptic Soy Broth as a substrate for growing the bacteria colonies. The *E. coli* cultures were then incubated at 37 ^{o} C for about 15-20 min while *Salmonella* cultures were incubated at 42 ^{o} C overnight. A ten-fold serial dilution (10^{-1}-10^{-4} for *E.* coli and 10^{-1}-10^{-7} for *Salmonella* spp.) was then

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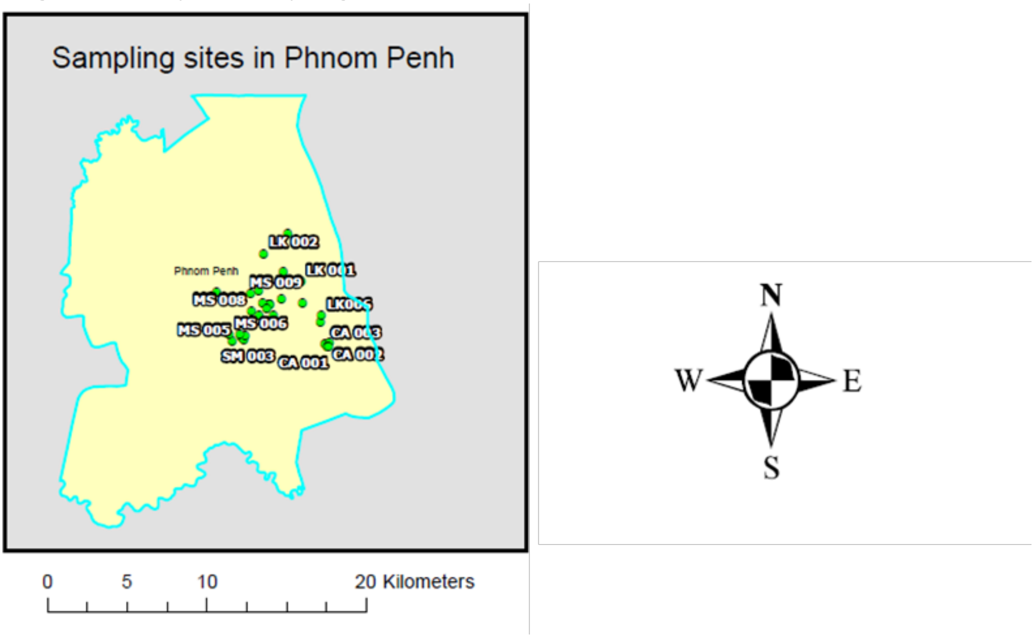
prepared from each sample and 25-50 µl of selected dilutions were spread on an agar plate.

Figure 1. Map of sampling sites in Phnom Penh



Analysis of the E. coli samples

A 50 µl aliquot of each sample (10^0-10^{-4}) was spread onto the surface of a Chromocult® Coliform Agar plate (Merck, Germany). The culture plates are then incubated at 37°C for 24 hours and the bacterial colonies inspected for the presence of dark blue to violet color (*E. coli*) and pink to red color (other coliforms). The concentration of each variable was then reported in colony-forming units per gram (CFUs/g). The sterile distilled water used to dilute the samples was also analyzed as negative control.

Analysis of the Salmonella samples

Following analysis for *E. coli,* 1 ml of the Tryptic Soy Broth culture was transferred into 9 mls of Rappaport Vassiliadis Broth, a selective culture broth

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for *Salmonella*, and incubated at 42°C for 24 hours. The next day, serial dilutions were prepared with sterile distilled water, before 25 µl aliquots (diluted from 10^{-1} to 10^{-4} for cucumber and 10^{-4} to 10^{-7} for lettuce and saw-leaf herb) were spread onto the surface of Salmonella Shigella Agar (Merck, Germany) plates, which were incubated at 37°C for 24 hours. Then, the bacterial colonies on each plate were inspected for the presence of a colorless colony with black center (*Salmonella*). The concentration of *Salmonella* was reported in CFUs/g. The sterile distilled water was again tested by a combination of membrane filtration and the spread plate method for use as a negative control.

Analysis of pathogen removal

The concentrations of bacteria in each of the samples were first expressed in CFUs/g. Then these values were transformed to a $\log_{10}$ reduction value (LRV). To investigate the effectiveness of pathogenic bacteria removal by washing the samples under running tap water, a decimal reduction in the bacterial population was calculated by comparing the colony counts obtained for the unwashed and washed samples. An analysis was then performed using Microsoft Excel.

Results and Findings

Samples were collected from four different types of markets including wholesale markets, roadside stalls, mobile vegetable carts, and supermarkets. Wholesale markets such as Chhbar Ampov, Steung Meanchey, and Doeum Kor are the largest vegetable distributors in Phnom Penh and supply produce to most roadside stalls and mobile vegetable carts. Thus, the level of bacterial contamination in each of these samples was expected to be reasonably

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consistent. In contrast, supermarkets source vegetables from different suppliers. Lettuce, saw-leaf herb, and cucumbers were selected for the study as they are raw vegetables used as preferred ingredients in Cambodian diets. As they are usually not sold or eaten before being washed or cooked, if they are contaminated with pathogenic bacteria, they are a probable infection pathway for foodborne diseases. Lettuce and saw-leaf herb were chosen to represent a broad range of green leafy vegetables, with a large surface area and small surface area, respectively, while cucumbers were selected to represent fruiting vegetables.

Bacterial Contamination in Lettuce

The results for the presence and concentration of *E. coli*, total coliforms and *Salmonella* in lettuce samples from the wholesale markets are presented in Figure 2 (a-d). *E. coli* and *Salmonella* was present in seven and six of the nine samples, respectively, while total coliforms were detected in every sample. This corresponds to an LRV for *E. coli,* total coliforms, and *Salmonella* ranging between 3.30 and 5.85 ($2x10^3$ and $7x10^5$ CFUs/g); 4.88 and 6.26 ($7.6x10^4$ to $1.81x10^6$ CFUs/g); and 4.6 and 7.25 ($4x10^4$ and $1.8x10^7$ CFUs/g), respectively (Figure 2a). No *E. coli* was detected in samples from the roadside stalls, however, eight and three of the nine samples contained total coliforms, and *Salmonella,* respectively. This corresponds to an LRV for total coliforms, and *Salmonella* ranging from 4.38 and 6.10 ($2.4x10^4$ to $1.25x10^6$ CFUs/g); and 6.03 and 9.82 ($1.08x10^6$ and $6.6x10^9$ CFUs/g), respectively (Figure 2b). Although the presence of pathogenic bacteria was less frequent in samples from roadside markets, when it was detected, the concentration was much higher. Like the roadside markets, no *E. coli* was detected in lettuce from the

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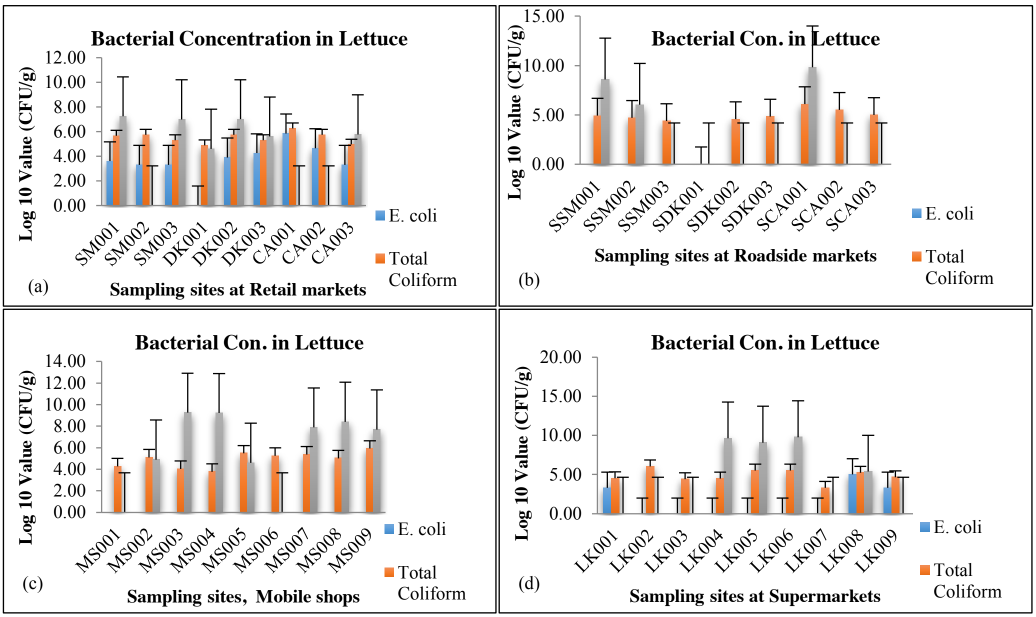
mobile vegetable carts. However total coliforms were present in all samples while seven of nine samples were contaminated with Salmonella. The LRV for values ranged between 3.78 and 5.49 ($6x10^3$ and $8.5x10^5$ CFUs/g); and between 4.60 and 9.26 ($4x10^4$ and $1.8x10^9$ CFUs/g) for total coliforms and *Salmonella*, respectively (Figure 2c). Mobile carts showed a high presence of salmonella, to a very high concentration.

Figure 2. Bacterial contamination in lettuce collected from (a) retail markets; (b) roadside stalls; (c) mobile street carts; and (d) supermarkets in Phnom Penh.



In contrast to expectations, all samples of lettuce collected from supermarkets were contaminated by *E. coli*, while total coliforms and *Salmonella* were present in three and four of the nine samples, respectively. The LRV for *E. coli,* total coliforms, and *Salmonella* ranged between 3.30 and 5.02 ($2x10^3$ CFU and $1.04x10^5$ CFUs/g); $2x10^3$ CFUs/g and $1.09x10^6$ CFUs/g; and $2.4x10^5$ CFU and $6x10^9$ CFUs/g, respectively (Figure 2d). The refrigeration of

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produce to appropriate temperatures is one of the most important factors in controlling the survival and growth of pathogens (Bolin et al., 1977). It may be that the temperature inside the produce section of supermarkets in Cambodia is supporting rather than reducing the survival and growth of bacteria.

Fresh fruits and vegetables, especially leafy greens vegetables eaten raw are being recognized as the main vehicles for transmission of human pathogens sourced from animals (Berger et al., 2010). While, the internal, uncut tissues of vegetables are sterile, they can become contaminated through the use of organic fertilizers, irrigation, contaminated soil, and post-harvest handling (Enabulele & Uraih, 2009; Gosh et al., 2004; Kłapeć & Borecka, 2012; Pachepsky et al., 2011; Ingham et al., 2004; Vital et al., 2014). A high prevalence of enterobacterial contamination in most of the open-air wholesale markets was found to be common. However, unexpectedly, there *E. coli* was not present in lettuce leaves collected from roadside markets or mobile vegetable stalls. This may be due to bacterial populations becoming stressed by chemical, physical, and biological factors. Examples of microbial stressors include changes in temperature, osmotic pressure, and nutrient availability (Capozzi et al., 2009). It is speculated that the bacteria present in the lettuce leaves, when sold at the retail market for distribution at the roadside markets, and mobile shops may be affected by higher temperatures and experience heat stress, resulting in a lower concentration of pathogenic microorganisms.

Bacterial Contamination in Saw-leaf Herbs

The presence and concentration of *E. coli*, total coliforms and *Salmonella* concentrations in the saw-leaf herb samples are presented in

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Figure 3 (a-d). Six and eight out of nine samples of saw leaf herb from the wholesale markets were contaminated with *E. coli* and total coliforms, respectively, while all samples collected from the market contained *Salmonella.* The LRV for *E. coli,* total coliforms, and *Salmonella* were between 3.30 and 5.30 ($2x10^3$ and $2x10^5$ CFUs/g), 4.94 and 5.60 ($8.8x10^3$ and $4x10^5$ CFUs/g), and 5.41 and 9.45 ($2.6x10^4$ and $2.8x10^9$ CFUs/g), respectively (Figure 3a). From the roadside stalls, total coliforms were present in all samples collected, while *E. coli* and *Salmonella* contamination was present in four and seven of nine samples, respectively. The LRV for *E. coli*, total coliforms and *Salmonella* ranged between 3.78 and 4.96 ($6x10^3$ and $9.10x10^4$ CFUs/g), $4x10^3$ and $2.41x10^5$ CFUs/g, and $2.2x10^5$ and $8.1x10^7$ CFUs/g, respectively (Figure 3b). This shows the presence of pathogenic bacteria and the LRV for saw-leaf herbs from roadside markets and retail markets were almost identical. However, unlike the roadside stalls, there was fewer samples containing *E. coli* and *Salmonella* for saw-leaf herbs purchased from mobile carts, with only two and four of nine samples being contaminated, respectively. Total coliforms were present in eight of the nine samples. The colony count for *E. coli*, total coliforms and *Salmonella* ranged between $2x10^3$ and $1.1x10^4$ CFUs/g; $2x10^4$ and $6x10^5$ CFUs/g; and $7.91x10^6$ and $7.5x10^7$ CFUs/g, respectively (Figure 3c). The results for mobile vegetable carts was similar to road stalls with 100% of samples containing total coliforms but only one and two of nine samples being contaminated by *E. coli* and *Salmonella*, respectively. However, the samples that were contaminated had high concentrations of *E. coli*, total coliform, and *Salmonella* species. The single *E. coli* sample had an LRV of 3.60 ($4x10^3$ CFUs/g); while the total coliform and *Salmonella* samples has an LRV ranging

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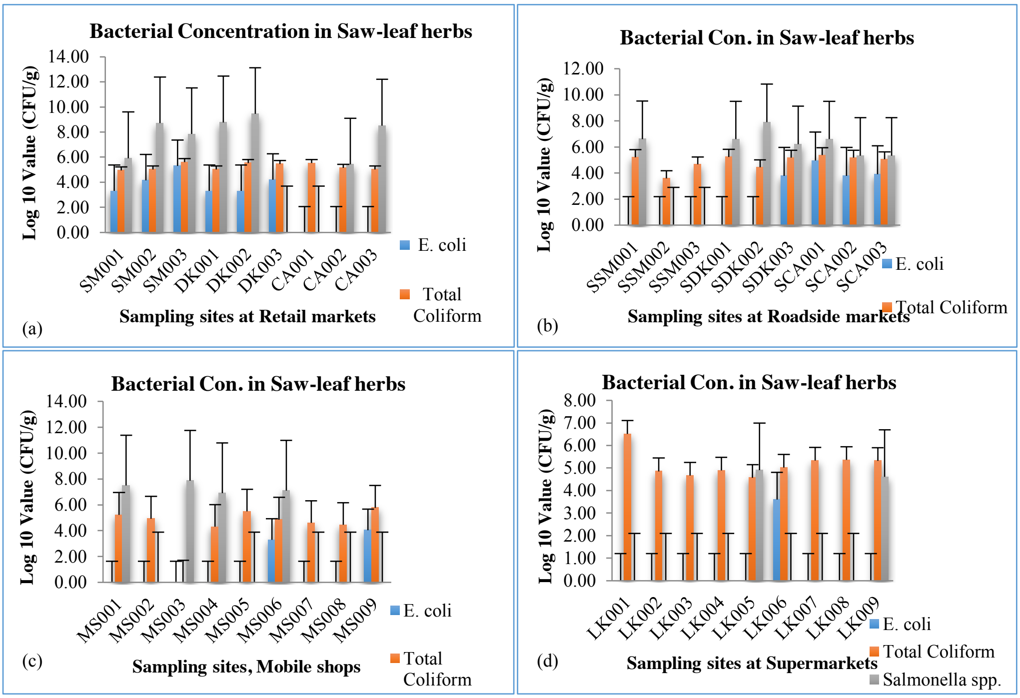
between 4.57 and 6.52 ($3.7x10^4$ and $3.30x10^6$ CFUs/g); and 4.60 and 4.90 (4 $x10^4$ and $8x10^4$ CFUs/g), respectively (Figure 3d).

Figure 3. Bacterial contamination in saw-leaf herbs collected from (a) retail markets; (b) roadside stalls; (c) mobile street carts; and (d) supermarkets in Phnom Penh



Bacterial Contamination in Cucumbers

The results for the presence and concentration of *E. coli*, total coliforms and *Salmonella* in samples of cucumbers purchased from various types of markets in Phnom Penh are presented in Figure 4 (a-d). Unlike lettuce and saw leaf herb, there was no salmonella contamination detected in cucumber samples purchased from the wholesale markets. One and four of the nine cucumber samples were contaminated by *E. coli* and total coliforms, respectively. The LRV for the sample contaminated with *E. coli* was 3.30 ($2x10^3$

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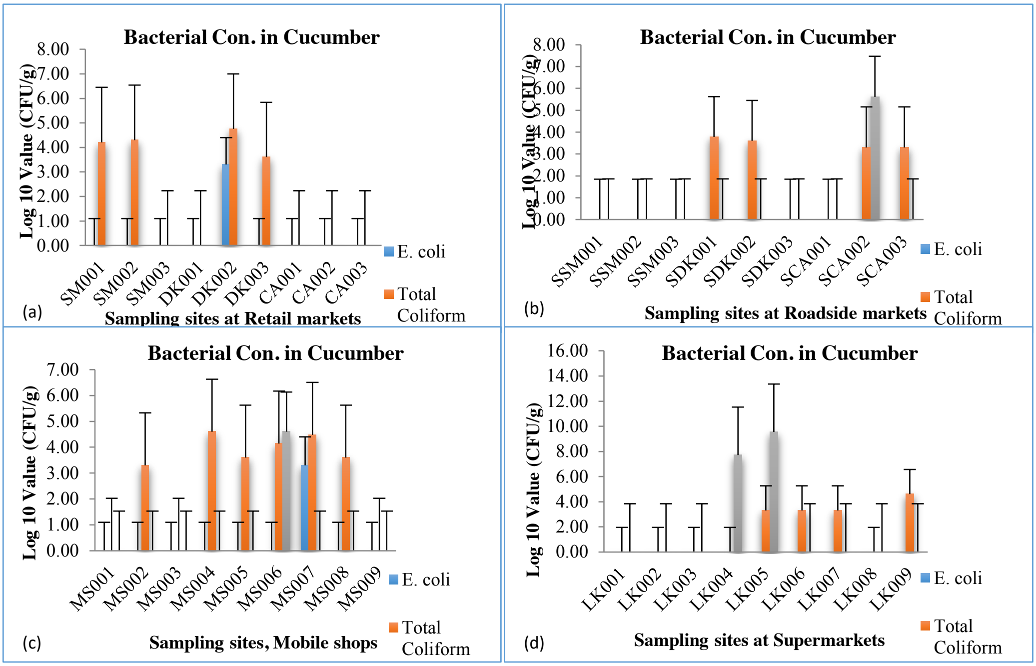
CFUs/g) and ranged between 3.60 and 4.76 ($4x10^3$ and 2×10^4CFUs/g) for the samples with total coliform presence indicated (Figure 4a).

Figure 4. Bacterial contamination in cucumbers collected from (a) retail markets; (b) roadside stalls; (c) mobile street carts; and (d) supermarkets in Phnom Penh.



From the roadside stall samples, no *E. coli* contamination was found in any of the nine cucumber samples. However total coliforms and *Salmonella* were present in four and one of the nine samples collected, respectively. The LRV for total coliforms ranged between 3.30 and 3.78 ($2x10^3$ and $6x10^3$ CFUs/g), and the sample contaminated with *Salmonella* had a LRV of 5.60 (4×10^4 CFUs/g) (Figure 4b). There was a low prevalence and concentration of pathogenic bacteria for cucumbers from the wholesale markets and roadside stalls in general.

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The LRV for *E. coli* and *Salmonella* ranged was 3.30 (2 x 10^3 CFUs/g) and 4.60 (4 x 10^4 CFUs/g), respectively, and ranged between 3.30 and 4.46 (2x10^3 and 4x10^4 CFUs/g) for total coliforms, which was higher than for the wholesale and roadside markets (Figure 4c). There was no *E. coli* present in cucumber samples collected from supermarkets. However, four and two of the nine samples collected were contaminated with total coliforms and *Salmonella* respectively. The LRV for total coliforms and *Salmonella* ranged between 3.30 and 4.60 (2x10^3 and 4x10^4 CFUs/g) and 7.70 to 9.53 (5x10^7 and 3x10^9 CFUs/g), respectively (Figure 4d).

Table 2. The total proportion of fresh vegetables contaminated with indicator species for bacterial pathogens.

Type of vegetables	**Proportion of total vegetable samples contaminated**		
	E. coli	total coliforms	*Salmonella*
Lettuce	30.7%	97.2%	55.7%
Saw-leaf herb	36.1%	97.2%	55.7%
Cucumber	5.7%	50%	11.1%

Bacterial Removal by Washing

Pathogenic bacteria, such as *E. coli* O157, have been described as bound to the surface of fresh vegetables rather than being contained within tissues (Berger et al., 2010). To test the effectiveness of pathogen removal from fresh vegetable samples, the presence and concentration of each indicator species for each sample was also analyzed before and after thorough washing with flowing tap water. The LRV of *E. coli* in the contaminated samples of lettuce from the wholesale markets was reduced by between 2.5%

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and 30% after washing for two of the nine samples while being completely removed in the other two. Most of the *E. coli* bacteria in the saw-leaf herb and cucumber samples were completely removed by thorough washing. *E. coli* in all of the vegetable samples from the roadside stalls (lettuce, saw-leaf herbs and cucumber) were completed eliminated, except for one lettuce sample. *E. coli* presence in all contaminated samples from both the mobile vegetable carts and supermarkets were totally removed after washing. However, washing the vegetables thoroughly under tap water was not found to be effective for removing total coliforms for all types of vegetables in this study (data not shown).

Unlike *E. coli* and total coliforms, the removal of *Salmonella* by thorough washing with tap water was not able to be assessed by this study. A comparison of the tap water with the control (distilled water) found that some of tap water samples used to wash the vegetables contaminated with *Salmonella* bacteria. Therefore, the analysis of these results provided highly skewed data and it was removed from the study. In future studies, experiments will be conducted using sterile distilled water to wash the vegetables to ensure errors are not introduced from other contamination sources.

Conclusion

Overall, this study has shown that majority of the lettuce and saw-leaf herbs sampled from the wholesale markets were contaminated with *E. coli,* while there fewer incidences of the presence of *E. coli* in these vegetables from other types of markets. Total coliforms and *Salmonella* were also present for these leafy vegetables from all types of markets: wholesale, roadside,

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mobile, and supermarkets. Unlike fresh leafy vegetables, the cucumber samples showed a low prevalence of bacterial contamination, thus presented a lower risk in terms of foodborne disease outbreaks. If fresh cucumber is contaminated, it is easier to reduce the pathogen levels by washing the cucumber with a clean water source. In summary, our data strongly suggests that the fresh leafy vegetables, particularly those with a large surface area, should be washed thoroughly prior to eating. This is also true for fruiting vegetables to remove bacteria and chemicals. This research may be used to raise the awareness of the likely bacterial contamination of fresh, raw vegetables and the risk of adverse health impacts if these vegetables are not washed prior to eating.

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References

Ananchaipattanan, C., Hosotani, Y., Kawasaka, S., Pongsawat, S., Bari, M. D. L., Isobe, S., and Inatsu, Y. (2012) Bacterial contamination in retail foods purchased in Thailand, *Food Sci. Technol. Res.*, *18*(5) pp. 705 – 712.

Berger, C.N., Sodha, S.V., Shaw, R.K., Griffin, P.M., Pink, D., Hand, P., and Frankel, G. (2010) Fresh fruit and vegetables as vehicles for the transmission of human pathogens, *Environ. Microbiol. 12* pp. 2385-2397.

Bolin, H.R., Stafford, A.E., King, A.D. Jr., and Huxsoll, C.C., (1977) Factors affecting the storage stability of shredded lettuce, *J. Food Sci.*, *42* pp. 1319-1321.

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Capozzi, V., Fiocco, D., Amodio, M. L., Gallone, A., and Spano, G. (2009) Bacterial stressors in minimally processed food, *Int. J. Mol. Sci.*, *10,* pp. 3076-3105.

Chau, H.L.Q., Thong, H.T., Chao, N.V., Hung, P.H.S., Hai, V.V., An, L. V., Fujeida, A., Ueru, T., and Akamatsu, M. (2014) Microbial and parasitic contamination on fresh vegetables sold in traditional markets in Hue city, Vietnam, *Journal of Food and Nutrition Research, 2*(12), pp. 959-964.

Chhay, C., Sadiq, M. B., Cho, T. Z. A., and Anal, A. K. (2018) Prevalence and analysis of antibiotic-resistant genes in *Escherichia coli* and *Salmonella* isolates from green leaf lettuce, *Chiang Mai J. Sci.*, *45*(10) pp. 1-13.

De Silva, G.D.D., Abayasekara, C.L., and Dissanayake, D.R.A, (2013) Freshly eaten leafy vegetables: A source of foodborne pathogen? *Ceytone Journal of Science, 42*(2), pp. 95-99.

Doyle, M.P., and Erickson, M.C. (2008) Summer meeting 2007 - The problems with fresh produce: An overview, *J. Appl. Microbiol.*, *105*, pp. 317-330.

Enabulele, S.A., and Uraih, N. (2009) Enterohaemorrhagic *Escherichia coli* O157: H7 prevalence in meat and vegetables sold in Benin City, Nigeria, *African Journal of Microbiology Research*, *3*(5), pp. 276-279.

Ghosh, M., Ganguli, A., and Mudgil, S. (2004) Microbiological quality of carrots used for preparation of fresh squeezed street vended carrot juices in India", *Journal of Food Agriculture and Environment*, *2*(2) pp. 143-145.

Gravani, R.B. (2009) The role of good agricultural practices in produce safety, in Fan, X., Niemira, B. A., Doona, C. J., Feeherry, F. E., Gravani, R. B. (Eds.), *Microbial safely of fresh produce*. Wiley-Blackwell and IFT Press, London, pp. 101-117.

© 2019 The Authors
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Hay, V. (2018) Prevalence of *Salmonella* species in raw animal meats and fresh vegetables in Kilometer No. 4 and Derm Kor markets in Phnom Penh, Undergraduate Thesis, Department of Bioengineering, Royal University of Phnom Penh.

Heaton, J.C., and Jones, K. (2008) Microbial contamination of fruit and vegetables and the behavior of enteropathogens in the phyllosphere: a review, *J. Appl. Micrbiol*, *104*, pp. 613-626.

Ingham, S. C., Losinski, J. A., Andrews, M. P., Breuer, J. E., Breuer, J. R., Wood, T. M., and Wright, T. H. (2004) *Escherichia coli* contamination of vegetables grown in soils fertilized with non-composted bovine manure: Garden-scale studies, *Applied and Environmental Microbiology*, *70*(11): 6420-6427.

Johnston, L. M., Jaykus, L., Moll, D., Martinez, M. C., Anciso, J., Mora, B., and Moe, C. L. (2005) A field study of the microbiological quality of fresh produce, *Journal of Food Protection, 68*(9), pp. 1840-1847.

Kheang, C. (2016) The assessment of *Escherichia coli* and *Salmonella* species contamination in fresh vegetables from Phnom Penh and Takhmao markets, Undergraduate Thesis, Department of Chemistry, Royal University of Phnom Penh.

Kłapeć, T., and Borecka, A. (2012) Contamination of vegetables, fruits and soil with geo-helminth eggs from organic farms in Poland, *Annals of Agricultural and Environmental Medicine*, *19* (3) pp. 421-425.

Lynch, M. F., Tauxe, R. V., and Hedberg, C. W. (2009) The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities, *Epidemiol. Infect., 137*, pp. 307-315.

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Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., and Taux, R. V. (1999) Food-related illness and death in the United States, *Emerging infectious diseases*, *5*(5) pp. 607-625.

Neufeld, D. S. G., Heng, S., Phoeurk, C., Glick, A., and Hernandez, C. (2010) Prevalence and persistence of organophosphate and carbamate pesticides in Cambodian market vegetables, *Asian Journal of Water, Environment and Pollution*, *7*(4) pp. 89-98.

Pachesky, Y., Shelton, D. R., McLain, J. E. T., Patel, J., and Mandrell, R. E. (2011) Chapter 2: Irrigation waters as a source of pathogenic microorganisms in produce: a review, *Advances in Agronomy*, *113* pp. 73-138.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., and Griffin, P. M., (2013) Attribution of foodborne illnesses, hospitalization, and deaths to food commodities by using outbreak data, United States, 1998-2008, *Emerging infectious diseases*, *19*(3) pp. 407-415.

Phoeurk, C. (2007) Determination of methyl parathion in kuang futsion and kale, Undergraduate Thesis, Department of Chemistry, Royal University of Phnom Penh.

Quiroz-Santiago, C., Rodas-Suárez, O. R., Carlos, R. V., Fernández, F. J., Quiñones-Ramirez, E., and Vázquez-Salinas, C. (2009) Prevalence of *Salmonella* in vegetables from Mexico, *Journal of Food Protection*, *72*(6), pp. 1279-1282.

Ruiz, J. A., Vargas, R. G., and Garcia-Villanova, R. (1987) Contamination on fresh vegetables during cultivation and marketing, *International Journal of Food Microbiology*, *4*(4) pp. 285-291.

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Santos, Y. O., Almeida, R. C. C., and Guimarães, A. M. (2010) Hygienic-sanitary quality of vegetables and evaluation of treatments for the elimination of indigenous *E. coli* and *E. coli* O157: H7 from the surface of leaves of lettuce (Lactuca sativa L.), *Ciênc. Tecnol. Aliment., Campinas*, *30*(4) pp. 1083-1089.

Slavin, J. L., and Lloyd, B. (2012) Health benefits of fruits and vegetables, *American Society for Nutrition*, *3* pp. 506-516.

Tambekar, D. H. and Mundhada, R. H. (2006) Bacteriological quality of salad vegetables sold in Amarvati city (India), *Journal of Bacteriological Sciences*, *6* pp. 28-30.

Vital, P. G., Dimasuay, K. G. B., Widmer, K. W., and Rivera, W. L. (2014) Microbiological quality of fresh produce from open-air markets and supermarkets in the Philippines, *The Scientific World Journal*, pp. 1-7.

World Health Organization (WHO) (2008) Microbiological hazards in fresh fruits and vegetables, Microbiological Risk assessment series, retrieved from www.who.int/foodsafety/pubications/fsmanagement/../eninJan2 010.