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Effect of Simulated Sanitizer Carryover on Recovery of *Salmonella* from Broiler Carcass Rinsates

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ABSTRACT

Numerous antimicrobial chemicals are currently utilized as processing aids with the aim of reducing pathogenic bacteria on processed poultry carcasses. Carryover of active sanitizer to a carcass rinse solution intended for recovery of viable pathogenic bacteria by regulatory agencies may cause false-negative results. This study was conducted to document the potential carryover effect of five sanitizing chemicals commonly used as poultry processing aids for broilers in a postchill dip. The effect of postdip drip time on the volume of sanitizer solution carryover was first determined by regression of data obtained from 10 carcasses. The five sanitizer solutions were diluted with buffered peptone water at 0-, 1-, and 5-min drip time equivalent volumes as determined by the regression analysis. These solutions were then spiked to 10^5 CFU/ml with a mixture of five nalidixic acid-resistant *Salmonella enterica* serovars, stored at 4°C for 24 h, and finally enumerated by plate count on brilliant green sulfa agar containing nalidixic acid. At the 0- and 1-min drip time equivalents, no *Salmonella* recovery was observed in three of the five sanitizers studied. At the 5-min drip time equivalent, one of these sanitizers still exhibited significant ($P \leq 0.05$) bactericidal activity. These findings potentially indicate that the currently utilized protocol for the recovery of *Salmonella* bacteria from postchill sanitizer interventions may lead to false-negative results due to sanitizer carryover into the carcass rinsate.

Key words: Broiler carcass; Buffered peptone; Carryover; *Salmonella*; Sanitizer

The U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) established the *Salmonella* Verification Program in 1996 as part of the "Pathogen Reduction; Hazard Analysis and Critical Control Point (PR/HACCP) Systems; Final Rule" *(14)*. Under this program, FSIS assesses industry performance and controls for reducing *Salmonella* contamination in raw meat and poultry products. As a result of this final rule, poultry processing facilities were encouraged to develop sanitation strategies to meet performance standards. As these standards have progressively become more stringent since 1996 *(15),* producers have gradually adopted a multihurdle approach *(12)* to reduce *Salmonella* bacteria at the different stages of processing. It is generally agreed that a multihurdle approach during poultry processing delivers the most effective means of reducing the prevalence of *Salmonella*. In this approach, various physical and chemical interventions may be utilized at different processing points to reduce *Salmonella* numbers. In their compliance guideline *(16),* FSIS recommends a postchill antimicrobial dip or spray as a final treatment for poultry carcasses. Many processors currently incorporate a postchill treatment *(10, 11)* using chemical antimicrobial treatments deemed safe and suitable by the FSIS *(17)*. At this last stage of a multihurdle process, antimicrobials can be used at higher concentrations without affecting product quality due to shorter contact times. Because of the higher concentrations, however, it is also the stage at which there is the most potential for sanitizer carryover. The antimicrobial chemicals used postchill include peroxyacetic acid (PAA) *(1),* acidified sodium chlorite (ASC) *(8),* cetylpyridinium chloride (CPC) *(9),* 1,3-dibromo-5,5-dimethylhydantoin (DBDMH) *(7),* and a mixture of citric and hydrochloric acid, all of which have demonstrated bactericidal action via various mechanisms.

Subsequent to the final antimicrobial treatment, carcasses may be removed from the line for routine testing according to the FSIS protocol *(18)*. This protocol calls for the carcass to be rinsed in a pre-enrichment solution of buffered peptone water (BPW) and the resultant liquid (rinsate) to be sealed, refrigerated, and shipped to an FSIS laboratory for *Salmonella* detection. BPW is used because it facilitates the recovery of sublethally injured *Salmonella* bacteria *(5)*. Even though it was not originally designed as a neutralizing broth, BPW may have a capacity for neutralization of some sanitizers, such as low-concentration oxidizers, due to the presence of reducing thiols in peptone, and moderately high- or low-pH solutions, due to the presence of phosphate buffer. However, some antimicrobials may not be neutralized by BPW, and the resulting carryover

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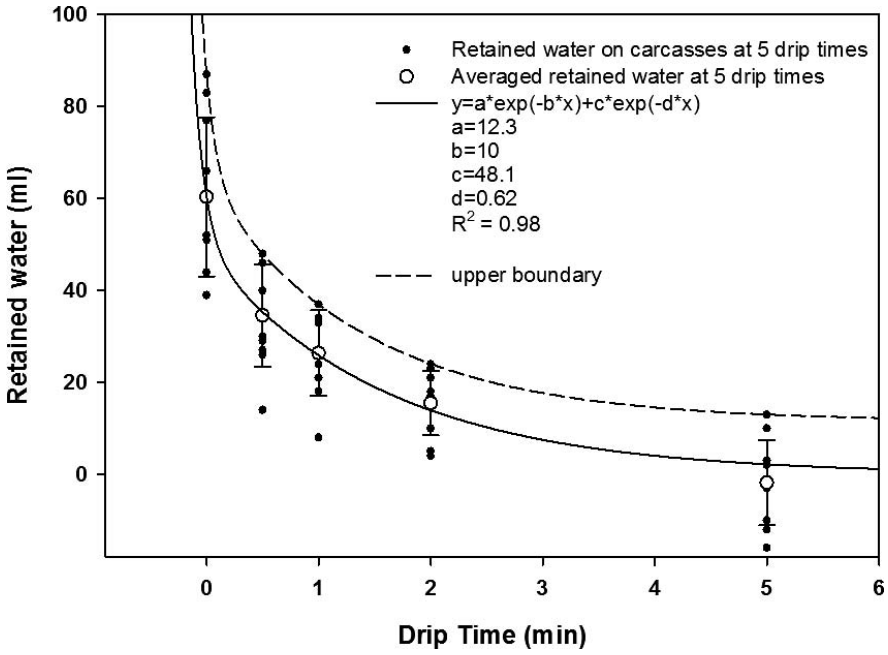

FIGURE 1. *The effect of drip times of postchill dipped broiler carcasses on the volumes of simulated intervention solution carryover.*

of an insufficiently neutralized sanitizer in carcass rinsate can potentially lead to false-negative detection of *Salmonella* bacteria present in the BPW. The goal of the present study was to determine whether the sanitizing chemicals routinely utilized by processors as postchill poultry carcass *Salmonella* interventions are sufficiently neutralized by BPW to prevent false-negative results. The sanitizers chosen for this study represent those most commonly used and have a range of sanitizing modes, i.e., oxidizers, acids, and membrane disruptors. The present research will be used to evaluate the effectiveness of the current protocol for utilizing BPW as a pre-enrichment broth for detection of *Salmonella* bacteria in rinsate collected from broiler carcasses.

MATERIALS AND METHODS

Effect of drip time on volume of water (simulated intervention solution) carryover. Ten male broilers at 42 days of age from the University of Georgia Poultry Research Facility (Athens, GA) were subjected to feed withdrawal for up to 12 h. All birds were transported less than 30 min to the USDA Agricultural Research Service U.S. National Poultry Research Center's pilot processing plant, weighed individually to ensure minimal variability in bird size, leg banded, and placed into shackles by their feet. The broilers were stunned using a brine stunner, slaughtered using an automatic rotary blade, severing both carotid arteries and the jugular vein, and bled for 2 min. All carcasses were scalded in a triple tank and defeathered for 30 s using a single-pass mechanical picker. The neck, feet, and viscera were manually removed from each carcass, and the thoracic inlet was cleared of any remaining tracheal, crop, or esophagus tissue. The weight of each carcass was recorded (prechill weight), following which the carcasses were chilled for 40 min in an air-agitated ice-water immersion chiller. Following chilling, the carcasses were hung in a shackle by a wing for 5 min to allow chill water and ice to drip from the carcass and reweighed (postchill weight). The carcasses were next subjected to a water dip treatment. The carcasses were hand dipped with agitation for 30 s in an 80-liter bucket filled with tap water. After 30 s, the carcasses were hung either by one leg ($n = 5$) or by one wing ($n = 5$) in a shackle suspended from a scale. Weights were recorded at 0, 0.5, 1.0, 2.0, and 5.0 min postdip. The difference between the postchill weight and the weight after the postdip drip time was defined as the volume of the intervention solution retained at that time interval, assuming 1 g equals 1 ml.

Carcass rinsates. Thirty-six broiler carcasses were chilled in ice water with air agitation for 40 min as described above. The chilled carcasses were subjected to a 60-s whole-carcass rinse in 400 ml of BPW using a mechanical shaking machine. Rinsates from the 36 carcasses were combined and divided into three equal volumes for replication.

Sanitizer stock solutions. CPC stock solution was prepared by adding 8.0 g of CPC (Sigma-Aldrich, St. Louis, MO) and 12.0 g of propylene glycol (Sigma-Aldrich) to 1 liter of deionized water. PAA stock solution was prepared by diluting 5.13 ml of 39% PAA in acetic acid (Sigma-Aldrich) to 1 liter using deionized water, for a final concentration of 2,000 ppm. ASC stock solution was prepared by dissolving 1.2 g of sodium chlorite (Pfaltz & Bauer, Waterbury, CT) in 100 ml of deionized water prior to adding 900 ml of a pH 2.2 acetic acid solution. The final concentration was 1,200 ppm of sodium chlorite, pH 2.3. The DBDMH stock solution was prepared by dissolution of 0.18 g of DBDMH (Albemarle Corp., Baton Rouge, LA) in 1 liter of deionized water, with a final concentration of 100 ppm of available bromine. The acid solution was prepared by diluting 5.0 ml of 10 M hydrochloric acid (Sigma-Aldrich) to 500 ml using deionized water. To this solution, 15 g of citric acid (Sigma-Aldrich) was added and dissolved, and the resulting solution diluted with deionized water to a final pH of 1.0. PAA, ASC, and DBDMH solutions were prepared 10 min prior to use.

Drip time-equivalent sanitizer-rinsate solutions. The calculated (based on regression) volumes of intervention solution retained postdip at the time intervals of 0, 1, and 5 min (see "Results and Discussion" and Fig. 1) were used to prepare approximately equivalent sanitizer-rinsate solutions. For the sake of efficiency and to ensure an adequate supply of rinsate, experiments were conducted using 200 ml of test solutions instead of 400 ml. For 0-min drip time equivalent solutions, 30 ml of each sanitizer stock solution was diluted to 200 ml using carcass rinsate;

TABLE 1. *Amounts of residual water remaining on broiler carcasses following postchill dip treatment when hung by either legs or wings for 0 to 5 min*[a]

Hang position	Mean (SD) amt (g) of water retained on carcasses after:				
	0 min	0.5 min	1 min	2 min	5 min
Wing ($n = 5$)	58.8 (19.9) A	31.6 (13.0) B	23.6 (10.5) BC	13.6 (6.7) C	−1.2 (10.5) D
Leg ($n = 5$)	62.0 (9.2) A	37.6 (9.2) B	29.2 (7.9) BC	17.4 (7.6) C	−2.4 (9.0) D
Combined	60.4 (17.3) A	34.6 (11.1) B	26.4 (9.3) BC	15.5 (7.0) C	−1.8 (9.3) D

[a] Values are pairwise multiple comparisons by the Holm-Sidak method. Values in columns and rows followed by different letters are significantly different ($P \leq 0.05$).

for the 1-min drip time equivalent solutions, 13 ml of each sanitizer stock solution was diluted to 200 ml using carcass rinsate; and for 5-min drip time equivalent solutions, 1 ml of each sanitizer stock solution was diluted to 200 ml using carcass rinsate. In each experiment, three replicates were performed for each sanitizer.

***Salmonella* isolate preparation and inoculation.** Isolates of *Salmonella enterica* serovars Kentucky, Enteritidis, Typhimurium var. 5, Heidelberg, and Hadar were used in the study. *Salmonella* Kentucky and *Salmonella* Enteritidis cultures were provided by the Bacterial Epidemiology and Antimicrobial Resistance Unit of the U.S. National Poultry Research Center, Athens, GA, and *Salmonella* Typhimurium, *Salmonella* Heidelberg, and *Salmonella* Hadar cultures were provided by the Eastern Laboratory of the FSIS in Athens, GA. Isolates with resistance to 100 ppm of nalidixic acid were selected for all serotypes. Fresh *Salmonella* cultures were prepared by incubating individual isolates in 200 ml of Trypticase soy broth (BD, Sparks, MD) for 18 to 24 h at 37°C in a gyratory water bath shaker at 125 rpm. Fifty-milliliter aliquots of cultures were centrifuged at room temperature for 10 min at 5,000 rpm in a Hermle Z300 centrifuge (Labnet International, Edison, NJ). The supernatant was decanted, and the pellets were suspended in 2 ml of 0.1% Bacto peptone (BD). Bacterial suspensions were added to 10 ml of 0.1% Bacto peptone in a Bellco Nephelo culture flask (Bellco Glass, Inc., Vineland, NJ) to achieve an optical density at 625 nm equivalent to 10^9 CFU/ml as measured by a Spectronic 20D+ spectrophotometer (Milton Roy, Wilmington, DE). A mixed culture of the five *Salmonella* isolates was prepared by combining a 1-ml aliquot of each serotype to produce a final volume of 5 ml containing 10^9 *Salmonella* bacteria per ml. The mixed culture was serially diluted in 0.1% peptone to produce a suspension containing 10^7 CFU/ml, and the number of CFU of *Salmonella* bacteria per milliliter was confirmed by spreading serial dilutions of the culture on brilliant green sulfa agar (BD) with 100 ppm of nalidixic acid (Sigma-Aldrich) and counting CFU after incubation at 35°C for 48 h. One milliliter of the *Salmonella* suspension was added to 99 ml of whole-carcass rinsate, and the inoculated rinsates were stored at 4°C for 24 h.

Enumeration. Following overnight cold storage to simulate shipping, serial dilutions of inoculated rinsates were surface plated in triplicate on brilliant green sulfa agar supplemented with nalidixic acid (100 ppm) and incubated aerobically at 35°C for 24 h. Colonies characteristic of *Salmonella* were counted; one colony from each sample was randomly selected and confirmed as *Salmonella* using triple sugar iron agar and lysine iron agar slants and *Salmonella* polyvalent O antiserum.

pH measurements. pH measurements were performed on all drip time equivalent sanitizer solutions using an Orion Star A111 pH meter (Thermo Scientific, Waltham, MA).

Statistical analysis and data regression. The numbers of *Salmonella* colonies counted were log transformed. The geometric means of the resulting counts were calculated and compared using Student's *t* test. One-way analysis of variance was performed on carcass residual water content following dip treatment at different times postdip, and subsequent data regression was performed. All statistical methods were performed using Sigmaplot 12.3 (Systat Software, Inc., San Jose, CA).

RESULTS AND DISCUSSION

No significant differences in mean retained water, as a percentage of carcass weight, were observed between carcasses hung by wings ($n = 5$) and those hung by legs ($n = 5$) at the five drip time intervals studied (0, 0.5, 1, 2, and 5 min; $P \leq 0.05$), as seen by the results in Table 1. Data on all 10 carcasses were thereafter treated as a single sample set ($n = 10$). Statistically significant ($P \leq 0.05$) differences in the amount of retained water as a function of drip time were observed except at the 1-min interval, which was not different from either the 0.5- or 2-min interval.

The effects of the drip times of postchill dipped carcasses on the volumes of simulated intervention solution carryover are presented in Figure 1. The mean amount of retained water at each drip time is shown, along with the individual data for each carcass. Due to the high observed variability at each time point, the data were fit to a bi-exponential regression equation in order to obtain solutions for the values to be used as drip time equivalents at 0, 1, and 5 min. These values are 60, 26, and 2 ml, respectively, to be added to 400 ml of BPW rinsate. Drip time equivalent solutions at 0, 1, and 5 min were next prepared for each of the five intervention solutions, as described in Materials and Methods. The solutions were then divided into separate aliquots, with 100 ml being used for pH testing both immediately and after 24 h of storage and 99 ml for inoculation with 1 ml of a 10^7 CFU/ml mixture of the five nalidixic acid-resistant *Salmonella* serovars. The resultant inoculated suspension was stored for 24 h, following which *Salmonella* bacteria were enumerated. The results of the enumeration and pH measurements are shown in Table 2.

No *Salmonella* bacteria were recovered from solutions with 0- or 1-min PAA drip time equivalents of 300 and 130 ppm, respectively. In addition, the pH measurements of both suspensions were significantly lower than that of the water control. At the 5-min drip time equivalent (10 ppm of PAA), however, the number of CFU per milliliter remaining after 24 h was indistinguishable from that in the water control, and the suspension pH was only slightly lower. No

TABLE 2. *Microbial counts and pH values for sanitizer solutions at different broiler carcass drip time equivalents 24 h after inoculation with a* Salmonella *suspension*[a]

Treatment[b]	Vol (ml/200 ml)	Initial concn (ppm)[c]	Drip time equivalent (min)	Log CFU/ml (95% CI)	*P* value (*t* test vs control)	pH	*P* value (*t* test vs control)
Water control	1	NA[d]	NA	5.3 (0.4)	NA	7.40	NA
PAA	30	300	0	0 (0.0)	<0.00001	6.64	<0.00001
PAA	13	130	1	0 (0.0)	<0.00001	7.06	<0.00001
PAA	1	10	5	5.4 (0.1)	0.53	7.36	0.003
CPC	30	1,200	0	0 (0.0)	<0.00001	7.40	0.725
CPC	13	520	1	0 (0.0)	<0.00001	7.41	0.492
CPC	1	40	5	4.0 (0.4)	0.003	7.41	0.374
ASC	30	180	0	0 (0.0)	<0.00001	3.60	<0.00001
ASC	13	78	1	0 (0.0)	<0.00001	4.03	<0.00001
ASC	1	6	5	5.2 (0.1)	0.89	6.70	<0.00001
Acid	30	15[e]	0	4.0 (1.3)	0.03	3.03	<0.00001
Acid	13	6.5[e]	1	5.5 (0.1)	0.38	4.60	<0.00001
Acid	1	1[e]	5	5.5 (0.0)	0.48	7.18	<0.00001
DBDMH	30	27	0	5.4 (0.0)	0.55	7.40	0.374
DBDMH	13	12	1	5.5 (0.0)	0.39	7.40	0.374
DBDMH	1	1	5	5.4 (0.1)	0.53	7.39	0.116

[a] The *Salmonella* inoculum was 10^5 CFU/ml.
[b] PAA, peroxyacetic acid; CPC, cetylpyridinium chloride; ASC, acidified sodium chlorite; DBDMH, 1,3-dibromo-5,5-dimethylhydantoin.
[c] By calculation based on dilution of sanitizer stock concentration.
[d] NA, not applicable.
[e] Value indicates concentration of free protons (ppm).

Salmonella bacteria were recovered from solutions with 0- or 1-min CPC drip time equivalents of 1,200 and 520 ppm, respectively. At the 5-min drip time equivalent (40 ppm of CPC), the number of CFU per milliliter remaining after 24 h was 5% of that in the control, indicating significant residual bactericidal activity. No *Salmonella* bacteria were recovered from solutions with 0- or 1-min ASC drip time equivalents of 180 and 78 ppm, respectively. For the 5-min drip time solution, containing 6 ppm of ASC, the number of CFU per milliliter remaining after 24 h was indistinguishable from that in the water control. The pH values of the ASC solutions were all significantly lower than that of the control solution, and in the case of the 0- and 1-min drip time equivalent solutions, the pH values remained at low-enough levels (3.60 and 4.03, respectively) to potentially sublethally injure or inhibit the growth of *Salmonella* bacteria *(3, 4, 13)*. The pH values of all CPC drip time equivalent solutions were indistinguishable from that of the control. The citric acid–hydrochloric acid solution showed no residual bactericidal activity in either the 1- or 5-min drip time equivalent solution (pH values of 4.60 and 7.18, respectively). The 0-min solution (pH 3.03), however, appeared to retain some bactericidal activity, with approximately 5% *Salmonella* numbers remaining compared to the number in the water control, though the CFU counts between the three replicates for this suspension had a much-higher 95% confidence interval than any of the other sanitizer CFU counts, making any conclusion subject to some uncertainty. DBDMH exhibited no residual bactericidal activity in any of the three drip time-equivalent suspensions, and the pH was unaffected in all three cases.

At the 0- and 1-min drip time equivalents, three of the five sanitizers studied (PAA, ASC, and CPC) displayed statistically significant ($P < 0.0001$) carryover activity, with 0% *Salmonella* detected. At the 5-min drip time equivalent, CPC still exhibits substantial and significant activity that may potentially lead to some false-negative indications for *Salmonella*. In addition, there appears to be a correlation between low pH and *Salmonella* recovery, as evidenced by the 0-min acid mixture, where recovery was only 5% compared to that in the water control. Low pH may also be a factor in the ASC bactericidal activity. These results are all based upon the sanitizer carryover values at 0, 1, and 5 min obtained from the regression equation in Figure 1. Also seen in Figure 1, however, is the upper boundary curve for retained water, based upon the highest observed value at each measured time interval. The implication of this curve is that the carryover effect may in some cases be much greater than that predicted by regression. In the case of the 5-min drip time retention, the upper boundary is 13 ml, or 6.5 times greater than the value of 2 ml used to prepare drip time equivalent solutions in the current study. Subsequent work on water carryover on postchill carcass dips *(2)* suggests that this carryover can be as high as 77 ml at the 5-min drip time interval for some carcasses hung by the leg, greater even than the 60-ml value used as the 0-min drip time equivalent in the present work. The extreme variability seen in water retention among carcasses is not completely understood but may be due to water remaining in the body cavity, even though the thoracic inlet was opened during manual evisceration, or to pockets of water collecting under the skin during agitation when dipped. In such extreme cases, the amount of sanitizer carryover poses a higher probability that false negatives will occur in the enumeration of *Salmonella* bacteria in the BPW rinsate, even when the

carcass is allowed to drip for 5 min or longer prior to rinsing in BPW.

Based on the results of the present study, BPW may not be capable of sufficiently neutralizing a variety of sanitizing solutions displaying different modes of bactericidal action in order to obtain reliable enumeration of *Salmonella* on postchill dipped chicken carcasses at the processing line. This, in combination with the wide variability in retained water observed between carcasses, suggests that current procedures for the isolation and identification of *Salmonella* on poultry carcasses *(18)* may need modification. Variability in water retention may be reduced by physical shaking of the carcass following the postchill dip and prior to rinsing in BPW in order to remove pockets of trapped intervention solution. There is also a need to develop a modified solution for use in facilitating the recovery of sublethally injured *Salmonella*. Several neutralizing broths are available, including Dey-Engley broth *(6),* incorporating a variety of chemical agents capable of neutralizing a range of antimicrobial chemicals for recovery of viable bacteria. In the case of poultry carcass examination, neutralizers for CPC, PAA, ASC, and acid are suggested as modifiers to BPW. Studies are currently in progress at the U.S. National Poultry Research Center to identify and validate the use of potential modifiers for neutralizing these different bactericides in BPW.

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