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Evaluation of the Sanitizing Capacity of a 2-ppm Ozonated Water Handheld Bottle Applied to Simulated Food-Contact Surfaces

ABSTRACT

Contaminated food-contact surfaces can transfer pathogenic organisms within domestic settings, potentially leading to foodborne illness. The objective of this study was to evaluate the efficacy of an ozonated water handheld spray bottle to eliminate bacteria on food-contact surfaces. *Escherichia coli, Listeria innocua, Pseudomonas* spp., and *Staphylococcus aureus* were used to inoculate polypropylene cutting boards and stainless steel knives. A 2-ppm ozonated water spray was applied to the inoculated surfaces and allowed to act for 30 or 60 s. Ozonated water was able to significantly reduce ($P < 0.05$) generic *E. coli* on cutting boards (0.7 ± 0.1 $\log_{10}$ CFU/cm^2). In addition, *L. innocua* was significantly reduced ($P < 0.05$) on polypropylene (0.9 ± 0.2 $\log_{10}$ CFU/cm^2) and stainless steel (1.1 ± 0.1 $\log_{10}$ CFU/cm^2). There were no significant reductions of *Pseudomonas* (0.5 ± 0.3 $\log_{10}$ CFU/cm^2) or *S. aureus* (0.2 ± 0.1 $\log_{10}$ CFU/cm^2). Significant reductions were only observed after 60-s treatments, and there was no significant effect of surface type on the reductions. Overall, low concentration–ozonated water is moderately effective at reducing nonpathogenic bacteria loosely attached to simulated clean food-contact surfaces.

INTRODUCTION

Foodborne illness outbreaks related to private home settings accounted for a minimum of 185 outbreaks between 2019 and 2020 in the United States alone, representing 4,071 illnesses cases *(8)*. Sources of microbial contamination in homes include naturally contaminated raw foods; animals or insects that bring in pathogens from the environment; and ill persons that may transfer pathogenic organisms through aerosols or the fecal–oral route, among others *(4, 12)*. Bacteria in the kitchen can survive, grow, spread to other surfaces, persist for long periods, and contaminate food and food-contact surfaces *(4)*. Studies of domestic kitchen surfaces and items have found high levels of contamination and different microbial populations, even when surfaces appear visibly clean *(10, 19)*. For example, Oxford et al. *(19)* sampled eight surface types in 160 households and found that 28% of surfaces or items had moderate-to-heavy bacterial

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loads, including kitchen cloths and kitchen taps. Borrusso and Quinlan *(5)* found that 45% of sampled homes (n = 100) tested positive for one of four pathogens—*Campylobacter*, *Listeria* spp., *Salmonella*, or *Staphylococcus aureus*—and 12% had more than one pathogen *(5)*.

Contaminated surfaces such as cutting boards, knives, and bowls can transfer bacteria to other raw or cooked foods *(22)*. It has been estimated that as many as 26% of consumers use unwashed surfaces during preparation of food and between 30 and 71% of consumers use the same cutting board to prepare raw meats and other foods *(15, 20)*. Contaminated cutting boards have been shown to transfer *Escherichia coli* O157:H7 from raw hamburgers to lettuce leaves and harbor *S. aureus* from contaminated ground beef *(22, 24)*. Similarly, *Salmonella* and *Campylobacter* spp. can be transferred from contaminated poultry products to salads via unwashed stainless steel surfaces *(15)*. Different surfaces, such as wood, laminated plastics, polypropylene, and stainless steel, can transfer bacteria to food at different rates *(18)*. Once in the home environment, bacteria may not be removed if household cleaners are only used casually or spontaneously *(19)*; therefore, the use of disinfectants in households may be necessary to minimize the risk of cross-contamination and pathogen transmission to humans *(22)*.

New sanitizing technologies must compete with traditional methods; therefore, it is important for new sanitation methods to not only be effective but also convenient, affordable, safe, and environmentally friendly *(22)*. Ozonated water has been proposed as a possible antimicrobial intervention in many food settings due to its generation on demand, its antimicrobial properties against a wide range of microorganisms, its minimal effect on nutritional and chemical properties, and its fast decomposition without leaving residues on food *(6, 7)*. Household ozonated water devices have become available for purifying air, treating drinking water, washing produce, and disinfecting surfaces *(25, 26)*; however, there is little evidence to validate their effectiveness. Thus, the objective of this study was to evaluate the efficacy of an ozonated water handheld bottle to reduce loosely attached bacteria inoculated on simulated clean food-contact surfaces.

MATERIALS AND METHODS

Surfaces

Polypropylene and stainless steel were used as model food-contact materials. Polypropylene cutting boards (surface area of 100 cm^2, Electron Microscopy Sciences, Hatfield, PA) were cleaned, wrapped in aluminum foil, and autoclaved before use. Stainless steel knives (surface area of 68 cm^2, 8″ Chef Knife, Cuisinart, East Windsor, NJ) were cleaned, dipped in 70% ethanol, and flame sterilized immediately before use.

Inoculum preparation and surface inoculation

One strain each of four different bacteria were used for the experiments: *E. coli* (ATCC 25922), *Listeria innocua* (ATCC 33090), *S. aureus* (ATCC 25923), and a *Pseudomonas* spp. strain (Loeffel Meat Laboratory Meat Spoilage Bacterial Collection, Department of Animal Science, University of Nebraska–Lincoln). Stock solutions of each bacterium were stored in tryptic soy broth (TSB; Remel, Lenexa, KS) with 20% glycerol at −80°C. For resuscitation, a 10-µl loopful of each strain was inoculated into 9 ml of the corresponding broth as follows: TSB for *E. coli* and *S. aureus*, brain heart infusion CBHI broth (Hardy Diagnostics, Santa Maria, CA) for *L. innocua*, and Luria–Bertani (LB) broth (Becton, Dickinson and Company, Sparks, MD) for *Pseudomonas* spp. All tubes were incubated at 37°C for 24 h followed by vortexing and dilution in 0.1% buffered peptone water (BPW; Sigma-Aldrich, St. Louis, MO) to approximately 10^5 log CFU/ml as determined by serial decimal dilutions plated onto the corresponding solid media (see Microbiological analysis) One-hundred microliter aliquots of each inoculum were spot inoculated onto the corresponding surface with a pipette tip, thereby ensuring drops of inoculum were spread throughout the entire surface area in a uniform manner. The bacterial suspensions were allowed to air dry for 30 min inside a biosafety cabinet.

Ozonated water spray treatment

Inoculated surfaces were treated with a commercially available spray bottle appliance (o3 Waterworks, Mooresville, NC). The spraying distance was 15 cm, and surfaces were sprayed evenly until wet, following the manufacturer's recommendations. The appliance generates ozone by using an electrolytic cell. Ozone concentration cannot be modified from that of the manufacturer's setting. The ozone concentration was 2.0 ± 0.3 ppm, as determined with a CHEMets® ozone measuring kit (K-7404, CHEMetrics, Midlands, VA). Ozonated water was allowed to act on cutting board surfaces for 30 or 60 s before sampling. Based on data from cutting boards, kitchen knives were sampled only after 60 s of exposure time. Sampling was carried out by swabbing the surface with a polyurethane sponge samplers moistened with D/E neutralizing broth (EZ-10DE-PUR, World Bioproducts, Libertyville, IL). Inoculated, untreated surfaces were used as controls and swabbed within 30 min of the air-drying period.

Microbiological analysis

Sponge samplers were hand massaged and squeezed to obtain 1 ml of sampling broth. Appropriate 10-fold dilutions were prepared in 0.1% BPW. For *E. coli*, dilutions were plated onto MacConkey agar (Neogen, Lansing, MI). For *Pseudomonas* spp., dilutions were plated onto LB agar (Becton, Dickinson and Company). For *L. innocua*, dilutions were plated on BHI agar (Becton, Dickinson and Company).

TABLE 1. Bacterial counts on simulated clean food-contact surfaces subjected to low concentration-ozonated water spray treatment

Bacteria	Surface type and treatment time (log_{10} CFU/cm² ± SD)								
	Polypropylene			Polypropylene			Stainless steel		
	Control	Ozone, 30 s	Reduction	Control	Ozone, 60 s	Reduction	Control	Ozone, 60 s	Reduction
E. coli	4.0 ± 0.1[a]	4.0 ± 0.3[a]	0.0 ± 0.3	5.2 ± 0.5[a]	4.6 ± 0.4[b]	0.7 ± 0.1	4.2 ± 1.0[a]	2.7 ± 0.2[a]	1.5 ± 0.8
L. innocua	4.5 ± 0.3[a]	4.4 ± 0.4[a]	0.1 ± 0.7	5.5 ± 0.3[a]	4.6 ± 0.4[b]	0.9 ± 0.2	5.1 ± 0.1[a]	4.0 ± 0.1[b]	1.1 ± 0.1
Pseudomonas spp.	4.4 ± 0.5[a]	4.3 ± 0.5[a]	0.1 ± 0.1	5.6 ± 0.4[a]	5.3 ± 0.6[a]	0.3 ± 0.2	5.6 ± 0.3[a]	5.1 ± 0.6[a]	0.5 ± 0.3
S. aureus	4.5 ± 0.1[a]	4.4 ± 0.1[a]	0.1 ± 0.2	5.2 ± 0.7[a]	5.0 ± 0.4[a]	0.2 ± 0.5	5.2 ± 0.1[a]	5.0 ± 0.1[a]	0.2 ± 0.1

[ab]Within a row, counts with different letters are significantly different ($P < 0.05$) from their respective untreated controls.

For *S. aureus*, dilutions were plated on tryptic soy agar (Becton, Dickinson and Company). Plates were incubated at 37°C for 18–24 h before counting colonies. Bacterial counts were recorded as log_{10} CFU/cm². The limit of detection was 0.2 log_{10} CFU/cm² (1.5 CFU/cm²).

Statistical analysis

Each bacterium and surface combination were analyzed separately. Mean log_{10} CFU/cm² values before and after treatment were compared through paired *t*-tests on Microsoft Excel® ($P < 0.05$). Experiments were conducted in triplicate, with one sample per material type per replicate.

RESULTS AND DISCUSSION

Absolute microbial counts on each surface type are presented in *Table 1*. Initial counts on inoculated polypropylene boards were between 4 and 5 log_{10} CFU/cm² for all bacteria. The ozone treatment for 30 s on cutting board surfaces did not show significant reductions ($P > 0.05$) for any of the bacteria tested *(Table 1)*; however, significant reductions ($P < 0.05$) in *E. coli* and *L. innocua* counts were observed on cutting boards with a 60-s exposure time to ozone. Significant reductions of *L. innocua* counts were also observed on stainless steel knives treated for 60 s. Previous studies in fresh produce and surfaces have reported that increasing exposure time to ozone leads to larger reductions *(1, 11, 16)*. Baumann et al. *(2)* observed up to a 4-log reduction of *L. monocytogenes* on stainless steel coupons treated with 1.0-ppm ozonated water for 1 min, when applying a continuous flow of fresh ozonated water. However, in noncontinuous applications, the inactivation process happens quickly at the beginning and tapers off as ozone is consumed and residual ozone becomes undetectable *(14)*. A similar limitation is the use of room temperature water for ozone generation because ozone is more stable and more effective in colder water (4°C) *(11)*.

Microbial reductions with ozonated water ranged from 0.2 ± 0.1 log_{10} CFU/cm² for *S. aureus* on both polypropylene and stainless steel to 1.5 ± 0.8 log_{10} CFU/cm² for *E. coli* on knives. These reductions are comparable with those described in published studies. For example, Marino et al. *(16)* found reductions of up to 2.0 log_{10} CFU/cm² of *P. fluorescens*, *S. aureus*, and *L. monocytogenes* on stainless steel coupons treated with ozonated water (0.5 ppm, 50 ml) for 60 s. Higher reductions of *Pseudomonas* on stainless steel coupons have been reported with higher ozone concentrations (20 ml of 5.0 ppm ozonated water, mixed with coupons in a blender for 1 min) *(13)*. Crapo et al. *(9)* used ozonated water spray (1.5 ppm, 60 s) to disinfect plastic cutting boards inoculated with *L. innocua* and found similar reductions to those achieved with a chlorine spray, indicating that ozonated water may be a viable alternative to the use of common household chemicals.

Overall, *L. innocua* and *E. coli* were more susceptible to ozonated water than *S. aureus* and *Pseudomonas*. Ozone is active against both gram-positive and gram-negative bacteria *(6)*. Restaino et al. *(21)* treated bacterial suspensions with 0.2-ppm ozonated water and observed 6-log reductions for gram-negative bacteria within 2 min, whereas gram-positive bacteria needed between 0 and 5 min to be inactivated. When comparing gram-positive bacteria in this study, *L. monocytogenes* was reduced faster than *S. aureus (21)*. Similarly, Almeida and Gibson *(1)* found that longer exposure times were necessary to inactivate *S. aureus* than *E. coli* and *L. innocua* on a stainless steel ice cream scoop dipped in ozonated water. In vitro tests by Białoszewski et al. *(3)* showed that *Pseudomonas* was slightly more resistant to ozonated water (1.3–1.5 ppm) than *S. aureus* and *E. coli*, which is similar to this study's observations. *S. aureus* is a potentially pathogenic microorganism, whereas *E. coli* and *L. innocua* are indicator organisms and *Pseudomonas* may cause food spoilage. All four bacteria can attach to food-contact surfaces and form biofilms. Biofilms can serve as a niche for the spread of pathogenic bacteria in the kitchen. Therefore, it is necessary to eliminate transient organisms as quickly as possible *(16)*.

Reductions were similar on polypropylene and stainless steel for *S. aureus* and *Pseudomonas*. *E. coli* and *L. innocua* reductions were slightly higher on stainless steel knives than on polypropylene cutting boards. Surface properties can influence ozonated water efficacy and reductions tend to be higher on smoother surfaces *(11, 17)*. For example, Khadre and Yousef *(13)* found that ozonated water was more active against *Pseudomonas* spp. on stainless steel (5.5-log reduction) than on multilaminated packaging material (4.6–5.5-log reduction). Similarly, Crapo et al. *(9)* used ozonated water (1.5 ppm, 60 s) to disinfect stainless steel or polyethylene surfaces covered with inoculated fish slime (10^4–10^6 CFU/cm^2). Aerobic plate count reductions were higher in stainless steel than on plastic surfaces, with decreases of 2–3 log CFU/cm^2 for stainless steel and 1 log CFU/cm^2 for plastic surfaces *(9)*.

The effectiveness of ozonated water can also be affected by the presence of organic matter on the surface to be disinfected, the presence of biofilms, and the initial bacterial load *(9, 16, 23)*; therefore, ozonated water sprays should not be used on their own, but alongside effective cleaning practices. Dynamic application of ozonated water has been found to be more effective than static application, and higher spray pressure may help dislodge more bacterial cells from surfaces *(16, 20)*. This can also be accomplished by wiping down ozone-treated surfaces with cloth or preferably, disposable paper napkins *(22, 25)*. Because of its rapid generation and low risk to operators, ozonated water sprays could become part of daily sanitizing as an additional cross-contamination preventive measure.

CONCLUSIONS

Ozonated water at 2 ppm was able to significantly reduce *E. coli* on polypropylene and *L. innocua* on polypropylene and stainless steel. An exposure time of 60 s was necessary to observe significant reductions. Overall, our data suggest that ozonated water spray can help reduce bacteria loosely attached to clean food-contact surfaces. Future experiments should focus on longer contact times and higher ozone concentrations, keeping in mind consumer safety and practicality of the sanitizing applications.

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In Memory

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