frontiers in Sustainable Food Systems

ORIGINAL RESEARCH
published: 25 March 2020
doi: 10.3389/fsufs.2020.00019

Comparing the Efficacy of Two Triple-Wash Procedures With Sodium Hypochlorite, a Lactic–Citric Acid Blend, and a Mix of Peroxyacetic Acid and Hydrogen Peroxide to Inactivate *Salmonella*, *Listeria monocytogenes*, and Surrogate *Enterococcus faecium* on Cucumbers and Tomatoes

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Specialty section:
This article was submitted to
Agro-Food Safety,
a section of the journal
Frontiers in Sustainable Food Systems

Received: 12 December 2019
Accepted: 17 February 2020
Published: 25 March 2020

Citation:
Li K, Chiu Y-C, Jiang W, Jones L,
Etienne X and Shen C (2020)
Comparing the Efficacy of Two
Triple-Wash Procedures With Sodium
Hypochlorite, a Lactic–Citric Acid
Blend, and a Mix of Peroxyacetic Acid
and Hydrogen Peroxide to Inactivate
Salmonella, Listeria monocytogenes,
and Surrogate Enterococcus faecium
on Cucumbers and Tomatoes.
Front. Sustain. Food Syst. 4:19.
doi: 10.3389/fsufs.2020.00019

This study was designed to evaluate two triple-wash procedures with commercial antimicrobials to inactivate foodborne pathogens and surrogate bacteria on cucumbers and tomatoes. Fresh, West Virginia locally grown cucumbers and tomatoes were dip-inoculated with *Salmonella* Typhimurium and Tennessee, *Listeria monocytogenes* (3-strain), and *Enterococcus faecium*. Produce was washed through two triple-wash steps (10 s each) including water dip, antimicrobial dip, and water dip (WAW), or water dip, water dip, and antimicrobial dip (WWA), followed by draining (2 min) on aluminum foil. A triple-water (WWW) process was also included as a water-only control. Tested treatments were (1) water; (2) sodium hypochlorite (SH, 100 ppm, pH 8.2); (3) acidified sodium hypochlorite (ASH, 100 ppm, pH 6.8 adjusted by citric acid); (4) lactic and citric acid blend (LCA, 2.5%); and (5) a H_2O_2-peroxyacetic acid mix [SaniDate-5.0 (SD) 0.0064, 0.25, and 0.50%]. Surviving bacteria were recovered on xylose lysine tergitol-4 (XLT-4, *Salmonella*), Modified Oxford (MOX, *L. monocytogenes*), and bile esculin agar (*E. faecium*). Data (two replicates, four samples/replicate) were analyzed using the mixed model procedure of SAS ($P = 0.05$). Counts of *Salmonella*, *L. monocytogenes*, and *E. faecium* on unwashed cucumbers and tomatoes were 5.42–6.23, 6.31–6.92, and 6.05 log colony-forming units (CFU)/produce, respectively. Triple-wash with water only reduced all three tested bacteria by 0.45–1.36 log CFU/fruit. Triple-wash by WWA with antimicrobials achieved additional reductions [least squares means (LsMeans)] of 0.38 log CFU/cucumber (*Salmonella*), 0.56 log CFU/cucumber (*E. faecium*), 1.48 log CFU/tomato (*Salmonella*), 1.09 log CFU/tomato (*L. monocytogenes*), and 0.71 log CFU/tomato more than the WAW procedure. Applying SD-0.25 and SD-0.50% solutions

in triple-washing cucumbers and tomatoes resulted in reductions ($P > 0.05$) similar to ASH and greater reductions ($P < 0.05$) than SH and LCA. *E. faecium* was less susceptible ($P < 0.05$) or there was no difference ($P > 0.05$) in comparison with *Salmonella* in most cases, except for tomatoes treated with WWA. The results of this study indicate that SD could be used as an alternative antimicrobial agent for chlorine water in triple-wash processing at local small produce plants. Future pilot plant validation studies and cost-effectiveness analyses are needed for applying SD solutions in triple-wash by WV local small produce growers.

Keywords: post-harvest wash, triple-wash, antimicrobials, pathogens, surrogate, cucumbers, tomatoes

INTRODUCTION

Among foodborne pathogens, *Salmonella* is the second most common cause of foodborne illnesses (Dewey-Mattia et al., 2018). A multi-state outbreak of *Salmonella* Poona on sliced cucumber from 2015 to 2016 caused more than 900 cases of infection, and six deaths were recorded according to the Centers for Disease Control and Prevention (CDC) (CDC, 2019). In 2006, an outbreak across 21 states in the United States caused 183 illnesses, of which 22 patients were hospitalized (CDC, 2006). In 2017, Denmark, Finland, Germany, Ireland, and the United Kingdom were affected by an outbreak of *Salmonella* on cucumbers (EFSA and ECDC, 2018). Another popular fresh produce commodity, tomatoes, has been associated with *Salmonella* outbreaks as well. Tomatoes were also inked to a recent *Salmonella* outbreak in Sweden, with 71 identified infections/illnesses (Colombe et al., 2019). In 2015, tomatoes served at Chipotle restaurants in Minnesota were reported to be contaminated with *Salmonella* (Minnesota Department of Health, 2015).

Listeria monocytogenes is another pathogen of concern reported by the United Fresh Produce Association (UFPA) due to the higher fatality rate of listeriosis (United Fresh Produce Association, 2018). In Iran, the prevalence of *L. monocytogenes* in sampled cucumbers was reported to be 18% (Hossein et al., 2013). Depending on the area, the prevalence of *L. monocytogenes* ranged from 3.8% (Li et al., 2017) to 17.5% (Strawn et al., 2013) on cucumbers sampled from a farmers market. Although outbreaks caused by *L. monocytogenes* on tomatoes are uncommon, tomatoes were recognized as a common food crop susceptible to foodborne pathogens (Honjoh et al., 2016).

The consumption of fresh produce (fresh vegetables and fruits) has increased to 345 pounds (per capita availability) in 2017 [(USDAERS, 2019)]. In the United Sates; however, there has been increasing concern regarding the microbial safety of farmers market–sold produce (Scheinberg et al., 2017). Fresh produce accounted for 46% of foodborne illnesses according to a comprehensive analysis by the CDC (Painter et al., 2013). In a recent study in West Virginia and Kentucky farmers markets, 18.6% of spinach, 10.9% of tomatoes, 18.5% of peppers, and 56.3% of cantaloupes tested positive for *Salmonella*, and 3.8% of the produce samples were positive for *Listeria* spp. (Li et al., 2017). Fresh produce may be eaten without further processing; therefore, cross-contamination during transportation, or handling becomes a serious issue to ensure produce safety. As the demand for locally produced foods has increased nationwide, with an estimated $20 billion target by 2019 (USDA, 2015), produce safety becomes a recurring problem, especially in regions where raw consumption of fresh produce is common practice.

In 1998, the United States Food and Drug Administration (FDA) published the "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" as the main guidelines for Good Agricultural Practices (GAP), which identified that antimicrobial chemicals in processing water may reduce the microbial load on the surface of produce (United States Food and Drug Administration, 1998). The West Virginia University (WVU) Extension Service Small Farm Center encourages small produce growers to apply a triple-wash process during their post-harvest processing if their produce is eaten raw or grown on the ground (Strohbehn et al., 2013). Although more evidence suggests that washing is more a cross-contamination preventative than a pathogen reduction step, the triple-wash process (water rinse, water rinse, and final antimicrobial dip) is still recommended for removing pathogens from food surfaces and for improving on-farm food safety with the assumption of clean water being used in each wash step (Strohbehn et al., 2013). The effectiveness of the triple-wash critically depends on the antimicrobial solutions used. Sodium hypochlorite (SH, referred to as chlorinated water) has been well-recognized and used extensively as an effective and low-cost sanitizer during the post-harvest washing process (Shen et al., 2013). The antimicrobial effect of chlorinated water against *Salmonella* was observed when spraying 200 ppm chlorinated water onto tomato surfaces (Bari et al., 2003). However, chlorinated water has obvious disadvantages including being easily degraded by organic matter and generating chlorine byproducts (Shen et al., 2016). Therefore, local produce growers are interested in learning the efficacy of new antimicrobial solutions. For example, Preston County Workshop Inc., a local small produce processor, is currently using SaniDate-5.0 [SD, a mix of peroxyacetic acid (PAA) and H_2O_2] in their triple-wash tanks to control foodborne pathogens on their produce. According to our recent internal survey from local small produce growers at the 2018 West Virginia Small Farm Conference produce safety training workshop, approximately half of the participants (9/20) currently choose water dip–antimicrobial dip–water dip (WAW), and the

other half (10/20) use the water dip–water dip–antimicrobial dip (WWA) procedure. Recently, there is growing recognition that post-harvest washing reduces cross-contamination with no expectation of achieving log count reductions of pathogens on produce (Gombas et al., 2017). Therefore, the concentration level to be used and the antimicrobial efficacy of the triple-wash procedures need to be investigated.

The efficacy of antimicrobial solutions during triple-washing should be tested in real local small produce commercial settings. This is because the dynamics of processing conditions applied by local produce growers could be controlled to a lesser extent than under laboratory conditions. To validate washing procedures, local small produce plants typically have a great aversion to using an actual microbial test pathogen in their processing lines; instead, they usually apply alternative methods such as ensuring a sufficient active sanitizer within the wash tank. The use of a pathogen surrogate is a possible valid approach, and the target surrogate needs to be validated in laboratory conditions first (Hu and Gurtler, 2017). *Enterococcus faecium*, a Gram-positive chain-shaped cocci, has been studied on our WVU poultry farm as a safer alternative for *Salmonella* during steam conditioning, antimicrobial inclusion, and standard/thermal aggressive pelleting during broiler feed manufacturing (Boney et al., 2018; Boltz et al., 2019). *Enterococcus faecium* has also been validated as a surrogate for *Salmonella* in almond pasteurization (Jeong et al., 2011). However, this *Salmonella* surrogate has not been validated on fresh produce, and no publications identified the ideal surrogate for *Salmonella* during the post-harvest produce washing process.

As outbreaks associated with *Salmonella* and *L. monocytogenes* from fresh produce are of concern, a more comprehensive evaluation of washing procedures should be carried out. Therefore, the objectives of this study were to evaluate two triple-wash procedures with three commercial antimicrobials to inactivate *Salmonella, L. monocytogenes*, and the surrogate *E. faecium* on WV locally grown cucumbers and tomatoes.

MATERIALS AND METHODS

Fresh Produce Sample Preparation and Background Microflora Elimination

Fresh cucumbers and tomatoes were purchased from WV Morgantown Farmers Market and stored overnight in a refrigerated cooler. Before each experiment, the population of natural microflora on produce surfaces was determined by adding one cucumber or tomato into 200 ml of buffered peptone water (BPW, Alpha Biosciences, Baltimore, MD, USA) with shaking for 30 s, followed by spread-plating onto tryptic soy agar (TSA, Alpha Biosciences, Baltimore, MD, USA) after 10-fold serial dilution and incubating at 35°C for 48 h. Results indicated that there were ∼5–6 log colony-forming units (CFU)/produce of background microbiota on cucumber and tomato surfaces. It was noticed that the microflora on cucumbers interfered with the results of the *E. faecium* experiment, as the selective medium used for the *E. faecium* experiment was not selective enough to preclude background microbiota. Therefore, cucumber samples were decontaminated before being subjected to *E. faecium* inoculation. To reduce microbiota on cucumbers in the *E. faecium* experiment, a pre-wash procedure was conducted. Fresh cucumbers were first rinsed with tap water and then submerged in a chlorinated solution (40 ml bleach in 2 L water) for a minute, followed by submerging in boiling water for 5 s. This surface disinfection method was verified by showing growth of no colonies on bile esculin agar (BEA) after shaking cucumbers with 200 ml BPW following by spread-plating. The decontaminated cucumber samples were air-dried in a biosafety cabinet before inoculation.

Preparation of Inoculum

Salmonella Typhimurium ATCC 14028, *Salmonella* Tennessee ATCC 10722, *L. monocytogenes* strains L2624 and L2625 (cantaloupe outbreak serotype 1/2b, donated by Dr. Joshua Gurtler, USDA-ARS, Wyndmoor, PA), and surrogate *E. faecium* ATCC 8459 were used in this study. Both *Salmonella* and *L. monocytogenes* strains were used in the previously reported farmers market produce safety projects (Li et al., 2017, 2018), and this strain of *E. faecium* has also been studied in WVU poultry meat projects (Lemonakis et al., 2017; Boltz et al., 2019). *Salmonella*, *L. monocytogenes*, and *E. faecium* retrieved from frozen stock cultures were streak-plated onto xylose lysine tergitol-4 agar (XLT-4, Hardy Diagnostics, Santa Maria, CA, USA), Modified Oxford agar (MOX, Hardy Diagnostics, Santa Maria, CA, USA), and BEA (Hardy Diagnostics, Santa Maria, CA, USA), respectively, and then incubated at 35°C for 48 h to generate single colonies. Before each experiment, a single colony was picked from XLT-4 (*Salmonella*), MOX (*L. monocytogenes*), and BEA (*E. faecium*) of each strain and was enriched in 10 ml tryptic soy broth (TSB; Alpha Biosciences, Baltimore, MD, USA) at 35°C for 24 h. Then, each individual bacterial suspension was centrifuged (5,000 × g) for 15 min (VWR Symphony 4417, VWR International, Radnor, PA). Each suspension was then centrifuged and washed in triplicate in 0.1% BPW followed by re-suspending in 10 ml of 0.1% BPW. The *Salmonella* and *L. monocytogenes* inoculum was made by combining the two strains of *Salmonella* with the two *L. monocytogenes* strains (Li et al., 2017, 2018). After creating the four-strain cocktail, the inoculum was diluted to ca. 6 log CFU/ml by adding the 40 ml cocktail into 3 L of 0.1% BPW solution for the dip inoculation process. The inoculum level of *E. faecium* was adjusted to 6.5 log CFU/ml by adding 40 ml of triplicate-washed strains into 3 L of 0.1% BPW.

Inoculation of Fresh Produce Samples

Tomatoes and cucumbers were inoculated by placing the product in a metal bowl containing 3 L of *Salmonella* or *Listeria* inoculum with gentle stirring for 5 min, followed by placement in a biosafety cabinet for 15 min to allow for pathogen attachment. According to our preliminary studies, *E. faecium* was inoculated onto tomato and cucumber samples by pipetting 1 ml of inoculum onto the samples, fully covering the surface using food-grade plastic wrap for 30 s, and then air-drying in a biosafety cabinet for 15 min.

Triple-Wash Produce With Antimicrobials

Before the triple-washing process, the inoculated produce (cucumbers and tomatoes) was tested for temperature using a scan thermometer (Exergen Corporation, Watertown, MA, USA), and the surface temperatures of both products were 46.76 ± 0.6°F (8.2°C). Inoculated samples were left unwashed (control) or triple-washed in three metal containers with 3 L of solution each. Each treatment contained six samples randomly and evenly split into two groups; each sample contained either one tomato or one cucumber. Two triple-wash procedures were applied to the samples, including WAW or WWA. Each step in the triple-wash procedure was completed by dipping the samples into the solutions with manual rotation for 10 s with agitation at ca. 200 rpm (Li et al., 2017). Treatments tested include: (i) tap water only (pH = 6.9, 15.4°C); (ii) SH [free available chlorine 100 ± 0.6 ppm, pH = 8.2 (SH), or pH = 6.8 (adjusted by 10% citric acid: acidified SH, ASH), 14.4°C; Birko, Henderson, CO, USA]; (iii) a lactic/citric acid blend (LCA, Veggiexide®, 2.5%, pH = 5.1, 15.4°C, Birko); and (iv) a H_2O_2-PAA mix (SD, 15.2°C; Arbico Organics, Tucson, AZ, USA) with concentrations of 0.0064% (pH 6.25), 0.25% (pH 5.52), and 0.50% (pH 3.75). Free chlorine concentration was measured using the N, N diethyl-1,4 phenylenediamine sulfate method with a chlorine photometer (CP-15, HF Scientific, Inc., Ft. Myers, FL). Temperatures of all wash solutions ranged from 57.9 to 59.7°F (14.4–15.4°C), which meets the U.S. FDA advisory recommendation that the wash should be 10°F higher than the produce (46.8°F) being washed (United States Food and Drug Administration, 2018). After triple-wash procedures, samples were drained and dried on aluminum foil for 2 min.

Microbiological Analysis

Each unwashed and washed produce sample was placed into a sterile sample bag (Nasco, Fort Atkinson, WI, USA) and rinsed in 200 ml of BPW, followed by vigorously shaking for 60 s to detach bacteria from the surface. Sample rinse solutions were then 10 or 100-fold serially diluted in 0.1% BPW and spread-plated (0.1 ml onto one plate or 1.0 ml equally divided onto three plates) on XLT-4, MOX, and BEA agar to enumerate *Salmonella*, *L. monocytogenes,* and *E. faecium* cells, respectively. All agar plates were than incubated at 35°C for 24 h (XLT-4) or 48 h (MOX and BEA) and were manually counted for CFU after the incubation period. Three different dilutions were used for spread-plating of each sample including "0" dilution by adding 1 ml of 200 ml BPW diluent equally split onto three agar plates (0.33 ml each). Numbers of colonies from each of the three plates were then combined following incubation; therefore, the detection limit was 2.3 log CFU/fruit (=$\log_{10}$ 200 CFU/fruit). The presumptive positive colonies of *Salmonella* and *L. monocytogenes* were confirmed by using an Oxoid latex agglutination test kit (Oxoid Ltd, Basingstoke, Hampshire, UK).

Data Analysis

The triple-wash studies were duplicated with four cucumbers and four tomatoes per treatment per repetition, with a total of eight samples per treatment for each bacterium. The experimental design was a randomized 2 × 6 factorial design with two triple-wash procedures (WAW or WWA) and six antimicrobial treatments. The survival and reduction of *Salmonella*, *L. monocytogenes*, and *E. faecium* were analyzed using the mixed model procedure of SAS (version 9.2, SAS Institute, Cary, NC), including individual factors of triple-wash procedures, antimicrobial treatments, and their interactions. The comparison of reductions between *Salmonella* and the surrogate *E. faecium* was also analyzed using the mixed model procedure. The reduction data were determined by a reduction ratio of $\log_{10}$ (N_0/N), which includes N_0, the average control plate counts, and N, the plate count of each individual antimicrobial treated sample (Adler et al., 2016). Means were separated by Tukey Honestly Significant Difference (HSD) at an $\alpha = 0.05$ significance level.

RESULTS

Comparison of WAW and WWA Procedures

In general, the least squares mean (LsMean) values across the six tested antimicrobial treatments (water-only wash was not included) indicated that triple-washing with WWA is more effective ($P < 0.05$) than WAW in reducing *Salmonella* (2.39 vs. 2.01 log CFU/cucumber) and *E. faecium* (2.16 vs. 1.60 log CFU/cucumber) on cucumbers, and reducing *Salmonella* (2.82 vs. 1.34 log CFU/tomato), *L. monocytogenes* (2.35 vs. 1.26 log CFU/tomato), and *E. faecium* (2.81 vs. 2.10 log CFU/tomato) on tomatoes. Although there is a statistical difference between the WWA and WAW processes, the differences in log reductions ranged from 0.38 to 1.44 log CFU/fruit, which are still relatively low. Applying WWA or WAW procedures on cucumbers showed no difference in reduction (1.39 vs. 1.35 log CFU/cucumber) for *L. monocytogenes*.

Efficacy of Triple-Wash With Antimicrobials Against *Salmonella*

Survival and reductions of *Salmonella* on cucumbers are shown in **Table 1**. All six antimicrobial treatments were more effective ($P < 0.05$) in reducing *Salmonella* than the water-only wash (1.26–1.36 log CFU/cucumber greater inactivation, **Table 1**). Compared to SH, ASH increased reductions of the pathogen by 0.41 ($P < 0.05$, WAW) and 0.32 log CFU/cucumber ($P = 0.06$, WWA). As expected, antimicrobials applied in the WAW procedure for cucumbers significantly ($P < 0.05$) reduced the *Salmonella* population (survivals of 3.36–4.17 log CFU/cucumber) more than the untreated control (5.80 log CFU/cucumber), with the reductions ranging from 1.63 (SD-0.0064%) to 2.44 (SD-0.50%) log CFU/cucumber (**Table 1**). Compared to WAW, SH, SD-0.0064%, and SD-0.25% applied in the WWA process achieved an additional ($P < 0.05$) reduction of *Salmonella* by 0.40 to 0.50 log CFU/cucumber (**Table 1**). However, there was no significant ($P > 0.05$) difference in reductions between WAW and WWA in ASH, LCA, and SD-0.50% washed samples (**Table 1**). Compared to SH and LCA, adding SD (0.0064, 0.25, 0.50%) into the triple-wash process showed similar or greater ($P < 0.05$) reductions (1.63–2.44 vs. 1.82–2.00 log CFU/cucumber for WAW, 2.09–2.66 vs. 2.14–2.43 log CFU/cucumber for WWA, **Table 1**). Compared to ASH, SD-0.25% and SD-0.50% showed similar ($P > 0.05$) reductions

TABLE 1 | Survival and reduction of *Salmonella* Typhimurium and Tennessee on cucumbers [log colony-forming units (CFU)/cucumber] by triple-wash procedure water dip–antimicrobial dip–water dip (WAW) or water dip–water dip–antimicrobial dip (WWA) in water, sodium hypochlorite (SH, 100 ppm, pH 8.2), acidified SH (ASH; SH, 100 ppm, pH 6.8 adjusted by citric acid), lactic and citric acid blend (LCA, Veggiexide®, 2.5%), and a peroxyacetic acid (PAA) and hydrogen peroxide mix [SaniDate-5.0 (SD), 0.0064, 0.25, and 0.50%].

Treatment	Survival		Reduction	
	WAW	WWA	WAW	WWA
Control	5.80 ± 0.28a	5.42 ± 0.33a	—*	—*
Water	4.54 ± 0.05b	4.06 ± 0.05b	1.26 ± 0.05cA	1.36 ± 0.05cA
SH 100 ppm	3.80 ± 0.44cd	3.00 ± 0.23cd	2.00 ± 0.44bA	2.43 ± 0.23abB
ASH 100 ppm	3.39 ± 0.38e	2.67 ± 0.42d	2.41 ± 0.38aA	2.75 ± 0.42aA
LCA-2.5%	3.98 ± 0.54cd	3.28 ± 0.26c	1.82 ± 0.54bA	2.14 ± 0.26bA
SD-0.0064%	4.17 ± 0.55bc	3.33 ± 0.43c	1.63 ± 0.55bcA	2.09 ± 0.43bB
SD-0.25%	3.62 ± 0.73de	2.76 ± 0.12d	2.18 ± 0.73abA	2.66 ± 0.20aB
SD-0.50%	3.36 ± 0.59e	2.79 ± 0.19d	2.44 ± 0.59aA	2.63 ± 0.19aA

—*Indicates reduction data are not available.
Mean values with different lowercase letters within a column are significantly different ($P < 0.05$).
Mean values with different capital letters within a row are significantly different ($P < 0.05$).

TABLE 2 | Survival and reduction of *Salmonella* Typhimurium and Tennessee on tomatoes (log CFU/tomato) by triple-wash procedure WAW or WWA in water, SH (100 ppm, pH 8.2), ASH (SH, 100 ppm, pH 6.8 adjusted by citric acid), LCA (Veggiexide®, 2.5%), and a PAA and hydrogen peroxide mix (SD, 0.00645, 0.25, and 0.50%).

Treatment	Survival		Reduction	
	WAW	WWA	WAW	WWA
Control	5.70 ± 0.19a	6.23 ± 0.42a	—*	—*
Water	4.96 ± 0.08b	5.45 ± 0.05b	0.74 ± 0.08eA	0.78 ± 0.05cA
SH 100 ppm	4.38 ± 0.31cd	3.09 ± 0.50d	1.32 ± 0.31bcA	3.14 ± 0.50aB
ASH 100 ppm	3.72 ± 0.44d	2.93 ± 0.73d	1.98 ± 0.44aA	3.30 ± 0.73aB
LCA-2.5%	4.80 ± 0.15b	2.95 ± 0.12d	0.90 ± 0.15deA	3.28 ± 0.12aB
SD-0.0064%	4.57 ± 0.25bc	4.51 ± 0.20c	1.13 ± 0.25cdA	1.72 ± 0.20bB
SD-0.25%	3.72 ± 0.39d	2.88 ± 0.28d	1.98 ± 0.39aA	3.35 ± 0.28aB
SD-0.50%	4.07 ± 0.1d	2.97 ± 0.32d	1.63 ± 0.13abA	3.26 ± 0.32aB

—*Indicates reduction data are not available.
Mean values with different lowercase letters within a column are significantly different ($P < 0.05$).
Mean values with different capital letters within a row are significantly different ($P < 0.05$).

ranging from 2.18 to 2.44 log CFU/cucumber (WAW) and 2.43–2.75 log CFU/cucumber (WWA).

As shown in **Table 2**, triple-washing tomatoes in antimicrobials by the WAW process significantly ($P < 0.05$) reduced *Salmonella*, with the survival populations ranging from 3.72 to 4.80 log CFU/tomato compared to 5.70 log CFU/tomato (unwashed control). All six tested antimicrobials were more effective ($P < 0.05$) at reducing the pathogen than the water-only wash, except for LCA. Again, ASH achieved additional ($P < 0.05$) reduction of 0.66 log CFU/tomato compared with the SH washed samples. SD-0.25% and SD-0.50% treated samples showed similar ($P > 0.05$) reductions (1.63–1.98 log CFU/tomato) compared to ASH (1.98 log CFU/tomato), and greater ($P < 0.05$) reductions than SH (1.32 log CFU/tomato) and LCA (0.90 log CFU/tomato). The reduction by SD-0.0064% was similar ($P > 0.05$) to SH and LCA but less ($P < 0.05$) than ASH. Compared to WAW, applying the WWA process increased ($P < 0.05$) the reduction levels from 1.32 to 3.14, 1.98 to 3.30, 0.90 to 3.28, 1.13–1.72, 1.98–3.35, and 1.63–3.26 log CFU/tomato for SH, ASH, LCA, SD-0.0064, SD-0.25, and SD-0.50%, respectively (**Table 2**).

TABLE 3 | Survival and reduction of *Listeria monocytogenes* on cucumbers (log CFU/cucumber) by triple-wash procedure WAW or WWA in water, SH (100 ppm, pH 8.2), ASH (SH, 100 ppm, pH 6.8 adjusted by citric acid), LCA (Veggiexide®, 2.5%), and a PAA and hydrogen peroxide mix (SD, 0.0064, 0.25, and 0.50%).

Treatment	Survival		Reduction	
	WAW	WWA	WAW	WWA
Control	6.31 ± 0.28a	6.92 ± 0.44a	—*	—*
Water	5.72 ± 0.37b	6.20 ± 0.13b	0.59 ± 0.37cA	0.72 ± 0.13cA
SH 100 ppm	4.67 ± 0.46c	5.87 ± 0.49c	1.64 ± 0.46abA	1.05 ± 0.46bcB
ASH 100 ppm	4.44 ± 0.72c	4.52 ± 0.43d	1.87 ± 0.72aA	2.41 ± 0.43aB
LCA-2.5%	5.08 ± 0.40c	6.17 ± 0.43c	1.23 ± 0.40bA	0.75 ± 0.43cB
SD-0.0064%	5.55 ± 0.46b	5.62 ± 0.39c	0.76 ± 0.46A	1.30 ± 0.39bB
SD-0.25%	4.56 ± 0.49c	5.64 ± 0.35c	1.75 ± 0.49aA	1.28 ± 0.35bA
SD-0.50%	4.75 ± 0.49c	5.76 ± 0.40c	1.56 ± 0.49abA	1.16 ± 0.40bA

—*Indicates reduction data are not available.
Mean values with different lowercase letters within a column are significantly different ($P < 0.05$).
Mean values with different capital letters within a row are significantly different ($P < 0.05$).

Efficacy of Triple-Wash With Antimicrobials Against *L. monocytogenes*

Survival and reductions of *L. monocytogenes* on cucumbers and tomatoes are shown in **Tables 3**, **4**, respectively. As shown in **Table 3**, triple-washing cucumbers in the six antimicrobials by the WAW process significantly ($P < 0.05$) reduced *L. monocytogenes*, with the survival populations ranging from 4.44 to 5.55 log CFU/cucumber compared to 6.31 log CFU/cucumber for the control (**Table 3**). For the WAW process, reductions of SH, ASH, LCA, SD-0.25%, and SD-50% were greater than the water control (0.59 log CFU/cucumber) except for SD-0.0064%, showing similar reduction (0.76 log CFU/cucumber). Among the tested antimicrobial treatments, no difference ($P > 0.05$) was found between the reductions (1.56–1.87 log CFU/cucumber) caused by SH, ASH, SD-0.25%, and SD-0.50%, which were greater ($P < 0.05$) than LCA (1.23 log CFU/cucumber) and SD-0.0064% (0.76 log CFU/cucumber) treatments. Applying the WWA process increased ($P < 0.05$) reductions from 1.64 to 2.25, 1.87 to 2.41, and 0.76 to 1.30 log CFU/cucumber for SH, ASH, and SD-0.0064% washed samples, respectively (**Table 3**). However, the WWA process did not increase reductions of the pathogen on cucumbers washed in LCA, SD-0.25%, and SD-0.50% as compared to the WAW process (**Table 3**).

TABLE 4 | Survival and reduction of *L. monocytogenes* on tomatoes (log CFU/tomato) by triple-wash procedure WAW or WWA in water, SH (100 ppm, pH 8.2), ASH (SH, 100 ppm, pH 6.8 adjusted by citric acid), LCA (Veggiexide®, 2.5%), and a PAA and hydrogen peroxide mix (SD, 0.0064, 0.25, and 0.50%).

Treatment	Survival		Reduction	
	WAW	WWA	WAW	WWA
Control	6.39 ± 0.16a	6.41 ± 0.16a	—*	—*
Water	5.96 ± 0.03b	5.99 ± 0.03b	0.43 ± 0.03dA	0.42 ± 0.03cA
SH 100 ppm	5.21 ± 0.30c	3.74 ± 0.39d	1.18 ± 0.30bcA	2.67 ± 0.39aB
ASH 100 ppm	4.42 ± 0.14d	4.03 ± 0.54d	1.97 ± 0.14aA	2.38 ± 0.54aA
LCA-2.5%	5.46 ± 0.15c	4.08 ± 0.20d	0.93 ± 0.15Ac	2.33 ± 0.20aB
SD-0.0064%	5.45 ± 0.97c	4.79 ± 0.16c	0.94 ± 0.97Abc	1.62 ± 0.16bB
SD-0.25%	4.88 ± 0.19d	3.76 ± 0.40d	1.51 ± 0.19abA	2.65 ± 0.40aB
SD-0.50%	4.67 ± 0.06d	3.93 ± 0.41d	1.72 ± 0.06aA	2.48 ± 0.41aB

—*Indicates reduction data are not available.
Mean values with different lowercase letters within a column are significantly different ($P < 0.05$).
Mean values with different capital letters within a row are significantly different ($P < 0.05$).

TABLE 5 | Survival and reduction of *Enterococcus faecium* on cucumbers (log CFU/cucumber) by triple-wash procedure WAW or WWA in water, SH (100 ppm, pH 8.2), ASH (SH, 100 ppm, pH 6.8 adjusted by citric acid), LCA (Veggiexide®, 2.5%), and a PAA and hydrogen peroxide mix (SD, 0.0064, 0.25, and 0.50%).

Treatment	Survival		Reduction	
	WAW	WWA	WAW	WWA
Control	6.05 ± 0.59a	6.05 ± 0.71a	—*	—*
Water	5.49 ± 0.27b	5.49 ± 0.27b	0.56 ± 0.27cA	0.56 ± 0.27eA
SH 100 ppm	5.25 ± 0.39b	5.00 ± 0.26c	0.80 ± 0.39bcA	1.05 ± 0.26dA
ASH 100 ppm	3.97 ± 0.49d	3.66 ± 0.41e	2.08 ± 0.49aA	2.39 ± 0.41bA
LCA-2.5%	5.13 ± 0.19b	4.42 ± 0.41d	0.92 ± 0.19bA	1.63 ± 0.41cB
SD-0.0064%	3.86 ± 0.52d	3.57 ± 0.60e	2.19 ± 0.52aA	2.48 ± 0.60bA
SD-0.25%	4.23 ± 0.30c	3.40 ± 0.57ef	1.82 ± 0.30aA	2.65 ± 0.57abB
SD-0.50%	3.78 ± 0.51d	3.09 ± 0.44f	2.27 ± 0.51aA	2.96 ± 0.44aB

—*Indicates reduction data are not available.
Mean values with different lowercase letters within a column are significantly different ($P < 0.05$).
Mean values with different capital letters within a row are significantly different ($P < 0.05$).

TABLE 6 | Survival and reduction of *E. faecium* on tomatoes (log CFU/tomato) by triple-wash procedure WAW or WWA in water, SH (100 ppm, pH 8.2), ASH (SH, 100 ppm, pH 6.8 adjusted by citric acid), LCA (Veggiexide®, 2.5%), and a PAA and hydrogen peroxide mix (SD, 0.0064, 0.25, and 0.50%).

Treatment	Survival		Reduction	
	WAW	WWA	WAW	WWA
Control	6.05 ± 0.36a	6.05 ± 0.51a	—*	—*
Water	5.60 ± 0.05b	5.60 ± 0.05b	0.45 ± 0.05e	0.45 ± 0.05e
SH 100 ppm	4.17 ± 0.75d	3.27 ± 0.46de	1.88 ± 0.75cA	2.78 ± 0.46cA
ASH 100 ppm	4.68 ± 0.23c	3.55 ± 0.12cd	1.37 ± 0.23dA	2.50 ± 0.12cdB
LCA-2.5%	4.71 ± 0.82c	3.49 ± 0.39cd	1.34 ± 0.82dA	2.56 ± 0.39cdB
SD-0.0064%	4.12 ± 0.47d	3.86 ± 0.62c	1.93 ± 0.47cA	2.19 ± 0.62dA
SD-0.25%	3.57 ± 0.50e	3.08 ± 0.41e	2.48 ± 0.50bA	2.97 ± 0.41bB
SD-0.50%	3.15 ± 0.67f	2.52 ± 0.44f	2.90 ± 0.67aA	3.53 ± 0.20aB

—*Indicates reduction data are not available.
Mean values with different lowercase letters within a column are significantly different ($P < 0.05$).
Mean values with different capital letters within a row are significantly different ($P < 0.05$).

The efficacy of antimicrobials in inactivating *L. monocytogenes* on tomatoes has not been widely studied. As shown in **Table 4**, significantly lower survival (4.42–5.46 log CFU/tomato) was observed on tomatoes washed in the six antimicrobials using the WAW process compared with the untreated control (6.39 log CFU/g) and water wash control (5.96 log CFU/g). SD-0.25% and SD-0.50% reduced the pathogen counts by 1.51–1.72 log CFU/tomato, which were slightly ($P > 0.05$) lower than ASH (1.97 log CFU/tomato) and greater ($P < 0.05$) than the reductions of SH (0.93 log CFU/tomato), LCA (1.18 log CFU/tomato), and SD-0.0064% (0.94 log CFU/tomato, **Table 4**). Compared to the WAW process, applying WWA procedures ($P < 0.05$) increased reductions of all six tested antimicrobial treatments by 0.49–1.40 log CFU/tomato (**Table 4**).

Comparison of *Salmonella* vs. Surrogate *E. faecium*

The response of surrogate *E. faecium* to antimicrobials on cucumbers and tomatoes is shown in **Tables 5**, **6**, respectively. Triple-wash with only water reduced ($P < 0.05$) the surrogate by 0.56 log CFU/cucumber and 0.45 log CFU/tomato compared to the unwashed control. ASH showed greater ($P < 0.05$) and similar ($P \geq 0.05$) reductions compared to SH for cucumbers and tomatoes, respectively. For the WAW process, reductions obtained after washing in SH, ASH, LCA, SD-0.0064, SD-0.25, and SD-0.50% ranged from 0.80 to 2.27 log CFU/cucumber (**Table 5**) and from 1.34 to 2.90 log CFU/tomato (**Table 6**). Like *Salmonella*, the application of the WWA process with the six tested antimicrobials resulted in additional ($P < 0.05$) reductions of 0.25–0.83 log CFU/cucumber and 0.49–1.22 log CFU/tomato, respectively, compared to the WAW process. For cucumbers, the application of SD-0.0064, SD-0.25, and SD-0.50% solutions showed similar ($P > 0.05$) reductions (1.82–2.27 log CFU/cucumber for WAW, 2.48–2.96 log CFU/cucumber for WWA) compared to ASH (2.08 log CFU/cucumber for WAW, 2.39 log CFU/cucumber for WWA), but better ($P < 0.05$) reductions than SH and LCA treatments in both the WAW (0.80–0.92 log CFU/cucumber) and WWA (1.05–1.63 log CFU/cucumber) processes (**Table 5**). For tomatoes, SD-0.25% and SD-0.50% created greater ($P < 0.05$) reductions (2.48–2.90 log CFU/tomato) than SH (1.88 log CFU/tomato), ASH (1.37 log CFU/tomato), and LCA (1.34 log CFU/tomato) treated samples using the WAW process. Applying the WWA process, SD-0.50% was the most effective ($P < 0.05$) in reducing the pathogen surrogate from tomatoes (3.53 log CFU/tomato, **Table 6**), followed by SD-0.25%, SH, LCA, and ASH, which showed similar reductions of 2.50–2.97 log CFU/tomato.

In this study, the mixed model procedure was applied to compare the reductions of *Salmonella* vs. the surrogate *E. faecium*, as shown in **Table 7** (cucumbers) and 8 (tomatoes). For

TABLE 7 | A comparison of the reduction of *Salmonella* and surrogate *E. faecium* on cucumbers (log CFU/cucumber) by triple-wash procedure WAW) or WWA in water, SH (100 ppm, pH 8.2), ASH (SH, 100 ppm, pH 6.8 adjusted by citric acid), LCA (Veggiexide®, 2.5%), and a PAA and hydrogen peroxide mix (SD, 0.0064, 0.25, and 0.50%).

Treatment	WAW		WWA	
	Salmonella	*E. faecium*	*Salmonella*	*E. faecium*
Water	1.26 ± 0.05A	0.56 ± 0.27B	1.36 ± 0.05A	0.56 ± 0.27B
SH 100 ppm	2.00 ± 0.44A	0.80 ± 0.39B	2.43 ± 0.23A	1.05 ± 0.26B
ASH 100 ppm	2.41 ± 0.38A	2.08 ± 0.49B	2.75 ± 0.42A	2.39 ± 0.41A
LCA-2.5%	1.82 ± 0.54A	0.92 ± 0.19B	2.14 ± 0.26A	1.63 ± 0.41B
SD-0.0064%	1.63 ± 0.55A	2.19 ± 0.52B	2.09 ± 0.43A	2.48 ± 0.60A
SD-0.25%	2.18 ± 0.73A	1.82 ± 0.30A	2.66 ± 0.20A	2.65 ± 0.57A
SD-0.50%	2.44 ± 0.59A	2.27 ± 0.51A	2.63 ± 0.19A	2.96 ± 0.44A

Mean values with different capital letters within a row under the WAW or WWA column are significantly different ($P < 0.05$).

TABLE 8 | A comparison of the reduction of *Salmonella* and surrogate *E. faecium* on tomatoes (log CFU/tomato) by triple-wash procedure WAW or WWA in water, SH (100 ppm, pH 8.2), ASH (SH, 100 ppm, pH 6.8 adjusted by citric acid), LCA (Veggiexide®, 2.5%), and a PAA and hydrogen peroxide mix (SD, 0.0064, 0.25, and 0.50%).

Treatment	WAW		WWA	
	Salmonella	*E. faecium*	*Salmonella*	*E. faecium*
Water	0.74 ± 0.08A	0.45 ± 0.05B	0.78 ± 0.05A	0.45 ± 0.05B
SH 100 ppm	1.32 ± 0.31A	1.88 ± 0.75B	3.14 ± 0.50A	2.78 ± 0.46B
ASH 100 ppm	1.98 ± 0.44A	1.37 ± 0.23B	3.30 ± 0.73A	2.50 ± 0.12B
LCA-2.5%	0.90 ± 0.15A	1.34 ± 0.82A	3.28 ± 0.12A	2.56 ± 0.39B
SD-0.0064%	1.13 ± 0.25A	1.93 ± 0.47B	1.72 ± 0.20A	2.19 ± 0.62A
SD-0.25%	1.98 ± 0.39A	2.48 ± 0.50A	3.35 ± 0.28A	2.97 ± 0.41A
SD-0.50%	1.63 ± 0.13A	2.90 ± 0.67B	3.26 ± 0.32A	3.53 ± 0.20A

Mean values with different capital letters within a row under the WAW or WWA column are significantly different ($P < 0.05$).

cucumbers, the reductions of *E. faecium* after the application of the WAW or WWA process with the six tested antimicrobial treatments were less (LsMeans 1.60 vs. 2.01 log CFU/cucumber for WAW, $P < 0.05$) or not different (LsMeans 2.16 vs. 2.39 log CFU/cucumber for WWA, $P > 0.05$) compared to *Salmonella*. Specifically, application of SH, ASH, and LCA indicated lower ($P < 0.05$) reductions, which ranged from 0.80 to 2.08 log CFU/cucumber (WAW) and 1.05–2.39 log CFU/cucumber (WWA) for *E. faecium* compared to *Salmonella* reduced by WAW (1.82–2.41 log CFU/cucumber) and WWA (2.14–2.75 log CFU/cucumber) (**Table 7**). SD-0.25 and SD-50% reduced *E. faecium* by 1.82–2.27 log CFU/cucumber (WAW) and 2.65–2.96 log CFU/cucumber (WWA), which were similar ($P > 0.05$) to the reductions of *Salmonella* (**Table 7**).

For tomatoes, the reduction of *E. faecium* from WAW with antimicrobials was greater ($P < 0.05$) than the reduction of *Salmonella* (LsMeans 2.10 vs. 1.39 log CFU/tomato) but similar ($P > 0.05$) to the reduction of *Salmonella* in WWA processed samples (LsMeans 2.80 vs. 2.81 log CFU/tomato). Specifically, *E. faecium* on tomatoes washed through WWA with SD-0.0064, SD-0.25, and SD-0.50% showed no significant difference ($P > 0.05$) compared to *Salmonella*, with reductions of 2.19–3.53 log CFU/tomato (*E. faecium*) compared to reductions of 1.72–3.35 log CFU/tomato (*Salmonella*) (**Table 8**).

DISCUSSION

Results from this study suggest that applying the WWA process during triple-wash is a better approach than WAW for reducing foodborne pathogens. This conclusion could possibly be explained by the fact that the residual sanitizers after the WWA process further inactivate pathogens on produce samples, since a neutralization step was absent from this study. The WWW control showed reductions of 0.5–1.2 log CFU/fruit across all tested pathogens on cucumbers and tomatoes in this study. These results are similar to those of Wang and Ryser (2014), who reported a 1.0 log CFU/g reduction of *Salmonella* from plain water wash for 15 s in a pilot-scale processing line.

As the physiochemical properties of cucumber and tomato surfaces are greatly different, it is plausible that the same sanitizer may not result in the same level of antimicrobial effect. Our results suggest that it is critical to consider the types of fresh produce when choosing a sanitizer to reduce foodborne pathogens, as the antimicrobial effect of one sanitizer may vary. Furthermore, the amount of organic load created by dust, soil, and debris from produce surfaces could impact the washing process.

Chlorine is a common sanitizer used in fresh produce processing due to the economic feasibility and the strong antimicrobial effect preventing cross-contamination (Shen et al., 2013). When chlorinated water is used as a sanitizer for fresh produce, the maximum concentration regulated by the U.S. FDA is 200 ppm. However, even 100 ppm chlorinated water demonstrates strong antimicrobial activity. An earlier study reported that tomatoes dipped in 100 ppm chlorinated water for 30 s showed significant reduction of *Salmonella*, and the level of reduction was not different between 30 s, 1, and 2 min treatments (Wei et al., 1995). A recent study by Sreedharan et al. (2017) showed that 100 ppm of free chlorine reduced *Salmonella* by > 4.5 log CFU/tomato in a model flume water for 30 s. It suggested that longer treatment time with chlorinated water was not necessary for fresh produce against *Salmonella*, which benefits actual produce processing plants. When immersing tomatoes into 200 ppm chlorinated water, its antimicrobial activity against *Salmonella* was significantly higher than 1 or 2 ppm ozonated water under different levels of turbidity of the water (Chaidez et al., 2007). Currently, there are no small produce growers (we contacted eight local growers in WV) that acidify the chlorine wash water before triple-washing their produce in WV (personal communication with Dr. Tom McConnell, Program Leader of the WV Small Farm Center). However, during the large-industry-scale produce washing process, adjusting the pH of chlorine solutions to 6.8 with citric acid is often conducted to

ensure that the protonated form of hypochlorite predominates in the wash solution (Luo et al., 2012). Therefore, chlorine solutions with near-neutral pH at 6.8 adjusted by citric acid were included as a test treatment in this study. The results of this study suggest that the antimicrobial efficacy of chlorinated water in the concentration of 100 ppm was significant against *Salmonella*, and the reductions were improved when the pH of SH was adjusted to 6.8 in most cases, except for *E. faecium* on tomatoes. A previous study by Wang and Ryser (2014) also reported that chlorine plus citric acid (pH 6.0) yielded a greater reduction (3.1 log CFU/g) of *Salmonella* on tomatoes than chlorine at alkaline status (2.1 log CFU/g). This is because hypochlorous acid, the most effective antimicrobial component, predominates in chlorine water at near-neutral pH, whereas the hypochlorite would be in the ionic form rather than the antimicrobial protonated state in an alkaline-pH solution (White, 2010). The antimicrobial effect of 100 ppm SH or ASH can also be maximized when the WWA triple-wash procedure is used on cucumbers and tomatoes, as the reduction of *Salmonella* on cucumbers and tomatoes was significantly higher in the WWA than the WAW process.

The antimicrobial activity of chlorinated water is not limited to *Salmonella. L. monocytogenes*., another common food pathogen, was also sensitive to chlorinated water treatment on the surface of fresh produce. On cucumbers, 1 or 2 min washing with 200 ppm chlorinated water demonstrated the same level of *Salmonella* reduction (Yuk et al., 2006). Spraying 200 ppm chlorinated water on tomatoes significantly reduced *L. monocytogenes* on tomatoes (Beuchat et al., 1998; Bari et al., 2003). Surprisingly, although a *L. monocytogenes* outbreak associated with cucumber has been reported (Meldrum et al., 2009; Ponniah et al., 2012; Hossein et al., 2013), the antimicrobial effect of chlorine water against *L. monocytogenes* is relatively lacking in the current literature. Considering the accessibility of chlorinated water, chlorinated water without, and with neutralizing pH was included in this study to better contribute to the current database of antimicrobial effects against *L. monocytogenes* on cucumbers (**Table 3**). Our results suggest that 100 ppm chlorinated water (SH) is an effective antimicrobial against *L. monocytogenes* on cucumbers, and neutralizing pH to 6.8 by citric acid and the WWA procedure maximize the antimicrobial effect of chlorinated water, as significantly higher reduction was observed. On tomatoes, although 100 ppm SH is still an effective sanitizer (**Table 4**), the antimicrobial efficacy of SH against *L. monocytogenes* was significantly increased when the pH was adjusted to 6.8 (ASH), which was similar to SD-0.25% and SD-0.50% and >SD-0.0064% and LCA.

Recently, there is growing interest for produce processors to apply antimicrobial chemicals rather than chlorine during produce washing, as chlorine water easily reacts with water constituents and generates chlorine byproducts after repeated replenishing with new chlorine solutions (López-Gálvez et al., 2012; Shen et al., 2016). Local small produce growers in WV are also losing interest in chlorine use due to the increased marketability of natural and organic fresh produce (personal communication with Dr. Tom McConnell, Program Leader of the WV Small Farm Center). LCA, a buffered mixture of lactic and citric acid solution, was introduced by the food chemical industry about a decade ago and was reported to be effective in reducing *Salmonella* on poultry carcasses, avoiding the discoloration of chicken meat caused by lactic acid solutions (Laury et al., 2009). The only study of LCA on produce demonstrated that spraying 2.5% LCA onto jalapeno peppers through a commercial cabinet reduced the natural flora, *Salmonella*, and the surrogate generic *Escherichia coli* by 1.3, 1.1, and 0.8 log CFU/g, respectively (Adler et al., 2016). The mechanism of LCA to inhibit bacterial survival is the combination effect of lactic and citric acids. Lactic acid decreases the ionic concentration within the bacterial cell membrane of the exterior cell wall, and citric acid can diffuse through the cell membrane, being a weak non-dissociated acid. The combination of both acids leads to an accumulation of the acid within the cell cytoplasm, acidification of the cytoplasm, disruption of the proton motive force, and inhibition of substrate transport (Vasseur et al., 1999). Results showed that similar reductions (<0.5 log CFU/g) were achieved by LCA compared to SH against *Salmonella*, *L. monocytogenes*, and the surrogate *E. faecium* for most tests in the current study. However, LCA was less effective than ASH for inactivating *Salmonella* on tomatoes and *E. faecium* on cucumbers.

SD is a mixed antimicrobial solution composed of 23% H_2O_2, 5.3% PAA, and 70% unknown ingredients, which has been recommended by the WV Small Farm Center to wash fresh produce processed from local small farms (Li et al., 2017), since the major wholesale buyer in WV Appalachian Harvest requires the use of SD as part of the post-harvest protocol for growers selling to their business, especially for the organic farming process (personal communication with Dr. Tom McConnell, Program Leader of the WV Small Farm Center). Like other oxidizing chemicals, SD oxidizes bacterial cells, denatures protein, and further disrupts the cell wall structure to kill or inhibit bacteria (Block, 2011). A previous study by Briñez et al. (2006) reported that a mix of H_2O_2 and PAA reduced nonpathogenic strains of *Staphylococcus*, *Listeria* spp., and *E. coli* by more than 5 log CFU/ml after 10 min contact even with organic matter present in solutions (Briñez et al., 2006). The results of the present study suggested similar ($P > 0.05$) reductions of *Salmonella*, *L. monocytogenes*, and *E. faecium* on cucumbers and tomatoes achieved by SD-0.25 and SD-50% when compared to ASH, which were greater ($P < 0.05$) than the reductions with SH and LCA solutions. The market price of a 5-gallon pallet of SD is $330 compared to $12 for SH and $108.5 for LCA. Therefore, agricultural economic cost-effectiveness analysis is needed to verify that SD is economically feasible for local small produce growers as an alternative antimicrobial solution to chlorine water.

Enterococcus faecium has been previously studied and validated as a potential *Salmonella* surrogate in almonds (Jeong et al., 2011), a balanced carbohydrate–protein meal (Bianchini et al., 2014), and pet foods (Ceylan and Bautista, 2015) during thermal activation processing. Our recent study also confirmed that *E. faecium* could be a non-pathogenic surrogate of *Salmonella* for in-plant antimicrobial validation studies on broiler carcasses (Lemonakis et al., 2017). To evaluate the suitability of choosing surrogate microorganisms

for foodborne pathogens when exposed to antimicrobials, the surrogate should behave equally well or better (resistant to interventions) compared to the target pathogen in challenge studies (Adler et al., 2016). Therefore, side-by-side comparisons of reduction levels of *Salmonella* and *E. faecium* after triple-washing through WAW or WWA with antimicrobials on cucumbers and tomatoes are presented in **Tables 7**, **8**. Results indicated that the *E. faecium* strain used in this study could potentially be a surrogate of *Salmonella* for validating triple-wash with commercial antimicrobials on cucumbers in local small produce processing settings; however, more studies are still needed to confirm its use on tomatoes as a *Salmonella* surrogate since opposite results were generated from the WAW compared with the WWA process. Other non-pathogenic bacteria such as generic *E. coli* (ATCC BAA-1427, ATCC BAA-1428, ATCC BAA-1429, ATCC BAA-1430, and ATCC BAA-1431) could be surrogates for *Salmonella* on different produce commodities including tomatoes (Adler et al., 2016). This may be surmised from our previous pilot plant trial, which showed that spraying 50 ppm SH or 1.0% LCA reduced the generic *E. coli* on jalapeno peppers by 0.8–1.0 log CFU/g, which was not different from the reductions of *Salmonella* (0.5–1.1 log CFU/g) (Adler et al., 2016).

CONCLUSIONS

Under the conditions of this study, the triple-wash WWA procedure was better than WAW at inactivating *Salmonella*, *L. monocytogenes*, and *E. faecium* on cucumbers and tomatoes. SD at concentrations of 0.25 and 0.50% was similar or better in antimicrobial efficacy compared to chlorine water without or with pH adjustment. *Enterococcus faecium* could be a potential *Salmonella* surrogate used for validation studies of antimicrobial treatments during post-harvest produce washing. The results from this study provide important information for local small produce growers who are interested in adopting the triple-wash procedure during post-harvest processing. Future studies are needed to validate the same procedure in commercial pilot plant settings, and cost-effectiveness analyses are necessary to evaluate whether SD is economically feasible for local small produce processors.

LIMITATIONS OF THIS STUDY

The authors recognize the following limitations of this study. First, this extension validation study is valuable for local, very small produce growers in WV, which do not represent large commercial-scale industry produce processing. Second, the extent of cross-contamination in the different wash regimes of the triple-wash process was not reported in this study. It is well-established that preventing cross-contamination is more critical than reduction of pathogens during the produce washing process (Gombas et al., 2017). A cross-contamination study of triple-wash in three washing tanks with or without antimicrobials should be included in future studies. Third, the cucumbers tested for *E. faecium* were pre-treated to remove background microbiota, which may not well represent the cucumbers' surface characteristics. An antibiotic marker should be introduced into *E. faecium* to solve this issue in our future related studies. Fourth, the absence of a neutralization step from the WWA process may promote further pathogen reduction by the residual sanitizer on produce samples.

DATA AVAILABILITY STATEMENT

The raw data of the whole study are recorded by hand in our lab notebooks and stored electronically in multiple electronic devices, which will be available from the corresponding author to any researchers who are interested in our results.

AUTHOR CONTRIBUTIONS

LJ created the idea of this study. KL, Y-CC, and WJ designed and conducted the experiments. KL and CS performed statistical analysis. KL, Y-CC, and CS drafted this manuscript. XE revised this manuscript.

FUNDING

This research was funded by the United States Department of Agriculture, the National Institute of Food and Agriculture, and the Agriculture and Food Research Initiative (AFRI) Critical Agricultural Research and Extension (CARE) (Grant # 2019-68008-29828).

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.