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PEER-REVIEWED ARTICLE

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Quantitative and Qualitative Assessments of *Enterobacteriaceae*, Coliforms, and Generic *Escherichia coli* on Fresh Vegetables Sold in Cambodian Fresh Produce Distribution Centers

ABSTRACT

Cambodia has introduced several initiatives to increase production and consumption of fresh produce throughout the country. Fresh produce, however, is often associated with foodborne disease; thus, understanding how food-borne pathogens enter and are transmitted throughout Cambodian production chains can help to ensure positive nutritional outcomes from increased produce consumption. This study was conducted to provide a quantitative (log CFU/g) and qualitative (prevalence) assessment of *Enterobacteriaceae*, coliforms, and *Escherichia coli* on tomato, cucumber, and lettuce sold through aproduce distribution center in Cambodia. Samples (n = 384) were collected over 6-month period (December 2019– May 2020) and screened for the presence of *Enterobacteriaceae*, coliforms, and *Escherichia coli* following methods adapted from the U.S. Food and Drug Administration's *Bacteriological Analytical Manual*. A significantly greater concentration of *Enterobacteriaceae* was observed on lettuce (4.71 ± 1.02 log CFU/g) than cucumber (3.44 ± 1.12 log CFU/g) and tomato (2.79 ± 1.02 log CFU/g) ($P < 0.05$). The indicator organisms were present at the highest percent prevalence ($P < 0.05$) in lettuce, followed by cucumber and tomato. The results of this study provide an initial assessment of *Enterobacteriaceae*, coliforms, and *E. coli* contamination in vegetables sold through Cambodian markets. These data highlight the necessity to establish and/or improve sanitation practices among the different points of the vegetable value chain in Cambodia.

INTRODUCTION

Sanitation is described as the process of creating and promoting hygienic and healthful conditions and the practices that help maintain such environments *(10)*. The *Enterobacteriaceae* is composed of 10 genera and 20 clinically significant species *(16)*. These microorganisms are also considered indicators for food quality and sanitation *(1, 3, 7)*. In general, the presence of *Enterobacteriaceae* in food does not indicate an apparent hazard to public health; however, it may indicate poor sanitation, hygiene, and handling practices *(10)*.

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Coliforms are part of *Enterobacteriaceae* and include genera such as *Citrobacter* and *Enterobacter* and species such as *Escherichia coli (7)*. These coliforms indicate the possible presence of pathogenic bacteria *(21, 22)*. This subgroup makes up approximately 10% of intestinal microflora in humans and animals, and it is often used as an indication of hygiene because of its association with fecal contamination *(1, 3)*. The presence of fecal contamination in food products and environments may lead to exposure of harmful bacteria such as pathogenic *E. coli* or *Salmonella (16)*. These harmful microorganisms are responsible for a large portion of diarrheal diseases in developing countries, such as Africa and Southeast Asia *(8)*.

Approximately one-third of diarrheal-related illness in developing counties are associated with the consumption of contaminated food *(8, 11, 22)*. For many of these countries, however, few studies exist that clearly identify specific etiological agents (e.g., pathogenic *E. coli*, *Salmonella*) responsible for foodborne disease outbreaks *(17)*. Nevertheless, fresh produce and other horticulture products have been linked to foodborne pathogens and outbreaks worldwide *(6, 15, 17)*, and Cambodian diets, such as many of those in South East Asia, contain many raw fruits and vegetables *(12)*. As in other countries in the Greater Mekong Subregion, qualitative assessments of food contamination rates in Cambodia are still limited and scarce. Understanding how microorganisms are transmitted along the food production chain might be key to developing intervention strategies for food safety.

Previous studies conducted within our research group have provided information about critical contamination points along the vegetable production chain, specifically at the informal market level. Samples of three vegetable types (e.g., lettuce, cucumber, and tomato) and environmental samples (e.g., food contact and non food-contact surfaces) were collected at the informal market and analyzed for *E. coli* and *Salmonella (4, 19)*. These studies revealed that the prevalence of *E. coli* and *Salmonella* ranged from 9.1 to 56.5% depending on the season and vegetable type *(4)*, and a vast diversity of *Salmonella enterica* serotypes were isolated from environmental samples *(19)*. In addition, another study from our research group evaluated the flow and food safety behaviors of stakeholders in the Cambodian vegetable value chain *(5)*. This study defined the flow of the vegetables and identified gaps across the value chain, including deficiencies in food safety practices in informal markets in Cambodia. Informal markets represent the main point for retail purchasing a variety of food products, including vegetables, fruits, meat, and household items, in Cambodia. Such markets have been described as locations that "escape effective health and safety regulations and are often untaxed and unlicensed" *(5)*.

Before reaching retail informal markets, many food products (e.g., produce) in Cambodia are gathered and collected in distribution centers before being redistributed to domestic retailers (e.g., informal markets) and/or international wholesalers (export of vegetables to Cambodia), such as Thailand and Vietnam. Produce distribution centers in Cambodia are a transition point between farmers and informal markets. At the distribution center level, bulk products such as produce, meat, and nuts as well as non food products are sold. The products that are sold at the distribution center can originate from local farmers, intermediaries, and international wholesalers (i.e., Vietnam). From the distribution center, these products may be purchased by collectors, informal market vendors, restaurant and hotel owners, and household consumers. The Cambodia vegetable value chain has been previously described by Sokhen et al. *(20)*.

The objective of this study was to better understand the role of distribution centers in the safety of produce farm-to-fork continuum by measuring indicator microorganisms that reflect sanitation and hygiene practices in a major Cambodian fresh produce distribution center.

MATERIALS AND METHODS

Produce distribution center

The samples described below were collected from the major and only distribution center in Battambang Province *(Fig. 1)*, located approximately 291 km northwest of Phnom Penh. The Battambang distribution center is the only distribution center in the northwestern region; therefore, it represents one of the main centralized centers in the Cambodia vegetable value chain.

Sample collection

From each vendor within the distribution center, samples of three vegetable types were collected—loose-leaf lettuce, cucumber, and tomato—for a total of 384 samples (tomato, n = 145; cucumber, n = 132; and lettuce, n = 107). From each vendor, multiple vegetables of each type were collected and combined for analysis (e.g., three cucumbers were purchased from vendor 1, and samples were taken from each cucumber to reach the desired weight amount [see "Sample preparation"]). Samples were collected over a 6-month period (December 2019–May 2020). Immediately after collection, vegetables were aseptically transferred into separate, sealable bags and then labeled and stored in ice. Vegetable samples were kept at 0 ± 2°C, and temperature was monitored using a (Cole-Parmer, Vernon Hills, IL) traceable digital thermometer during transport to the laboratory at Royal University of Agriculture-Phnom Penh. Samples were received at the lab within 24 h of collection for microbial analysis.

Sample preparation

The surfaces of lettuce leaves, tomato skins, and cucumber skins were aseptically excised from each sample with a sterile scalpel blade, and a 10-g portion from each was weighed and transferred to individual filter bags (Whirl-Pak, Nasco, Fort Atkinson, WI). Buffered peptone water (BD, Sparks, MD) was added to the bag (90 mL), and samples were hand

Figure 1. Vegetable distribution center at Battambang Province in Cambodia.

stomached for 2 min. Samples were serially diluted in 0.1% peptone water (BD) and plated to determine viable cell counts as described below.

Detection and quantification of *Enterobacteriaceae*, coliforms, and generic *E. coli*

Diluted samples were transferred to *Enterobacteriaceae* Petrifilm and *E. coli*/Coliform Petrifilm (3M, Minneapolis, MN) and incubated at 35 ± 2°C for up to 48 h. *Enterobacteriaceae* prevalence was assessed by the identification of red colonies with yellow zones and/or gas bubbles on *Enterobacteriaceae* Petrifilm plates. The prevalence of coliforms and generic *E. coli* was assessed by the presence of red and blue colonies with gas and blue colonies with gas on the *E. coli*/Coliform Petrifilm plates, respectively.

Statistical analysis

Samples were plated in duplicate, and average log CFU/g for each sample was calculated and used in data analysis. Statistical differences were evaluated using SAS software PROC GLIMMEX (SAS Institute Inc., Cary, NC). A completely randomized design was used to compare values across vegetables (tomato, cucumber, lettuce). Differences were considered statistically significant at $P < 0.05$.

RESULTS

A significant level ($P < 0.05$) of *Enterobacteriaceae* and coliforms was observed on lettuce, compared with cucumber and tomato *(Fig. 2A)*. Overall, the highest ($P < 0.05$) concentration of *Enterobacteriaceae* was observed on lettuce (4.71 ± 1.02 log CFU/g) followed by cucumber (3.44 ± 1.12 log CFU/g) and tomato (2.79 ± 1.02 log CFU/g). Comparable trends were observed for coliform concentration *(Fig. 2B)*, with lettuce having the highest concentration of coliforms (4.36 ± 1.23 log CFU/g) followed by cucumber (3.28 ± 1.32 log CFU/g) and tomato (2.69 ± 1.45 log CFU/g). Vegetable type did not have a significant effect ($P > 0.05$) on concentration of *E. coli* *(Fig. 2C)*: lettuce, 2.71 ± 0.97 log CFU/g; cucumber, 2.28 ± 0.95 log CFU/g; and tomato, 2.18 ± 0.16 log CFU/g). Presence of typical colonies of *Enterobacteriaceae*, coliforms, or generic *E. coli* was considered to be positive by using *Enterobacteriaceae* or *E. coli*/Coliform Petrifilm and used to calculate percent prevalence. Overall, the highest ($P < 0.05$) prevalence of *Enterobacteriaceae* was observed on lettuce (98.1% ± 5.44%)

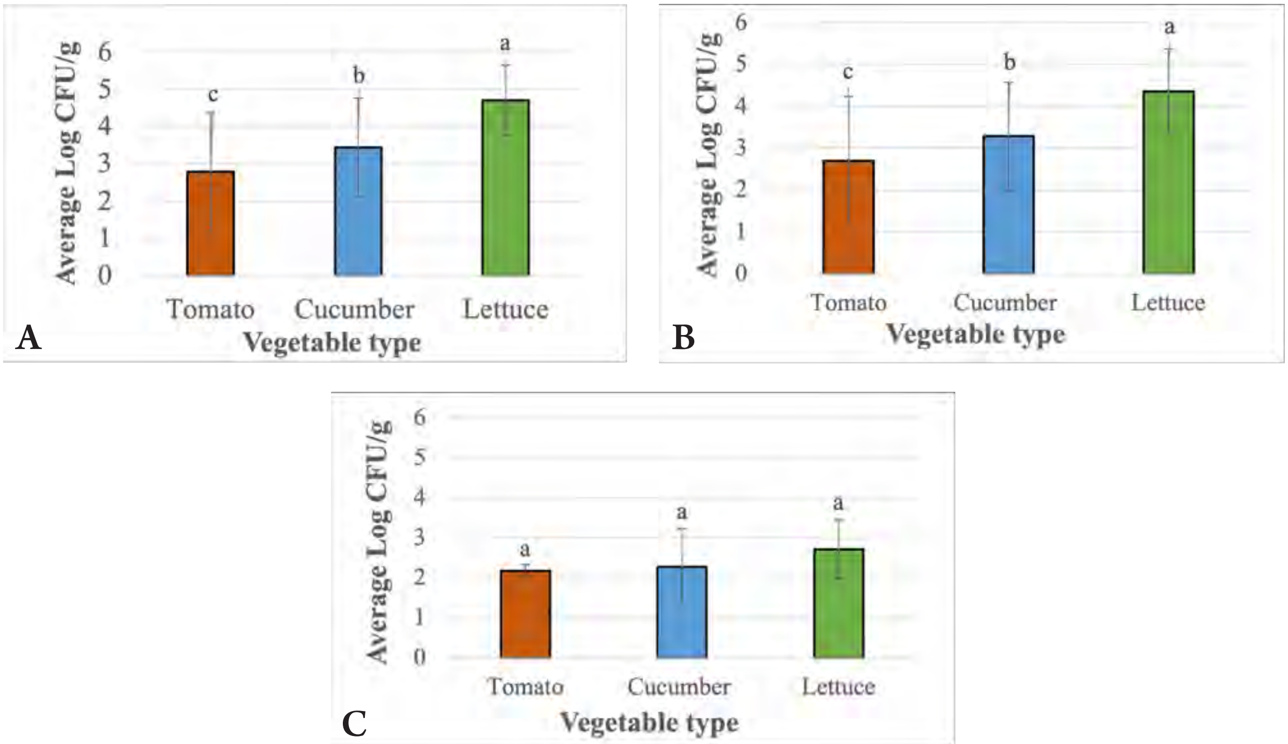

Figure 2. Concentration of *Enterobacteriaceae* (A), coliform (B), and generic *E. coli* (C) on tomato, cucumber and lettuce expressed as average Log CFU/g. Different letters indicate statistical difference between vegetable type ($P < 0.05$).

followed by cucumber (86.6% ± 13.08%) and tomato (65.8% ± 12.64%). A similar trend was observed for prevalence of coliforms, with lettuce (96.8% ± 8.16%) having the overall highest prevalence ($P < 0.05$) followed by cucumber (84.9% ± 10.86%) and tomato (62.6% ± 15.44%). Generic *E. coli* was observed at a lower prevalence: the highest prevalence ($P < 0.05$) was on lettuce (28.9% ± 12.64%) followed by cucumber (9.0% ± 6.97%) and tomato (1.2% ± 1.9%).

DISCUSSION

The *Enterobacteriaceae* is composed of 10 genera and 20 clinically significant species *(16)*. In general, the presence of *Enterobacteriaceae* in food does not indicate an apparent hazard to public health; however, it may indicate poor sanitation, hygiene, and handling practices *(1, 3, 7, 10)*.

Coliforms are members of the *Enterobacteriaceae* and include genera such as *Citrobacter* and *Enterobacter* and species such as *E. coli (7)*. Their presence in food often indicates fecal contamination and the possible presence of pathogenic bacteria *(1, 3, 21, 23)*. For vegetables, the use of raw manure, poor hygiene of food handlers, and contaminated biological soil amendments have been shown to play a role in the transfer of pathogens to vegetables *(2, 13, 18)*. Notably, pathogenic *E. coli* is responsible for the majority of diarrheal diseases in developing countries, such as Africa and Southeast Asia *(8)*.

The concentrations of *Enterobacteriaceae*, coliforms, and generic *E. coli* described here indicate that effective sanitary practices are not likely in place at the produce distribution center in Cambodia. Desiree et al. *(5)* previously described behaviors that contribute to cross-contamination at multiple stages along the value chain, including the distribution center. Inadequate practices such as infrequent washing practices, improper handling of cut vegetables, and unsanitary practices during sale were observed at the distribution center in Cambodia. Furthermore, several transportation vehicles were allowed in the market *(Fig. 1)* and produce was accumulated and stored directly on floors, increasing the risk for bacterial and pest contamination. As seen in similar informal markets, vegetables were frequently stored and sold at ambient temperature and directly exposed to the environment without any protection against insects and rodents *(14, 17)*. Intervention strategies such as educational training on basic food safety, handling of vegetables, and hygiene and sanitation practices, along with investments in infrastructure, should be implemented to prevent and reduce the contamination of vegetables across the value chain.

For all three indicator microorganisms (*Enterobacteriaceae*, generic *E. coli*, and coliforms), the highest prevalence was observed on lettuce, followed by cucumber and tomato.

Produce characteristics, such as composition, surface morphology, pH, and moisture availability, as well as differences in growing conditions, transportation, and storage procedures, play a significant role in bacterial colonization *(9)*. Similarly, Desiree et al. *(4)* found a higher level of coliforms and *E. coli* on lettuce than tomato and cucumber collected from informal markets in Cambodia. The vegetable value chain, as previously described by Desiree et al. *(5)*, is complex and affected by several factors at different points. For example, at the distribution center level, food safety challenges include lack of hygiene and sanitation practices, food safety regulations, and infrastructure as well as the absence of potable water and proper storage conditions *(5, 17)*. Strategies to improve food safety interventions at the distribution level might effectively control the burden of microbial disease. For example, using disposable gloves when handling produce; washing hands frequently, especially after handling money or using restrooms; keeping produce off the ground; and cleaning food-contact surfaces frequently could help control microbial contamination at the distribution level and improve safety of vegetables available to Cambodians.

CONCLUSIONS

Results from this study provide an initial assessment of *Enterobacteriaceae*, coliforms, and generic *E. coli* carriage rates in Cambodian vegetables at distribution centers and highlight the need to establish and/or improve sanitation interventions within and between the different points of the food supply chain. Future research should include a survey to collect information from the source of the vegetables and the characteristics of the distribution center as well as evaluate the presence and concentrations of specific foodborne pathogens in the Cambodian vegetable value chain. Furthermore, the identification of sustainable intervention strategies to reduce microbial contamination across the Cambodian vegetable value chain is necessary.

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