PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 44, No. 6, p. 420–428
https://doi.org/10.4315/FPT-24-020

Vannith Hay,[1†] Jessie Vipham,[2] Nora M. Bello,[3]
Daniel L. Boyle,[4] Sara Gragg,[5] and Valentina Trinetta[1,2*]

[1]Food Science Institute, Kansas State University, 1530 Mid-campus Drive North, Manhattan, KS 66506, USA

[2]Dept. of Animal Sciences and Industry, Kansas State University, 1424 Claflin Road, Manhattan, KS 66506, USA

[3]Agricultural Research Service–Northeast Area, U.S. Dept. of Agriculture, 10300 Baltimore Avenue, Beltsville, MD 20705, USA

[4]Division of Biology, K-State Microscopy Facilities, Kansas State University, 1717 Claflin Road, Manhattan, KS 66506, USA

[5]Dept. of Food Science, University of Wisconsin–Madison, 1605 Linden Drive, Madison, WI 53706, USA

Efficacy of Cleaning and Sanitizing Methods in Reducing *Salmonella* on Banana Leaves and Bamboo Baskets, Common Surfaces Found in Cambodian Fresh Food Markets

ABSTRACT

The lack of proper cleaning and sanitizing practices on natural food-contact surfaces can lead to foodborne illness outbreaks. This study was conducted to evaluate the efficacy of cleaning and sanitizing methods for reducing *Salmonella* inoculated onto banana leaf and bamboo basket surfaces. Surfaces were inoculated with a *Salmonella* cocktail and assigned to a combination treatment of cleaning and sanitizing methods. Three cleaning methods were evaluated (wiping, scrubbing, or no cleaning) alone or in combination with 200 ppm of bleach or 400 ppm of peracetic acid (either applied for 3 or 5 min) or no sanitizing. Regardless of cleaning method or contact time, peracetic acid reduced *Salmonella* on banana leaves to a level below the limit of quantification (<1.44 log CFU/cm^2). The efficacy of sanitizing with bleach for banana leaves differed depending on the cleaning method (P = 0.044). Bleach applied for 5 min with wiping was most effective and reduced *Salmonella* on banana leaves to 2.85 log CFU/cm^2 (95% confidence interval: 2.52, 3.18). For bamboo baskets, the most effective treatment was sanitizing with peracetic acid for 5 min regardless of cleaning method (P < 0.0001). This study offers insights for controlling *Salmonella* on food-contact surfaces commonly used in Cambodian fresh food markets.

INTRODUCTION

Salmonella is one of the major pathogens linked to foodborne illnesses worldwide and is increasingly prevalent in low- and middle-income countries (LMICs) due to an increased demand for nutrient-dense foods originating from informal value chains *(1, 11, 15, 27, 30)*. This microorganism often has been isolated from various animal source foods and fresh produce *(7, 21, 23)*. *Salmonella* is predominantly found in poultry, eggs, pork, and dairy products *(3)*. In the last few decades, *Salmonella* also has been associated with fresh fruits and vegetables *(4)*. A similar phenomenon has been reported in LMICs, where

*Author for correspondence: Phone: +1 785.532.1667; Email: vtrinetta@ksu.edu

†Present address: Center of Health Research and Policy Support, National Institute of Public Health, Lot 80, Samdech Penn Nouth Street (289), Toul Kork, Phnom Penh, 120408, Cambodia.

fresh and perishable foods are primarily sold in informal markets *(10)*.

Fresh food markets (or informal markets) are a large collection of open-air stalls where fruits, vegetables, and animal source foods are sold *(22)*. In LMICs, fresh food markets are the daily destination of millions of consumers because food products are often less expensive and fresher than foods in supermarkets *(29)*. However, vendors often sell animal source foods, fresh fruits, and fresh vegetables at the same stand, favoring potential cross-contamination due to extensive food handling during market operations. Food items are often displayed at ambient temperature and are directly exposed to the open-air environment, thereby increasing the risk of microbial contamination *(19)*. In several studies, microbial contamination of food products sold in fresh food markets has been documented *(2, 20, 26, 28, 33)*. In Cambodia, various food products sold in fresh food markets were reported to be contaminated with foodborne pathogens, including *Campylobacter* spp., *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus (7, 15, 21, 32)*. Lay et al. *(15)* reported that raw poultry sold in fresh food markets in Phnom Penh (Cambodia) had a high prevalence of *Salmonella* (88% of 152 samples) and *Campylobacter* spp. (81% of 129 samples). In a study conducted in two northeast provinces of Cambodia, Desiree et al. *(7)* suggested that approximately 28% and 22% of 312 vegetable samples collected from fresh food markets were contaminated with *Salmonella* and *E. coli*, respectively. Environmental contamination is also a concern in these settings *(23, 31)*. Schwan et al. *(31)* reported that food-contact surfaces sampled in fresh food markets could be more than twice as likely to be contaminated with *Salmonella* compared with non food-contact surfaces in the dry season; the estimated probabilities of detection (95% confidence interval) were 0.41 (0.25, 0.59) and 0.17 (0.08, 0.32), respectively ($P = 0.002$). In these studies, the presence of *Salmonella* on these food-contact surfaces was associated with poor personal hygiene and poor sanitizing practices among vendors *(23, 31)*.

Banana leaves and bamboo baskets are natural and sustainable food-contact surfaces commonly used to display, store, and transport food products in Cambodian fresh food markets. Banana leaves are typically harvested from trees grown in rural areas and are minimally processed by simply rinsing with water or wiping with cloths to maintain their freshness; thus, they could serve as vehicles for pathogens transmission. Banana leaves are favored for food use due to their local abundance and growing global concerns over the environmental impacts of plastics. Bamboo baskets, cardboard, grass mats, styrofoam boxes, lotus flower leaves, and banana leaves are commonly used in fresh food markets in Cambodia to store, display, and transport food products. Chua and Dykes *(6)* reported that foodborne pathogens are capable of attaching to banana leaf surfaces despite their high wax content (3% of leaf), which could reduce attachment *(6, 36)*. Bamboo baskets can harbor diverse *Salmonella* serotypes; Rissen, Weltevreden, and Altona are the most commonly isolated *(31)*. Schwan et al. *(32)* found that *Salmonella* serotype abundance differed depending on the surface type. These researchers evaluated the diversity of serotypes on floors (13 serotypes) baskets *(11)*, mats *(5)*, scales *(5)*, and lotus flower leaves *(4)*. Baskets, scales, and lotus leaves were the top three surfaces with the highest diversity of *Salmonella* serotypes. These findings suggest that food-contact surfaces such as bamboo baskets and banana leaves used to store, display, and transport food products in fresh food markets in Cambodia might be a vehicle of food contamination.

Cleaning and sanitation can play an important role in preventing foodborne illnesses and reducing foodborne outbreaks *(16)*. Improved sanitation also has positive effects on human health and national economies. According to a 2012 study conducted by the World Health Organization, every dollar invested in sanitation had a $5.50 return in reduced health costs, decreased premature deaths, and increased productivity *(12)*. Often in LMICs, poor hygiene and sanitation practices and insufficient food safety infrastructures can increase the risk of bacterial contamination of food and lead to foodborne outbreaks. Maintaining a clean and sanitary food environment is essential for reducing the prevalence of foodborne pathogens.

The objectives of this study were to evaluate the efficacy of cleaning and sanitizing methods for reducing *Salmonella* experimentally inoculated onto banana leaf and bamboo basket surfaces and to investigate the attachment of *Salmonella* on these food-contact surfaces.

MATERIALS AND METHODS

Salmonella strains

Three serotypes of *Salmonella enterica* subsp. *enterica* isolated from food and non food-contact surfaces in fresh food markets during a previous research study conducted in Cambodia *(31)* were used in this study: Rissen, Corvallis, and 4,[5],12,:i:-. *Salmonella* stock cultures were maintained at −80°C in a CryoCare Organism Preservation System (Key Scientific, Stamford, TX) until used for experiments.

Inoculum preparation

A single frozen bead of each *Salmonella* strain was transferred into 10 ml of tryptic soy broth (TSB; BD Difco, Sparks, MD) and grown overnight (16 to 18 h) at 37°C on an orbital shaker at 70 rpm. A loopful of *Salmonella* culture was then streaked on tryptic soy agar (TSA; BD Difco) and incubated at 37°C for 24 h. A single colony from the streak plate was inoculated into10 ml of freshly prepared TSB and incubated overnight at 37°C on an orbital shaker at 70 rpm. Overnight cultures were centrifuged at 4,000 rpm for 10 min. Supernatant was discarded, and the pellet resuspended in 1 ml of phosphate-buffered saline (VWR Chemicals, Solon, OH). *Salmonella* strains were equally combined to form a 3-ml multistrain *Salmonella* cocktail. The initial population was verified by plating on TSA.

Sample preparation and inoculation

Banana leaves were collected from banana trees locally grown in an outdoor farm in Hutchinson, KS and transported at ca. 20°C (car air-conditioning system) in polyethylene bags to Kansas State University. Leaves were stored at −80°C until processed. Bamboo baskets were purchased from a local vendor and stored at room temperature in the laboratory.

Experiments were performed on 3- by 3-cm^2 samples of banana leaves and 1.2- by 3-cm^2 samples of bamboo baskets. Samples of both surfaces were prepared with a sterile template under aseptic conditions. Samples were placed into a sterile petri dish and exposed to UV-C light for 15 min to eliminate background microflora. Surfaces used for wiping and scrubbing were also UV sterilized. Samples were inoculated by applying ten 10-µl drops of the *Salmonella* cocktail onto each sample. Banana leaves were inoculated on the underside of the leaf. Bacteria were allowed to attach for 60 min following inoculation.

Treatment description and allocation

Samples obtained from a single banana leaf were randomly assigned to treatments, such that a leaf served as a blocking structure in the experimental design. Similarly, samples obtained from an individual bamboo basket were randomly assigned to treatments, such that a basket served as a blocking structure. Six banana leaves and six bamboo baskets were used in this study.

Each treatment was defined by the combination of cleaning and sanitizing methods. Three cleaning methods were evaluated: (i) wiping with a dry paper towel vertically and horizontally five times in each direction, (ii) scrubbing with a dry kitchen sponge vertically and horizontally five times in each direction, and (iii) no cleaning. At each change of direction a different part of the dry paper towel and dry kitchen sponge was used to avoid cross-contamination. After cleaning, five sanitizing methods were used: (i) 200 ppm of disinfecting bleach (Clorox Company, Oakland, CA) applied for 3 min, (ii) 200 ppm of disinfecting bleach applied for 5 min; (iii) 400 ppm of peracetic acid solution (PAA; Shield-Brite 15.0, Pace International, Wapato, WA) applied for 3 min; (iv) 400 ppm of PAA applied for 5 min; and (v) no sanitizing. The sanitizing method was performed by immersing the sample into a 50-ml test tube containing 20 ml of PAA or bleach solution. Sanitizer solutions were freshly prepared from bulk ingredients before each application, and the concentration of each sanitizer was measured with commercial test kits (Peracetic Acid Test Kit and Chlorine Test Kit, ChemWorld, Kennesaw, GA).

Neutralization and enumeration of *Salmonella* cells

Upon completion of each treatment, samples were transferred into 10 ml of Dey and Engley neutralizing broth (Hardy Diagnostics, Santa Maria, CA). Controls were submerged into 10 ml of buffered peptone water (BD Difco). To recover cells, samples were vortexed for 30 s. Serial dilutions were then made in 0.1% peptone water (Bacto, Sparks, MD) and plated on TSA in duplicate. Plates were incubated for 24 h in 37°C. For plates with colonies in the countable range (25 to 250), colonies were counted, and data were recorded as log CFU/cm^2. Before starting the experiments, a neutralizing assay was performed for each sanitizer to ensure complete neutralization

***Salmonella* attachment determined by scanning electron microscopy**

Samples (1 by 1 cm^2) of banana leaves and bamboo baskets were prepared and inoculated as described above for microscopic examination. Inoculated samples were stained with SYTO9 and SYTOX red (Thermo Fisher Scientific, Eugene, OR) and fixed with 10% formalin as previously reported by Mendez et al. *(18)*. Samples were analyzed by scanning electron microscopy (SEM; Hitachi S-3500N, Tokyo, Japan) in the Microscopy Facility, Division of Biology, at Kansas State University.

Statistical analysis

A general linear mixed model was used to evaluate the effect of cleaning and sanitizing methods on *Salmonella* counts, which were averaged over duplicate count plates for each sample and expressed as log CFU/cm^2. A separate analysis was conducted on data obtained from banana leaves and from bamboo baskets. In both cases, the linear predictor included the fixed effects of cleaning, sanitizing methods, and their two-way interaction. For banana leaves, the statistical analysis excluded data corresponding to sanitizing with PAA because *Salmonella* counts were below the limit of quantification (LOQ; <1.44 log CFU/cm^2) for all samples. The random effect of leaf or basket was incorporated into the model as an overarching blocking structure. Restricted maximum likelihood was used to estimate variance components, and the Kenward-Roger's approach was used for estimation of degrees of freedom and standard error correction. Model assumptions were checked and considered to be reasonably met. Estimated least square means and 95% confidence intervals (CIs)were presented at the appropriate level of inference in the factorial treatment structure. Relevant pairwise comparisons between treatment groups were conducted using either a Tukey-Kramer or a Bonferroni adjustment, as appropriate in each case, to avoid inflation of the type I error rate due to multiple comparisons. Estimates of log reductions relative to the negative control (i.e., no cleaning and no sanitizing) were reported to express the relative number of *Salmonella*

TABLE 1. Estimated *Salmonella* counts on banana leaf surfaces following treatment with different cleaning and sanitizing methods

Cleaning	Sanitizing	Mean count (log CFU/cm²)[a]	95% CI (log CFU/cm²)[b]
Wiping	No sanitizing	5.73[A]	5.59, 5.87
	Bleach 3 min	4.15[B]	3.82, 4.47
	Bleach 5 min	2.85[C]	2.52, 3.18
	PAA 3 min	<1.44	
	PAA 5 min	<1.44	
Scrubbing	No sanitizing	5.75[A]	5.61, 5.90
	Bleach 3 min	4.02[B]	3.69, 4.35
	Bleach 5 min	3.58[B]	3.25, 3.90
	PAA 3 min	<1.44	
	PAA 5 min	<1.44	
No cleaning	No sanitizing	5.75[A]	5.61, 5.90
	Bleach 3 min	4.01[B]	3.68, 4.33
	Bleach 5 min	3.45[B]	3.19, 3.78
	PAA 3 min	<1.44	
	PAA 5 min	<1.44	

[a]Means followed by different letters are significantly different within each cleaning method (Bonferroni adjusted $P < 0.05$).

[b]95% confidence interval.

cells that were eliminated by treatment and tested using Dunnett's adjustment for multiple comparisons.

RESULTS

Salmonella counts and reductions on banana leaf surfaces

Estimated *Salmonella* counts on banana leaves for each treatment combination of cleaning and sanitizing method are shown in *Table 1*. For the negative control (i.e., no cleaning and no sanitizing), *Salmonella* counts were estimated at 5.75 log CFU/cm² (95% CI: 5.61, 5.90).

Sanitizing with PAA for either 3 or 5 min resulted in a reduction of observed *Salmonella* counts on banana leaf surfaces below the LOQ (<1.44 log CFU/cm²) for all samples evaluated regardless of cleaning method. Therefore, further statistical analyses of the efficacy of sanitizing banana leaf surfaces with PAA could not be conducted. Reduction estimates can be approximated only to their lower bound (i.e., magnitude of reduction was at least of a magnitude obtained by the difference between the LOQ and the *Salmonella* counts on the negative control).

For the remaining treatments, a significant interaction was observed whereby *Salmonella* counts on banana leaf surfaces were dependent on the combination of cleaning and sanitizing method utilized ($P = 0.0440$). When banana leaf surfaces were cleaned by wiping, sanitizing with bleach for 5 min yielded the lowest *Salmonella* counts (2.85 log CFU/cm²; 95% CI: 2.52, 3.18) relative to no sanitizing (5.73 log CFU/cm²; 95% CI: 5.59, 5.87), followed by bleach sanitizing for 3 min (4.15 log CFU/cm²; 95% CI: 3.82, 4.47).

When banana leaf surfaces were cleaned by scrubbing, bleach sanitizing yielded a significant decrease in *Salmonella* counts relative to no sanitizing method (5.75 log CFU/cm²; 95% CI: 5.61, 5.90), although no evidence for differences were observed between duration of bleach sanitizing for 3 versus 5 min (4.02 log CFU/cm²; 95% CI: 3.69, 4.35 versus 2.85 log CFU/cm²; 95% CI: 2.52, 318; $P = 0.06$).

Conversely, in the absence of sanitizing, there was no evidence of any effect of cleaning method by itself (i.e., wiping, scrubbing, or no cleaning) on reducing *Salmonella* ($P > 0.99$).

Table 2 presents estimates for log reductions in *Salmonella* counts on banana leaf surfaces for each treatment combina-

TABLE 2. Estimated reductions in *Salmonella* counts on banana leaf surfaces relative to the negative control (i.e., no cleaning and no sanitizing) following treatment with different cleaning and sanitizing methods

Cleaning	Sanitizing	Mean count reduction (log CFU/cm^2)[a]	95% CI (log CFU/cm^2)[b]
Wiping	PAA 5 min	>4.31	
	PAA 3 min	>4.31	
	Bleach 5 min	2.90*	2.1, 3.39
	Bleach 3 min	1.61*	1.12, 2.10
Scrubbing	PAA 5 min	>4.31	
	PAA 3 min	>4.31	
	Bleach 5 min	2.18*	1.69, 2.67
	Bleach 3 min	1.73*	1.25, 2.22
	No sanitizing	0.01	−0.26, 0.26
No cleaning	Bleach 3 min	1.75*	1.26, 2.24
	Bleach 5 min	2.30*	1.81, 2.79
	PAA 3 min	>4.31	
	PAA 5 min	>4.31	

[a]Asterisk indicates significant differences relative to the negative control (Dunnett's adjusted $P < 0.05$).
[b]95% confidence interval.

TABLE 3. Estimated reductions in *Salmonella* counts on bamboo basket surfaces (averaged across cleaning methods) relative to negative control (i.e., no cleaning and no sanitizing) following sanitizing treatment

Sanitizing	Mean count reduction (log CFU/cm^2)[a]	95% CI (log CFU/cm^2)[b]
PAA 5 min	2.26*	1.98, 2.54
Bleach 5 min	1.46*	1.15, 1.76
PAA 3 min	1.36*	1.08, 1.64
Bleach 3 min	1.14*	0.83, 1.45

[a]Asterisk indicates significant differences relative to the negative control (Dunnett's adjusted $P < 0.05$).
[b]95% confidence interval.

tion relative to the negative control used for reference (5.75 log CFU/cm^2; 95% CI: 5.61, 5.90). Compared with the negative control, reductions of *Salmonella* were observed for every treatment combination ($P < 0.0001$) except when cleaning was used without a sanitizing method. No significant difference ($P > 0.05$) was observed between cleaning by wiping followed by bleach for 5 min and no cleaning followed by bleach for 5 min.

Salmonella counts and reductions on bamboo basket surfaces

No interaction between cleaning and sanitizing methods was found for *Salmonella* counts on bamboo basket surfaces ($P = 0.9691$); however, regardless of cleaning method, sanitizing methods had different effects ($P < 0.0001$).

Table 3 presents estimates for log reductions in *Salmonella* counts on bamboo basket surfaces by treatment relative to the negative control (i.e., no sanitizing averaged across cleaning methods; 5.37 log CFU/cm^2; 95% CI: 5.29, 5.45). Compared with the control, a significant reduction in *Salmonella* counts was observed for every sanitizing method regardless of the cleaning method it was combined with. The use of PAA for 5 min resulted in the largest reduction (2.26 log CFU/cm^2; 95% CI: 1.98, 2.54), followed by bleach for 5 min (1.46 log CFU/cm^2; 95% CI: 1.15, 1.76). PAA and bleach applied for 3 min also yielded a reduction in *Salmonella* counts on bamboo basket surfaces, although the reduction was less at 1.36 log CFU/cm^2 (95% CI: 1.08, 1.64) and 1.14 log CFU/cm^2 (95% CI: 0.83, 1.45), respectively.

Attachment of *Salmonella* on bamboo basket and banana leaf surfaces determined by SEM

The attachment of *Salmonella* on banana leaf and bamboo basket surfaces was visualized by SEM. *Fig. 1A* shows a typical surface structure of a banana leaf, with stomata and wax platelets distributed over the leaf surface. Wax platelets were abundant and formed peaks and valleys, thereby creating air pockets and increasing surface roughness. These structures made it difficult to clearly observe the attachment of *Salmonella* cells on banana leaf surfaces *(Fig. 1B)*. Nevertheless, *Salmonella* cells were observed with higher magnification *(Fig. 1C)*.

Fig. 2A shows a typical bamboo basket surface under SEM, revealing the roughness of the surface. *Salmonella* cells attached strongly to the surface and formed lumps on the grooves of bamboo epidermal cells (arrow in *Fig. 2B* and *Fig. 2C*), although no *Salmonella* cells were observed around trichomes or stomata.

DISCUSSION

In the present study, we investigated the attachment of *Salmonella* cells to banana leaf and bamboo basket surfaces and evaluated the efficacy of cleaning and sanitizing methods for reducing *Salmonella* counts on these food-contact surfaces commonly used in Cambodian fresh food markets.

On banana leaf surfaces, findings indicate that sanitizing with PAA reduced *Salmonella* counts to below the detection limit within 3 min of application. Beyond PAA, reduction of *Salmonella* counts on banana leaf surfaces was dependent on the combination of cleaning and sanitizing method used; the most effective combination was wiping plus the use of bleach for 5 min. Although applying bleach for at least 3 min was less effective than the 5-min application, a 3-min application was more effective than use of no sanitizing method, regardless of cleaning method.

Wiping or scrubbing with cloths or a kitchen sponge are common household practices used to physically remove food residues from surfaces. In this study, traditional cleaning methods alone (without sanitizing), such as wiping with a dry paper towel or scrubbing with a dry kitchen sponge, had no significant effect on *Salmonella* counts on banana leaves or bamboo baskets. In contrast, Kusumaningrum et al. *(14)* reported that wiping with a kitchen sponge reduced *Salmonella* Enteritidis from initial levels of 5.8 ± 0.6 log CFU/100 cm^2 to <1.2 log CFU/100 cm^2 on stainless steel, achieving a ca. 4.5-log reduction. Sun-Young et al. *(34)* found that wiping with a dry paper towel reduced *E. coli* O157:H7 cells attached to wood cutting boards to 2.16 ± 1.09 log CFU/100 cm^2, much lower than the control (3.31 ± 0.32 log CFU/100 cm^2). Kim et al. *(13)* observed that Vibrio parahaemolyticus counts were reduced to below the detection limit (1 log CFU per unit area) from 3.58 ± 0.27 log CFU per unit area when the wood cutting boards were wiped with a dry paper towel. Although these studies indicated that cleaning may reduce pathogen presence, we reconcile the apparent conflict with our results by noting that our study used different, previously untested food-contact surfaces. This inconsistency also may be explained by the variation in the amount of pressure applied to dry paper towels or kitchen sponges during cleaning, which was not monitored in our study.

Our results indicate that a combination of cleaning and sanitizing may be necessary to reduce microbial contamination, particularly for banana leaves. In several studies conducted on other food-contact surfaces such as low-density polyethylene, Formica dining tables, and apple packing equipment surfaces, the results were consistent with ours; sanitizing efficacy was enhanced by the cleaning method applied *(17, 24, 25)*. Røssvoll et al. *(24)* found that the efficacy of chlorine-based disinfection was improved to a ca. 1- to 2-log reduction when combined with cleaning by wiping with a water-moist cloth or brushing with running water. Similarly, Masuku et al. *(17)* found that the effect of sanitizing was significantly improved by the use of wipe clothes combined with silver hydrogen citrate compared with wiping with a cloth by itself. In another study of the efficacy of cleaning and sanitizing methods against Listeria innocua on apple packing equipment surfaces, the use of a degreaser or detergent to clean rollers and conveyor belt followed by

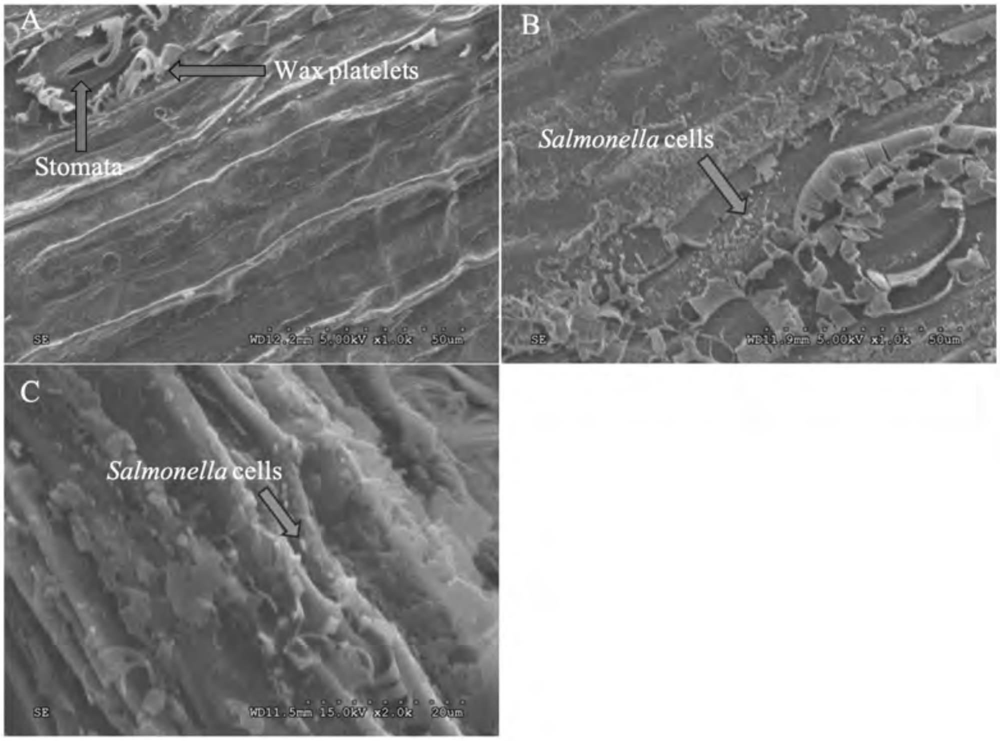

FIGURE 1. Images of banana leaf surfaces under scanning electron microscopy: A, control sample (not inoculated) at magnification of 50 µm; B, inoculated sample at magnification of 50 µm; C, inoculated sample at magnification of 20 µm.

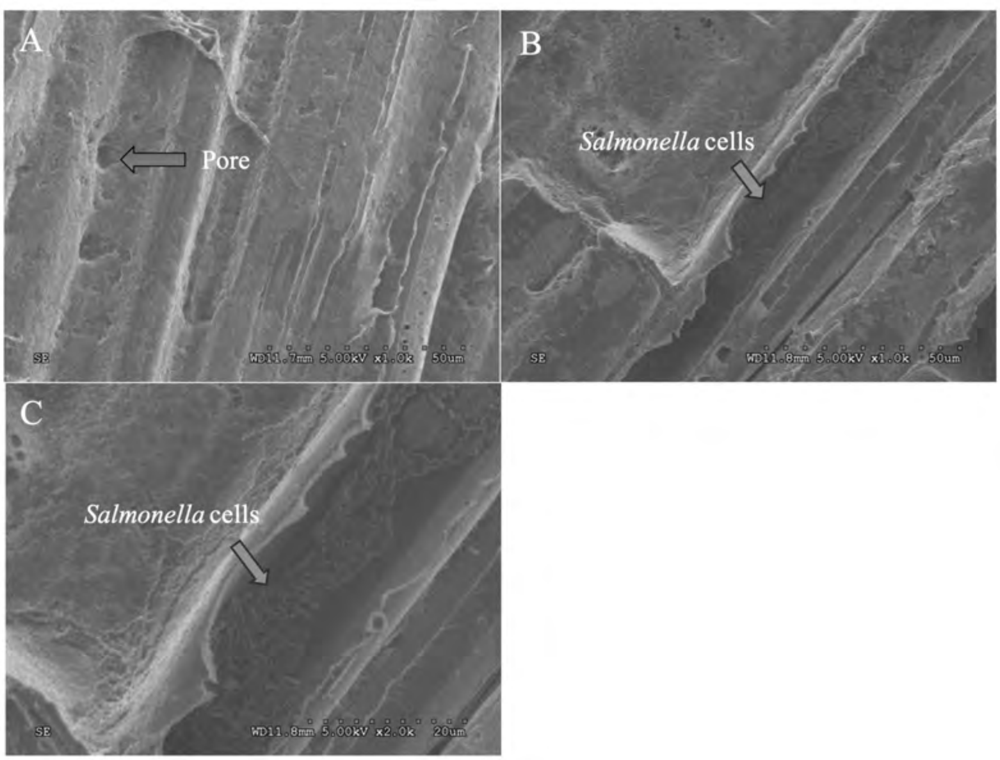

FIGURE 2. Images of bamboo basket surfaces under scanning electron microscopy: A, control sample (not inoculated) at magnification of 50 µm; B, inoculated sample at magnification of 50 µm; C, inoculated sample at 20 µm.

sanitizer application resulted in the greatest reduction in microbial contamination *(25)*. These study results indicate that effective interventions for reducing pathogens on food-contact surfaces require the combination of cleaning and sanitizing methods to ensure maximum pathogen reduction.

In the present study, bleach treatment only partially reduced *Salmonella* populations on banana leaf surfaces. When compared with sanitizing with PAA, the lower efficacy observed for bleach on banana leaves may be due to the complex microstructure of epidermal cells on the leaf surface combined with the presence of wax platelets. The high abundance of wax platelets, as observed by SEM, might have created a large surface area of organic matter likely to react with free chlorine, causing chlorine depletion and therefore reducing the efficacy of the bleach *(9, 35, 37)*. The limited reduction of *Salmonella* observed when using either bleach or PAA sanitizers on bamboo baskets might also be explained by the morphological structure of the basket surface *(Fig. 2)*. The porous architecture of the bamboo basket surface may provide physical protection to *Salmonella* cells, limiting penetration of the sanitizer and reducing its efficacy *(5, 8)*. Chiu et al. *(5)* also observed that *Vibrio* was more persistent on bamboo and wood cutting boards than on plastic cutting boards following treatment with electrolyzed oxidizing water.

Although these results contribute to an understanding of the importance of cleaning and sanitizing methods for common food-contact surfaces in Cambodian fresh food markets, we acknowledge limitations. To our current knowledge, PAA is not readily available in Cambodian fresh food markets for commercial retail use, although it is typically available for purchase by large food manufacturing industries. Bleach is widely available and relatively cheap. Therefore, the use of bleach as a sanitizing method is a strategy for reducing *Salmonella* contamination associated with fresh and perishable food products in fresh food markets in Cambodia.

These results were obtained from an experimental study conducted in a controlled laboratory environment. We anticipate additional challenges to the efficacy of cleaning and sanitizing methods when applied in the context of real-world situations, such as in fresh food markets. Relevant considerations beyond the type of food-contact surface used include but are not limited to the presence of organic soil and concomitant biological and chemical contaminants and the availability of potable water and appropriate concentrations of sanitizer solutions. Access to clean, potable water is still limited in most fresh food markets in Cambodia. Further efforts should be focused on improving access to clean water and sanitizers to ensure the feasibility of implementing cleaning and sanitizing practices in fresh food markets in Cambodia.

CONCLUSIONS

Our findings reveal that proper cleaning and sanitizing practices can significantly reduce the survival of *Salmonella* on banana leaf and bamboo basket surfaces, highlighting the importance of maintaining a hygienic environment in fresh food markets. Overall, sanitizing with PAA was most effective against *Salmonella* on both types of surfaces; sanitizing with bleach was less effective. When using bleach on banana leaf surfaces, the efficacy against *Salmonella* depended upon the cleaning method used and was most effective when bleach applications for at least 5 min were combined with cleaning by wiping. Under some circumstances, longer contact time enhanced sanitizer activity. The ability of foodborne pathogens such as *Salmonella* to adhere to surfaces has important implications for the efficacy of cleaning and sanitizing methods. The complex morphological structure of these natural surfaces might affect the efficacy of cleaning and sanitizing for microbial reduction. This study offers an initial contribution to understanding how to effectively use cleaning and sanitizing methods in Cambodian fresh food markets. Market vendors may use this information to make informed decisions about effective practices for cleaning and sanitizing surfaces used to sell food products in these settings.

ACKNOWLEDGMENT

This research was funded by USAID Feed the Future Innovation on Food Safety.

REFERENCES

1. Abate, D., and N. Assefa. 2021. Prevalence and antimicrobial resistance patterns of *Salmonella* isolates in human stools and animal origin foods in Ethiopia: a systematic review and meta-analysis. *Int. J. Health Sci. (Qassim)* 15:43–55.
2. Anal, A. K., G. Perpetuini, A. Petchkongkaew, R. Tan, S. Avallone, R. Tofalo, H. Van Nguyen, S. Chu-Ky, P. H. Ho, and T. T. Phan. 2020. Food safety risks in traditional fermented food from south-east Asia. *Food Control* 109:106922.
3. Andino, A., and I. Hanning. 2015. *Salmonella enterica*: survival, colonization, and virulence differences among serovars. *Sci. World J.* 2015:520179.
4. Carstens, C. K., J. K. Salazar, and C. Darkoh. 2019. Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. *Front. Microbiol.* 10:2667.
5. Chiu, T.-H., J. Duan, C. Liu, and Y.-C. Su. 2006. Efficacy of electrolysed oxidizing water in inactivating *Vibrio parahaemolyticus* on kitchen cutting boards and food contact surfaces. *Lett. Appl. Microbiol.* 43:666–672.
6. Chua, P. T. C., and G. A. Dykes. 2013. Attachment of foodborne pathogens to banana (*Musa* sp.) leaves. *Food Control* 32:549–551.
7. Desiree, K., C. L. Schwan, V. Ly, L. Hok, N. M. Bello, L. Nwadike, R. K. Phebus, and J. L. Vipham. 2021. Investigating *Salmonella enterica, Escherichia coli*, and coliforms on fresh vegetables sold in informal markets in Cambodia. *J. Food Prot.* 84:843–849.
8. Frank, J. F. 2001. Microbial attachment to food and food contact surfaces. *Adv. Food Nutr. Res.* 43:319–370.
9. Fransisca, L., and H. Feng. 2012. Effect of surface roughness on inactivation of *Escherichia coli* O157:H7 87-23 by new organic acid–surfactant combinations on alfalfa, broccoli, and radish seeds. J. *Food Prot.* 75:261–269.

10. Grace, D. 2015. Food safety in low and middle income countries. *Int. J. Environ. Res. Public Health* 12:10490–10507.

11. Grace, D. 2023. Burden of foodborne disease in low-income and middle-income countries and opportunities for scaling food safety interventions. *Food Sec.* 15:1475–1488.

12. Hulton, G. 2012. Global costs and benefits of drinking-water supply and sanitation interventions to reach the MDG target and universal coverage. World Health Organization, Geneva.

13. Kim, S.-Y., T. Li, J.-Y. Heo, Y.-M. Bae, I.-K. Hwang, S.-Y. Lee, and B. Moon. 2012. Efficacies of cleaning methods for decontaminating *Vibrio parahaemolyticus* on the surfaces of cutting boards cross-contaminated from grated fish fillet. *J. Food Saf.* 32:459–466.

14. Kusumaningrum, H. D., R. Paltinaite, A. J. Koomen, W. C. Hazeleger, F. M. Rombouts, and R. R. Beumer. 2003. Tolerance of *Salmonella* Enteritidis and *Staphylococcus aureus* to surface cleaning and household bleach. *J. Food Prot.* 66:2289–2295.

15. Lay, K. S., Y. Vuthy, P. Song, K. Phol, and J. L. Sarthou. 2011. Prevalence, numbers and antimicrobial susceptibilities of *Salmonella* serovars and *Campylobacter* spp. in retail poultry in Phnom Penh, Cambodia. *J. Vet. Med. Sci.* 73:325–329.

16. Marriott, N. G., M. W. Schilling, and R. B. Gravani. 2018. Principles of food sanitation. Springer, New York.

17. Masuku, S. M., D. Babu, E. M. Martin, O. K. Koo, C. A. O'Bryan, P. G. Crandall, and S. C. Ricke. 2012. Cleaning and decontamination efficacy of wiping cloths and silver dihydrogen citrate on food contact surfaces. *J. Appl. Microbiol.* 113:89–95.

18. Mendez, E., D. K. Walker, J. Vipham, and V. Trinetta. 2020. The use of a CDC biofilm reactor to grow multi-strain *Listeria monocytogenes* biofilm. *Food Microbiol.* 92:103592.

19. Muramatsu, S., M. Uchino, S. Sorm, Y. Tanikoka, D. Oka, Y. Muramatsu, T. Nakajima, M. Sekido, T. Nakamura, C. Chay, and M. Mihara. 2021. Evaluation of bacterial contamination levels in pickles sold at wet market in Cambodia. Part 2. Detection of several food-poisoning bacteria of 48 samples from Phnom Penh. *Int. J. Environ. Rural Dev.* 11:121–126.

20. Nguyen, T. K., H. T. Bui, T. A. Truong, D. N. Lam, S. Ikeuchi, L. K. T. Ly, Y. Hara-Kudo, T. Taniguchi, and H. Hayashidani. 2021. Retail fresh vegetables as a potential source of *Salmonella* infection in the Mekong Delta, Vietnam. *Int. J. Food Microbiol.* 341:109049.

21. Phoeurk, C., S. Tieng, S. Tan, S. Moeung, S. Cheu, P. Chean, V. Hay, C. Say, L. Lim, and L. Kann. 2019. Prevalence and concentration of *Escherichia coli* and *Salmonella* species in fresh vegetables collected from different types of markets in Phnom Penh. Cambodia *J. Basic Appl. Res.* 1:76–96.

22. Roesel, K., and D. Grace. 2014. Food safety and informal markets: animal products in sub-Saharan Africa. Routledge, New York.

23. Rortana, C., H. Nguyen-Viet, S. Tum, F. Unger, S. Boqvist, S. Dang-Xuan, S. Koam, D. Grace, K. Osbjer, and T. Heng. 2021. Prevalence of *Salmonella* spp. and *Staphylococcus aureus* in chicken meat and pork from Cambodian markets. *Pathogens* 10:556.

24. Røssvoll, E., S. Langsrud, S. Bloomfield, B. Moen, E. Heir, and T. Møretrø. 2015. The effects of different hygiene procedures in reducing bacterial contamination in a model domestic kitchen. *J. Appl. Microbiol.* 119:582–593.

25. Ruiz-Llacsahuanga, B., A. M. Hamilton, K. Anderson, and F. Critzer. 2022. Efficacy of cleaning and sanitation methods against *Listeria innocua* on apple packing equipment surfaces. *Food Microbiol.* 107:104061.

26. Salleh, N. A., G. Rusul, Z. Hassan, A. Reezal, S. H. Isa, M. Nishibuchi, and S. Radu. 2003. Incidence of *Salmonella* spp. in raw vegetables in Selangor, Malaysia. *Food Control* 14:475–479.

27. Samad, M. A., L. Eberson, R. Begum, M. G. S. Alam, F. Talukdar, R. Akter, S. Dang-Xuan, G. Sharma, S. Islam, and N. A. Siddiky. 2023. Microbial contamination and antibiotic resistance in marketed food in Bangladesh: current situation and possible improvements. *Antibiotics* 12:555.

28. Sapugahawatte, D. N., C. Li, P. Dharmaratne, C. Zhu, Y. K. Yeoh, J. Yang, N. W. S. Lo, K. T. Wong, and M. Ip. 2022. Prevalence and characteristics of *Streptococcus agalactiae* from freshwater fish and pork in Hong Kong wet markets. *Antibiotics* 11:397.

29. Sary, S., X. Shiwei, Y. Wen, S. Darith, and S. Chorn. 2019. Household food consumption in rural, Cambodia almost ideal demand system analysis. *J. Phys. Conf. Ser.* 1176:042077.

30. Schwan, C. L., T. J. Dallman, P. W. Cook, and J. Vipham. 2022. A case report of *Salmonella enterica* serovar Corvallis from environmental isolates from Cambodia and clinical isolates in the UK. *Access Microbiol.* 4:000315.

31. Schwan, C. L., K. Desiree, N. M. Bello, L. Bastos, L. Hok, R. K. Phebus, S. Gragg, J. Kastner, and J. L. Vipham. 2021. Prevalence of *Salmonella enterica* isolated from food contact and nonfood contact surfaces in Cambodian informal markets. *J. Food Prot.* 84:73–79.

32. Schwan, C. L., A. Molitor, L. Hok, P. Ebner, J. L. Vipham, and V. Trinetta. 2022. Quantitative and qualitative assessments of *Enterobacteriaceae*, coliforms, and generic *Escherichia coli* on fresh vegetables sold in Cambodian fresh produce distribution centers. *Food Prot. Trends* 42:107–112.

33. Sripaurya, B., R. Ngasaman, S. Benjakul, and K. Vongkamjan. 2019. Virulence genes and antibiotic resistance of *Salmonella* recovered from a wet market in Thailand. *J. Food Saf.* 39:e12601.

34. Sun-Young, L., K. Su-Yeon, D. Lee, H. Yu-Jin, B. Young-Min, C. Na-Young, and M. BoKyung. 2011. Comparison of sanitizing methods for reduction of attached microorganisms on various kitchen cutting boards. *J. Korean Soc. Appl. Biol. Chem.* 54:926–932.

35. Wang, H., H. Feng, W. Liang, Y. Luo, and V. Malyarchuk. 2009. Effect of surface roughness on retention and removal of *Escherichia coli* O157:H7 on surfaces of selected fruits. *J. Food Sci.* 74:E8–E15.

36. Yanagida, T., N. Shimizu, and T. Kimura. 2005. Extraction of wax and functional compounds from fresh and dry banana leaves. *Jpn. J. Food Eng.* 6. https://doi.org/10.11301/jsfe2000.6.79

37. Yi, J., J. H. J. Leveau, and N. Nitin. 2022. Role of multiscale leaf surface topography in antimicrobial efficacy of chlorine-based sanitizers. *J. Food Eng.* 332:111118.

This study is made possible by the generous support of the American people through the United States Agency for International Development (USAID). The contents are the responsibility of the authors and do not necessarily reflect the views of USAID or the United States Government. Program activities are funded by USAID under Cooperative Agreement No. 7200AA19LE00003.