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Peroxyacetic Acid and Chlorine Reduce *Escherichia coli* in Agricultural Surface Water for Potential Produce Postharvest Uses

ABSTRACT

An increase in foodborne illnesses associated with fruits and vegetables has been observed in recent years, with several outbreaks linked to contaminated agricultural water. The effectiveness of peroxyacetic acid (PAA) and chlorine (Cl) at reducing *Escherichia coli* in rain barrel and creek water was evaluated in this study. Rain barrel and creek water (12 and 32°C overnight) were inoculated with ~5 log CFU/ml of an *E. coli* cocktail, reequilibrated to 12 and 32°C (30 min), and treated with 25 ± 2 ppm of Cl, 75 ± 5 ppm of PPA, or a water control (W). Samples were collected 0, 5, 10, 60, 1,440, and 2,880 min after treatment, neutralized in Dey–Engley broth, and enumerated using the Food and Drug Administration-approved IDEXX Colilert method, as well as *E. coli*/coliform (EC) Petrifilm, and enriched for the presence or absence of *E. coli*. *E. coli* was not detected in 12 and 32°C creek and rain barrel water 0 min after treatment with PAA and 60 min after treatment with Cl using Colilert. *E. coli* was not detected in samples treated with PAA or Cl at any time point using EC Petrifilm. These data allow growers and extension educators to explore the use of these treatments in surface water sources for postharvest uses in produce.

INTRODUCTION

An estimated one in six Americans becomes ill with a foodborne illness each year, which leads to 120,000 hospitalizations and 3,000 deaths *(8)*. Produce and nuts are linked to approximately half of these illnesses *(31)*. To proactively reduce or prevent the burden caused by foodborne outbreaks associated with fresh produce, the Food and Drug Administration (FDA) Food Safety Modernization Act (FSMA) includes the Produce Safety Rule (PSR), which establishes mandatory science-based minimum standards for the safe growing, harvesting, packing, and holding of fruits

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and vegetables grown for human consumption *(15)*. The use of untreated surface water during harvest or postharvest can lead to microbial attachment and internalization on or in the produce, as well as cross-contamination of other produce that weren't initially contaminated; therefore, the PSR prohibits the use of untreated surface water during harvest and postharvest activities *(25)*. The FSMA PSR requires water that is used in postharvest handling of produce to have no detectable generic *E. coli* per 100 ml of water *(15)*. Therefore, the FSMA PSR requires surface water both to be treated and to have no detectable generic *E. coli* per 100 ml of water before use in produce postharvest handling.

Surface water sources, such as rivers, harvested rainwater, ponds, and creeks, are used by growers because they are generally readily available. However, surface water is open to the environment, which makes it susceptible to chemical and microbial contamination from various point and nonpoint sources. Other factors, such as changing rainfall and temperature patterns, have been associated with an increasing number of fecal pathogens, such as *Salmonella*, in surface water sources *(19)*, as well as the changing physicochemical characteristics of surface water *(10)*. Rain-harvested water provides an alternative source of water for growers to use, but rain catchment methods might also collect fecal matter deposited on the roof from which the rain water is collected, making rain catchment water susceptible to microbial contamination *(18, 23)*.

Chlorine (Cl) and peroxyacetic acid (PAA) are two FDA- and Environmental Protection Agency (EPA)-approved chemical sanitizers frequently used by growers and the agriculture industry for water treatment *(1, 27, 33)*. The efficacy of these chemical interventions is affected by the physicochemical characteristics of water (e.g., organic content), pathogen target, treatment concentration, and contact time *(17, 36)*. An increase in organic matter in the water reduces the free available concentration of the antimicrobial interventions, which would otherwise be available to reduce microbial contamination in rinse water *(6)*. Biofilm formation, strong attachment of bacteria on the produce surface, and internalization of microorganisms by produce also reduce the effectiveness of chemical treatments *(16)*. Because fresh produce is mostly consumed raw, a thermal kill step is lacking. The interventions used by the produce industry postharvest are generally inefficient at reducing pathogens on contaminated produce. Thus, it is crucial to use water with no detectable generic *E. coli* per 100 ml of water during harvest and postharvest handling of produce to avoid potential microbial contact and cross-contamination *(16)*. Overall, chemical treatments, such as Cl and PAA, are more commonly used for killing microorganisms in rinse water and thus preventing microbial contact and cross-contamination during the washing of produce *(5)*. Therefore, this study focused on demonstrating the efficacy of Cl and PAA at treating surface water to generate microbially safe water that can be used postharvest in the produce industry.

Haley et al. *(20)* reported that *E. coli* concentrations were statistically greater in surface water sources in comparison to groundwater sources in Kansas and Missouri, emphasizing the importance of effective treatment of surface water if it is to be safely (<1 generic *E. coli* MPN/100 ml) and effectively used for postharvest purposes. Furthermore, as previously stated, untreated surface water cannot legally be used as agricultural water during or after harvest for farms covered by the FSMA PSR *(15)*. Therefore, the objective of this study was to evaluate the effectiveness of Cl and PAA at reducing *E. coli* in a rain barrel and creek water to satisfy the "no detectable generic *E. coli*" requirement outlined by the FSMA PSR for water to be safely used for postharvest rinsing of produce *(15)*. A previous study conducted by our team evaluated the efficacy of these treatments in simulated (lab-prepared) water using the Petrifilm (3M, Saint Paul, MN) enumeration method, and the data suggested that Cl and PAA may be effective at treating surface water for use postharvest *(22)*. The current study expands upon our previous work by evaluating Cl and PAA efficacy in natural water sources using not only the Petrifilm enumeration method but also the Colilert (IDEXX Laboratories, Westbrook, ME) method (FDA-approved microbial water testing method). This study serves as a validation of the two treatments for potential use in agricultural surface water to be used postharvest.

MATERIALS AND METHODS

The methods used in this study are based largely on the FDA/EPA protocol that describes methods for evaluating antimicrobials as interventions for preharvest agricultural water used in the produce industry *(34)*. One major difference is that this study used natural water sources (creek and rain barrel), whereas the FDA/EPA protocol outlines the use of artificially prepared agricultural water. Our research team recently published on the efficacy of Cl and PAA at reducing *E. coli* in simulated agricultural water *(22)*, and the present study is an extension of that work by using natural water sources with the addition of Colilert enumeration. Other notable differences between this study and the FDA/EPA protocol include the use of generic *E. coli* and direct plating using Petrifilm. Fig. 1 summarizes the experimental design followed for this study.

Bacterial strains

Three strains (ATCC 8739, ATCC 13706, and ATCC 23631) of nonpathogenic *E. coli* from the American Type Culture Collection (ATCC) that are recommended for water testing were used in this study (2–4). Before each replication, a 10-µl loop of stock culture from each frozen strain was streaked for isolation on nutrient agar (NA) plates (Difco, Sparks, MD) and incubated at 37°C for 24 ± 2 h. A single

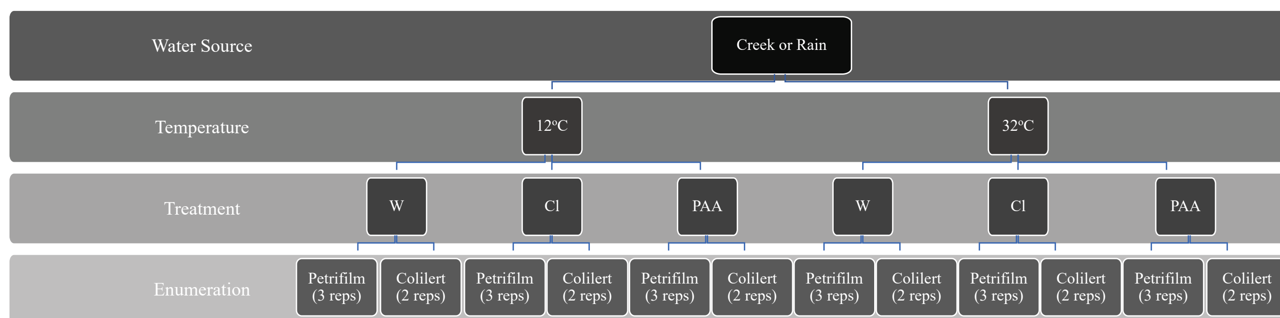

FIGURE 1. Overview of experimental design, including water source, temperature, treatment, enumeration, and number of replications completed.

isolated *E. coli* colony from the NA plates was then used to make the working inoculum, as described in detail later. The appearance of each strain as pure culture was documented before the beginning of the study by plating on *E. coli*/coliform (EC) Petrifilm, where the colonies grew blue to purplish with gas bubbles.

Inoculum preparation

An isolated colony of each ATCC strain was added to a separate 10-ml brain heart infusion (BHI) broth (Thermo Scientific Oxoid, Hants, UK) tube. The three tubes were incubated at 37°C for 24 ± 2 h. A pellet of pure 10^8–10^9 CFU/ml *E. coli* was then separated from the broth by separately centrifuging the tubes at 4,300 × g for 15 min at 4°C. The supernatant broth was discarded and 10 ml of phosphate-buffered dilution water (PBDW; EMD Millipore, Billerica, MA) was used to rehydrate each pellet. Preliminary studies confirmed that these methods result in consistent concentrations for each strain. The concentration of each strain was enumerated, which resulted in 9 ml remaining for each strain following enumeration. The remaining equal volumes (9 ml each) of the three strains were then mixed to form a cocktail solution that was used as the working inoculum (~27 ml total). The working inoculum was enumerated at the beginning and the end of the trial to ensure that the *E. coli* population was consistent throughout the inoculation trial. To enumerate the concentrations of the strains individually and the used cocktail, subsequent dilutions were made in PBDW and plated in duplicate on EC Petrifilms; the EC Petrifilms were incubated at 37°C for 48 ± 4 h. Individual strain colonies (blue and purplish with gas bubbles) were counted on the EC Petrifilms, and they were used as a reference for proper enumeration of generic *E. coli* (i.e., appearance of each inoculum strain on an EC Petrifilm) in the inoculated study. The rain barrel and filtered creek water native *E. coli* populations were also enumerated preinoculation using Colilert Quanti-Tray/2000 (IDEXX Laboratories, Westbrook, ME).

Water sampling, preparation, and inoculation

A 6.5-liter batch of rain barrel water was collected from a rain barrel owned by a local produce grower in northeast Kansas. The rain barrel was a 50-gal covered barrel stored outside, collecting rainwater from the gutters of a garage with a metal roof. A sterilized carboy with a 9-liter capacity was placed directly under the spigot of a rain barrel (covered with a lid) and used to collect the rainwater directly. Before collection, the external surface of the rain barrel spigot was sterilized, and the water was run for 1 min in the carboy before collection. From a creek (mostly rainfed) used for produce irrigation at the Olathe Kansas Horticulture Research and Extension Center, 6.5 liters of creek water was pumped through a sand filter before it was collected directly into a 10-liter sterile carboy. Following each collection, a portion (three subsamples) of each water type was enumerated for naturally occurring coliform and *E. coli* populations using Colilert *(Table 1)*. Water samples were collected on three separate occasions in April 2022. Both water types were also analyzed for turbidity, pH, electroconductivity, and total dissolved solids (TDS) *(Table 2)*. Then, each batch of source water was separated into two water samples, each with a volume of 3 liters. One 3-liter bottle of each water type was stored overnight (8–10 h) at 32 and 12°C, as described in the FDA/EPA protocol *(34)*, to simulate cool season and warm season scenarios.

Before inoculation the following day, each water sample was plated on EC Petrifilm, as described later, to ensure that naturally occurring populations did not change during the overnight temperature equilibration. Each 3-liter sample bottle per water source and temperature (e.g., rain at 32°C and creek at 12°C) was inoculated with 1 ml of the working inoculum to achieve a target concentration of ca. 5 log CFU/ml. The *E. coli* concentration of each 3-liter bottle of water was confirmed after inoculation by plating on EC Petrifilm, as described later. Each inoculated bottle of water was then equally distributed in three subsamples of 990 ml and placed back at their respective temperature for approximately 30 min for temperature equilibration.

TABLE 1. Naturally occurring generic *E. coli* populations in filtered creek water and water collected from a rain barrel

Replication	Water source[a]	Subsample[b]	*E. coli* (log MPN/100 ml)[c]	Mean *E. coli* (log MPN/100 ml)
1	Filtered creek	1	1.3	1.2
		2	1.2	
		3	1.2	
	Rain barrel	1	<1	0.0
		2	<1	
		3	<1	
2	Filtered creek	1	2.3	2.2
		2	2.2	
		3	2.3	
	Rain barrel	1	<1	0.0
		2	<1	
		3	<1	
3	Filtered creek	1	1.8	1.9
		2	1.8	
		3	2.0	
	Rain barrel	1	<1	0.0
		2	<1	
		3	<1	

[a]Water was collected from sources in Kansas.

[b]Three subsamples of each water type were enumerated using Colilert Quanti-Tray/2000 for each replication.

[c]Generic *E. coli* not detected is indicated as <1 MPN/100 ml.

TABLE 2. Physical parameters of sand-filtered creek and rain barrel water collected from sources in Kansas

Replication[a]	Water source	Mean pH[b]	Mean electrical conductivity (µS/cm)	Mean turbidity (NTU)	Mean TDS (mg/ml)
1	Filtered creek	8.3 (0.01)	716.0 (12.77)	2.3 (0.13)	397.3 (33.56)
	Rain barrel	7.0 (0.05)	120.9 (4.42)	0.5 (0.01)	52.3 (3.06)
2	Filtered creek	8.1 (0.02)	464.3 (7.51)	5.0 (0.23)	422.3 (71.32)
	Rain barrel	6.9 (0.09)	241.2 (270.90)[c]*	0.8 (0.09)	180.7 (236.13)[c]*
3	Filtered creek	8.2 (0.02)	509.5 (10.02)	4.5 (0.07)	488.0 (2.08)
	Rain barrel	6.9 (0.02)	66.1 (6.79)	1.1 (0.13)	53.7 (2.89)

[a]For each replication, three subsamples were analyzed for pH, electrical conductivity, turbidity, and TDS.

[b]The average of each parameter is reported for each replication. The standard deviation of each mean is provided in parentheses.

[c]Results from replication 2 differed substantially from those of replications 1 and 3, resulting in a large standard deviation.

Antimicrobial treatment preparation

SaniDate 15 (PAA; BioSafe Systems, East Hartford, CT) at a concentration of 75 ± 5 ppm of PAA, and Ultra Clorox germicidal bleach (Cl; Clorox Professional Products, Oakland, CA), at a concentration of 25 ± 2 ppm of free available Cl, were used for the treatments, with sterile deionized (DI) water used as a control (W). These concentrations were determined by EPA product labels and the approved concentration limits for use with produce *(32, 35)*. Before each replication, titrations were completed following manufacturer instructions to confirm stock solution concentrations that achieve the required 75 ± 5 ppm of PAA and 25 ± 3 ppm of free available Cl in the final 1,000-ml treated water samples (treatment details follow). Sterile aluminum foil-covered flasks were used to prepare treatments to ensure the treatments were not affected by light exposure. A free and total Cl high-range portable photometer (HI96734; Hanna Instruments, Woonsocket, RI) was used to validate the free available Cl concentrations. The PAA treatment concentration was validated using a peracetic acid test kit (BioSafe Systems, East Hartford, CT) following the manufacturer's instructions. The concentrations of both treatments were confirmed at the beginning and the end of each trial day to ensure the same treatment concentration was used throughout the duration of the trial. The PAA and Cl concentrations were not confirmed in each water sample throughout the trial.

Antimicrobial application and microbial analysis

For time point 0, the sample was collected immediately after a 10-ml aliquot of the appropriate treatment solution (Cl, PAA, and W) was added into each 990-ml sample bottle and swirled to equally distribute the sanitizer for 10 s (hereafter called $t = 0$). Water sampling for *E. coli* enumeration from each treated bottle was also performed 5, 10, 60, 1,440, and 2,880 min after treatment. Enumeration using EC Petrifilm was previously described in a similar study conducted by our research team *(22)* and was one of two methods used for enumeration in this study. However, because the EC Petrifilm is not an FDA-approved testing method for agricultural water, the IDEXX Colilert Quanti-Tray/2000 was also used to provide an FDA-approved method in comparison to the previously published EC Petrifilm method. For the EC Petrifilm method, 1 ml of each treated sample was neutralized in 9 ml of Dey–Engley (DE) neutralizing buffer in tubes (Difco, Sparks, MD) and 5 ml of the treated sample was neutralized in 45 ml of DE neutralizing buffer in Whirl-Pak bags (Nasco, Madison, WI). Dilutions were prepared using DE-neutralized tubes in 9 ml of PBDW and plated for enumeration in duplicate on EC Petrifilm, and colonies (blue and purplish with gas bubbles) were counted after Petrifilm incubation at 37°C for 48 ± 4 h. The limit of detection (LOD) for the EC Petrifilm method was 5 CFU/ml. For Colilert enumeration, at each sampling point, 100 ml of each treated (Cl and PAA) sample was neutralized in a transparent, nonfluorescing glass bottle containing 0.2 ml of 10% sodium thiosulfate (Sigma Aldrich, St. Louis, MO) and then Colilert reagent was added and samples were shaken to mix for 30 s. For the W (control) samples, 10 μl from each treated sample was diluted in 99.99 ml of sterile DI water containing the Colilert reagent and 0.2 ml of 10% sodium thiosulfate. The use of 0.2 ml of 10% sodium thiosulfate was validated following the neutralizer or control validation procedure of the FDA/EPA protocol *(34)*. Then, all samples were individually poured in a Colilert Quanti-Tray/2000, sealed, and incubated at 35°C for 24 h. The LOD for Colilert is one organism per 100 ml of water. For enrichment and recovery of *E. coli*, 50 ml of 2× BHI broth was added to the 50-ml DE-neutralized sample (5 ml of sample + 45 ml of DE neutralizing buffer) in Whirl-Pak bags, resulting in a 1× BHI broth dilution, and then the bags were incubated at 37°C for 24 ± 2 h. After incubation, the enriched bags were streaked on MacConkey agar (MAC; Thermo Scientific Remel, Lenexa, KS) to determine the presence or absence of *E. coli*, and MAC plates with pink colonies following incubation at 37°C for 18–24 h were interpreted as positive for *E. coli*. The enriched samples were considered to have an LOD of 1 cell per 5 ml, or 20 cells in a 100-ml water sample. Sample bottles were returned to their respective temperatures after the 10-min time point and were taken out for a short period (~5 min or less) for sample collection at the subsequent time points.

Statistical analysis

The experimental procedures were replicated three times; however, two replications of data are represented for the Colilert method because counts were too numerous for the W (control) samples for one replication. Statistical analyses were conducted with Statistical Analysis Software (SAS version 9.4; SAS Institute, Cary, NC). Data for each combination of water source (creek and rain barrel) and enumeration method (Colilert and EC Petrifilm) were analyzed separately (e.g., creek water enumerated using Petrifilm was analyzed separately from creek water enumerated using Colilert). All data were subjected to linear mixed modeling using the PROC MIXED procedure, with a significance level of 0.05. Because repeated measures analysis was used in this study, the best covariance structure for the model was determined. The least squares means (LS means) were calculated and the Tukey–Kramer adjustment for multiple comparisons was used to determine statistical significance between individual treatments. The main effects of time, temperature, and treatment, as well as the three-way interaction (time × temperature × treatment) and all two-way interactions, were evaluated for statistical significance.

TABLE 3. *E. coli* survival in creek water using EC Petrifilm and analyzed by time, antimicrobial treatment, and temperature[a]

		LS means ± SEM *E. coli* survival (log CFU/ml)[b–d]					
Temperature	Treatment	0 min	5 min	10 min	60 min	1,440 min	2,880 min
	Cl	0.0 ± 0.05[Ab]	0.0 ± 0.12[Ab]	0.0 ± 0.05[Ab]	0.0 ± 0.07[Ab]	0.0 ± 0.10[Ac]	0.0 ± 0.24[Ab]
12°C	PAA	0.0 ± 0.05[Ab]	0.0 ± 0.12[Ab]	0.0 ± 0.05[Ab]	0.0 ± 0.07[Ab]	0.0 ± 0.10[Ac]	0.0 ± 0.24[Ab]
	W	5.3 ± 0.05[Aa]	5.3 ± 0.12[Aa]	5.3 ± 0.05[Aa]	5.2 ± 0.07[Aa]	5.0 ± 0.10[Aa]	4.3 ± 0.24[Aa]
	Cl	0.0 ± 0.05[Ab]	0.0 ± 0.12[Ab]	0.0 ± 0.05[Ab]	0.0 ± 0.07[Ab]	0.0 ± 0.10[Ac]	0.0 ± 0.24[Ab]
32°C	PAA	0.0 ± 0.05[Ab]	0.0 ± 0.12[Ab]	0.0 ± 0.05[Ab]	0.0 ± 0.07[Ab]	0.0 ± 0.10[Ac]	0.0 ± 0.24[Ab]
	W	5.2 ± 0.21[Aa]	5.0 ± 0.12[Aa]	5.3 ± 0.05[Aa]	5.2 ± 0.07[Aa]	4.1 ± 0.10[Bb]	3.7 ± 0.24[Ba]

[a]The time × treatment × temperature interaction was significant ($P = 0.0275$). Therefore, data are displayed according to time, treatment, and temperature.

[b]*E. coli* not detected is indicated as 0.0 log CFU/ml.

[c]Values with different uppercase letters in the same row indicate significant differences between temperatures for a treatment.

[d]Values with different lowercase letters in the same column indicate significant differences between treatments at a temperature.

TABLE 4. *E. coli* survival in rain barrel water using EC Petrifilm and analyzed by time, antimicrobial treatment, and temperature[a]

		LS means ± SEM *E. coli* survival (log CFU/ml)[b–d]					
Temperature	Treatment	0 min	5 min	10 min	60 min	1,440 min	2,880 min
	Cl	0.0 ± 0.06[Ab]	0.0 ± 0.07[Ab]	0.0 ± 0.08[Ab]	0.0 ± 0.11[Ab]	0.0 ± 0.19[Ac]	0.0 ± 0.22[Ac]
12°C	PAA	0.0 ± 0.06[Ab]	0.0 ± 0.07[Ab]	0.0 ± 0.08[Ab]	0.0 ± 0.11[Ab]	0.0 ± 0.19[Ac]	0.0 ± 0.22[Ac]
	W	5.3 ± 0.06[Aa]	5.2 ± 0.07[Aa]	5.2 ± 0.08[Aa]	5.2 ± 0.11[Aa]	4.9 ± 0.19[Aa]	4.6 ± 0.22[Aa]
	Cl	0.0 ± 0.06[Ab]	0.0 ± 0.07[Ab]	0.0 ± 0.08[Ab]	0.0 ± 0.11[Ab]	0.0 ± 0.19[Ac]	0.0 ± 0.22[Ac]
32°C	PAA	0.0 ± 0.06[Ab]	0.0 ± 0.07[Ab]	0.0 ± 0.08[Ab]	0.0 ± 0.11[Ab]	0.0 ± 0.19[Ac]	0.0 ± 0.22[Ac]
	W	5.0 ± 0.06[Aa]	5.0 ± 0.07[Aa]	5.1 ± 0.08[Aa]	5.0 ± 0.11[Aa]	3.3 ± 0.19[Bb]	1.6 ± 0.22[Cb]

[a]The time × treatment × temperature interaction was significant ($P < 0.0001$). Therefore, data are displayed according to time, treatment, and temperature.

[b]*E. coli* not detected is indicated as 0.0 log CFU/ml.

[c]Values with different uppercase letters in the same row indicate significant differences between temperatures for a treatment.

[d]Values with different lowercase letters in the same column indicate significant differences between treatments at a temperature.

RESULTS

Water quality

Table 1 summarizes the naturally occurring generic *E. coli* population in the creek and rain barrel water sources used in this study. The physical parameters associated with the creek and rain barrel water sources used in this study are highlighted in *Table 2*.

Petrifilm method

When enumerating using Petrifilm, the time × temperature × treatment interaction was significant for creek water ($P = 0.0275$) and rain water ($P < 0.0001$); therefore, all results are discussed according to time, temperature, and treatment. At each temperature, *E. coli* was not detected in creek water samples treated with either Cl or PAA at any of the

TABLE 5. *E. coli* survival in creek water using the Colilert method and analyzed by time, antimicrobial treatment, and temperature[a]

		LS means ± SEM *E. coli* survival (log MPN/ml)[b–d]					
Temperature	**Treatment**	**0 min**	**5 min**	**10 min**	**60 min**	**1,440 min**	**2,880 min**
	Cl	2.8 ± 0.21^{Ab}	1.0 ± 0.47^{Ab}	1.0 ± 0.59^{Ab}	0.0 ± 0.01^{Bc}	0.0 ± 0.01^{Bc}	0.0 ± 0.07^{Bc}
12°C	PAA	0.0 ± 0.21^{Ac}	0.0 ± 0.47^{Ab}	0.0 ± 0.59^{Ab}	0.0 ± 0.01^{Ac}	0.0 ± 0.01^{Ac}	0.0 ± 0.07^{Ac}
	W	5.1 ± 0.21^{Aa}	5.2 ± 0.47^{Aa}	5.2 ± 0.59^{Aa}	5.2 ± 0.01^{Aa}	5.0 ± 0.01^{Aa}	4.3 ± 0.07^{Aa}
	Cl	2.5 ± 0.21^{Ab}	1.0 ± 0.47^{Ab}	1.2 ± 0.59^{Ab}	0.0 ± 0.01^{Bc}	0.0 ± 0.01^{Bc}	0.0 ± 0.07^{Bc}
32°C	PAA	0.0 ± 0.21^{Ac}	0.0 ± 0.47^{Ab}	0.0 ± 0.59^{Ab}	0.0 ± 0.01^{Ac}	0.0 ± 0.01^{Ac}	0.0 ± 0.07^{Ac}
	W	5.0 ± 0.21^{Aa}	5.0 ± 0.47^{Aa}	5.1 ± 0.59^{ABCa}	5.0 ± 0.01^{Ab}	3.9 ± 0.01^{Bb}	2.7 ± 0.07^{Cb}

[a]The time × treatment × temperature interaction was significant ($P = 0.0015$). Therefore, data are displayed according to time, treatment, and temperature.

[b]*E. coli* not detected is indicated as 0.0 log MPN/ml.

[c]Values with different uppercase letters in the same row indicate significant differences between a specific temperature and a treatment combination at each sampling point.

[d]Values with different lowercase letters in the same column indicate significant differences between each temperature and treatment combination at a specific sampling time point.

TABLE 6. *E. coli* survival in rain barrel water using the Colilert method and analyzed by time, antimicrobial treatment, and temperature[a]

		LS means ± SEM *E. coli* survival (log MPN/ml)[b–d]					
Temperature	**Treatment**	**0 min**	**5 min**	**10 min**	**60 min**	**1,440 min**	**2,880 min**
	Cl	0.0 ± 0.18^{Bb}	1.0 ± 0.18^{Ab}	0.0 ± 0.18^{Bb}	0.0 ± 0.18^{Bb}	0.0 ± 0.18^{Bc}	0.0 ± 0.18^{Bb}
12°C	PAA	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ac}	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ac}	0.0 ± 0.18^{Ab}
	W	5.1 ± 0.18^{Aa}	5.0 ± 0.18^{Aa}	5.1 ± 0.25^{Aa}	5.2 ± 0.18^{Aa}	4.9 ± 0.18^{Aa}	4.6 ± 0.18^{Aa}
	Cl	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ac}	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ac}	0.0 ± 0.18^{Ab}
32°C	PAA	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ac}	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ab}	0.0 ± 0.18^{Ac}	0.0 ± 0.18^{Ab}
	W	4.6 ± 0.18^{Aa}	4.8 ± 0.18^{Aa}	4.8 ± 0.25^{ABCa}	4.6 ± 0.18^{Aa}	3.0 ± 0.18^{Bb}	0.0 ± 0.18^{Cb}

[a]The time × treatment × temperature interaction was significant ($P < 0.0001$). Therefore, data are displayed according to time, treatment, and temperature.

[b]*E. coli* not detected is indicated as 0.0 log MPN/ml.

[c]Values with different uppercase letters in the same row indicate significant differences between a specific temperature and a treatment combination at each sampling point.

[d]Values with different lowercase letters in the same column indicate significant differences between each temperature and treatment combination at a specific sampling time point.

sampling points, and both were significantly different from W (control). *E. coli* in the W sample declined throughout storage at both 12 and 32°C *(Table 3)*. This reduction in population was significant at the 1,440- and 2,880-min time points in water samples stored at 32°C. At the 1,440-min time point, *E. coli* populations in the 12°C W sample (5.0 log CFU/ml) were significantly greater than in the W sample stored at 32°C (4.1 log CFU/ml).

Table 4 summarizes rain barrel water data and shows that *E. coli* was not detected in creek water samples treated with either Cl or PAA at any of the sampling points, for either storage temperature, and that both were significantly different from W. *E. coli* in the W sample declined throughout storage at both 12 and 32°C. At the 1,440- and 2,880-min time points, this reduction in population was significant in water stored at 32°C. At the 1,440- and 2,880-min time points, *E. coli* populations in the 12°C W sample were significantly greater than in the W sample stored at 32°C. None of the Cl- and PAA-treated 5-ml water enrichments were positive for *E. coli* on the MAC plates.

Colilert method

Using the Colilert detection method, the time × temperature × treatment interaction was significant for creek water ($P = 0.0015$) and rain water ($P < 0.0001$), and all results are discussed according to time, temperature, and treatment as a result. *Table 5* shows that *E. coli* populations were not recovered using the Colilert method at any time point from 12 or 32°C creek water samples treated with PAA. At sampling point 0, Cl significantly reduced *E. coli* in creek water stored at both temperatures compared with the W sample. By the 60-min sampling point, *E. coli* was not recovered from 12 or 32°C creek water samples treated with Cl. *E. coli* in the W sample declined throughout storage at both 12 and 32°C, and this reduction was significant in creek water stored at 32°C. *E. coli* populations in the 12°C W sample were significantly greater than in the 32°C W sample at the 1,440- and 2,880-min sampling points.

E. coli was not recovered at any sampling point from 12 and 32°C rain barrel water treated with PAA *(Table 6)*. With the exception of 1 log MPN/ml of *E. coli* recovered at 5 min, *E. coli* was not recovered from 12°C rain barrel water treated with Cl. Populations of *E. coli* in the W sample declined throughout 12 and 32°C storage, and this decline was significant in 32°C rain barrel water, resulting in no detection of *E. coli* by the 2,880-min sampling point.

DISCUSSION

The objective of this study was to evaluate the effectiveness of 25 ppm of Cl and 75 ppm of PAA at reducing *E. coli* in rain barrel and creek water to a level of no detectable generic *E. coli* per 100 ml of water to determine whether surface water can be effectively treated for postharvest use in produce according to the FSMA PSR *(15)*. When using Petrifilm as the enumeration method, the application of PAA (75 ± 5 ppm) and Cl (25 ± 3 ppm) resulted in no detectable *E. coli* in 12°C and 32°C creek and rain barrel water at the 0-min sampling point, which suggests that the 10-s mixing period was sufficient for reducing ~5 log CFU/ml of *E. coli*. Like the Petrifilm method, *E. coli* was also not detected from PAA-treated creek and rain barrel samples (12 and 32°C) at the 0-min sampling point using the Colilert method. As a general comparison, the Colilert method recovered generic *E. coli* from 12°C rain barrel water treated with Cl through the 5-min sampling point and from 12 and 32°C creek water treated with Cl through the 60-min sampling point. The discrepancy in *E. coli* recovery for Cl-treated water is likely because Petrifilm plates hold 1 ml of sample, whereas the Colilert method is based on a 100-ml sample and the larger sample size improves the likelihood of recovering generic *E. coli* when microbial populations are low. The combination of Petrifilm and Colilert data suggests that PAA is more efficient at reducing *E. coli* in surface water sources at the concentrations tested. The efficacy of PAA has been reported by several other studies as being more stable in the presence of organic matter and dissolved substances in comparison to Cl *(13, 28)*. When used in produce wash water sources with varied levels of organic matter, most of the Cl is reportedly associated with small molecular substances, and 50% of the Cl is reported to be used in the first 5 min *(37)*. Winward et al. *(38)* also reported that the protection provided to microorganisms by water particles significantly decrease as initial Cl concentration increases. These two studies suggest rapid killing of suspended microorganisms in the water if the concentration of Cl is high enough to withstand Cl demand. McFadden et al. *(26)* also demonstrated that PAA required a shorter lag time than Cl to inactivate *E. coli*, so if the *E. coli* population in the water source was resistant or attached to particles, it might explain why PAA achieved inactivation of *E. coli* more rapidly than Cl. The present study also shows that 25-ppm Cl treatment provided enough residual Cl treatment to continually decrease *E. coli* populations until they were not detected.

Table 1 shows that more *E. coli* was recovered from creek water than rain barrel water, and *Table 2* highlights notable differences in physical parameters associated with each water source. The difference in microbial presence and survival in these two sources of water could be hypothesized to be associated with the physicochemical characteristics of each water source. For instance, the average turbidity and TDS of the creek water were higher than those of the rain barrel water *(Table 2)*. The higher turbidity and TDS of creek water compared with rain barrel water could have influenced the bacterial protection by particles, which then made *E. coli* more recoverable in creek water compared with rain barrel water. Turbidity is directly correlated to the increase of total suspended solids, and both have been shown by other researchers to have a significant effect on the efficacy of

treatment by protecting the microorganisms from treatments and harsh environmental conditions *(12, 14, 24, 26)*. The creek water initially harbored, on average, a greater *E. coli* population compared with the rain barrel water *(Table 1)*; however, this population difference was small compared with the added inoculum (~5 log CFU/ml). In addition, the microbial recovery difference in creek water compared with rain barrel water in all treatments is <0.4 MPN/ml, which is likely too small to be of biological relevance. PAA has been shown to be more effective, even when in the presence of higher organic matter content, and is not greatly affected by TDS compared with Cl treatments, which have been reported to be greatly affected by the physicochemical characteristics of water *(26, 28)*. The latter could explain the overall better efficacy of PAA compared with Cl in both rain barrel and creek water sources. However, different concentrations of PAA and Cl were used, and concentration affects efficacy.

This study also evaluated the effect of water temperature on the efficacy of PAA and Cl in reducing generic *E. coli* populations. The data presented in *Tables 3–6* suggest that temperature did not affect the efficacy of Cl and PAA in reducing *E. coli* populations in the creek and rain barrel water. This is in agreement with the findings of Hassaballah et al. *(21)*, who reported no significant impact of temperature (4°C compared with room temperature) on the efficacy of PAA and Cl in wastewater treatment. This allows growers to use these two validated treatment concentrations for postharvest in both cool and warm temperature seasons.

Another important finding was the reduction of *E. coli* in the W (control) samples over time. This reduction was statistically similar in both rain barrel and creek water sources stored at 12°C, regardless of the enumeration method used. Conversely, *E. coli* populations significantly died off in the creek and rain barrel water stored at 32°C; however, rain barrel water was generally associated with greater microbial die-off than was creek water. The roof material from which the rainwater is collected is known to influence the chemical properties of the final collected water *(30)*. One could hypothesize that this chemical catchment might include chemicals that reduce the microbial load of the water, which manifests in increased die-off over time. More research studies would be necessary to test this hypothesis.

Finally, the microbial reductions achieved by this study may have been influenced by the *E. coli* ATCC strains used in the inoculum, as well as the naturally occurring *E. coli* populations (~2 logs CFU/100 ml) present in the water sources, which is not necessarily a reflection of the entire bacterial load in the agricultural surface water sources. For instance, Evans et al. *(11)* found negligible coliform counts compared with the total bacterial population of 77 roof-collected rainwater runoff samples, which indicates that other bacterial types, including pathogens, may be present in amounts different from and more significant than coliforms. Different microorganisms or strains of the same microorganisms may also have different resistance to the PAA and Cl treatments because of different defense mechanisms genotypically, as well as developed phenotypic resistance induced by environmental exposure *(7, 9, 29)*.

Haley et al. *(20)* tested 247 surface water samples from Kansas and Missouri and reported the average generic *E. coli* levels to be 158.7 MPN/100 ml, which calculates to 2.2 log MPN/ml. Thus, if these surface water sources were treated as described in this study and used for postharvest purposes in the produce industry, the potential for both 75 ppm of PAA and 25 ppm of Cl to reduce >3 logs of *E. coli* in rain barrel and creek water sources in the first 10 s of contact at both 12 and 32°C is promising. This suggests that 75 ppm of PAA and 25 ppm of Cl may be effective chemical intervention treatments for various surface water sources during different seasons.

CONCLUSIONS

The results presented herein suggest that at the tested doses, both PAA and Cl are effective at reducing *E. coli* to nondetectable levels (in a 5-ml sample subjected to enrichment) within 60 min of treating rain barrel and creek water at temperatures of 32 and 12°C. As described in our recent publication *(22)*, this is equivalent to a nearly 6 log reduction. Although the EC Petrifilm method indicated that Cl achieved no detectable generic *E. coli* in 12 and 32°C creek and rain barrel water at the 0-min sampling point, the Colilert method suggests that complete elimination of generic *E. coli* was more rapid with PAA. Overall, both treatments were effective at satisfying the FSMA PSR requirement of no detectable generic *E. coli* in agricultural water used postharvest. However, the EC Petrifilm method was based upon 1 ml of sample per plate (plated in duplicate with a LOD of 5 CFU/ml), followed by a 5-ml enrichment method (LOD of one cell in 5 ml), in comparison to the 100-ml sample required by the FSMA PSR and used by the Colilert method (LOD of one cell in 100 ml). This study suggests that PAA and Cl can be used as effective interventions for treating rain catchment water, creek water, and potentially other surface water to provide a water source that can be safely used postharvest in produce following 60 min of treatment. The present study did not have time points between 10 and 60 min. Therefore, future studies should evaluate *E. coli* populations between 10 and 60 min after treatment to determine whether the treatment time can effectively be reduced from 60 min. Future studies should also consider enriching the remaining water sample at the conclusion of the study to demonstrate complete inactivation by the PAA and Cl treatments.

Although these data provide promising results, the data are limited to generic *E. coli*, two temperatures, two treatment concentrations, and two water sources. A large amount of variation likely exists within a single type of water source

and the water recovered from one creek to the next, and even water recovered from the same creek throughout different times of the year and different sampling locations will likely vary greatly. Another consideration for future research would be to isolate naturally occurring *E. coli* from a water source and use these isolates to prepare the inoculum. Similarly, the data presented herein cannot be extrapolated to pathogens or other microorganisms. Additional research is necessary to fully understand the efficacy of PAA and Cl in treating surface water for postharvest use in fresh produce.

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In Memory

IAFP was notified of the passing of member **Charles D. Price.** The Association extends our deepest sympathy to his family and colleagues. IAFP has sincere gratitude for his contribution to food safety.