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Validation of Individual and Multiple-Sequential Interventions for Reduction of Microbial Populations during Processing of Poultry Carcasses and Parts

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ABSTRACT

Changes in aerobic plate counts (APC), total coliform counts (TCC), *Escherichia coli* counts (ECC), and *Salmonella* incidence on poultry carcasses and parts and in poultry processing water were evaluated. Bacterial counts were estimated before and after individual interventions and after poultry carcasses were exposed to multiple-sequential interventions at various stages during the slaughter process. Individual and multiple-sequential interventions were evaluated at three processing plants: (i) plant A (New York wash, postevisceration wash, inside-outside bird washes 1 and 2, chlorine dioxide wash, chlorine dioxide wash plus chlorine chiller, chiller exit spray, and postchiller wash), (ii) plant B (New York wash, inside-outside bird washes 1 and 2, trisodium phosphate wash, and chlorine chiller), and (iii) plant C (trisodium phosphate wash and chlorine chiller). The majority of individual interventions effectively or significantly ($P < 0.05$) reduced microbial populations on or in carcasses, carcass parts, and processing water. Reductions in APC, TCC, and ECC due to individual interventions ranged from 0 to 1.2, 0 to 1.2, and 0 to 0.8 log CFU/ml, respectively. Individual interventions reduced *Salmonella* incidence by 0 to 100% depending on the type of process and product. Multiple-sequential interventions resulted in significant reductions ($P < 0.05$) in APC, TCC, ECC, and *Salmonella* incidence of 2.4, 2.8, and 2.9 log CFU/ml and 79%, respectively, at plant A; 1.8, 1.7, and 1.6 log CFU/ml and 91%, respectively, at plant B; and 0.8, 1.1, and 0.9 log CFU/ml and 40%, respectively, at plant C. These results enabled validation of in-plant poultry processing interventions and provide a source of information to help the industry in its selection of antimicrobial strategies.

Birds arrive at processing facilities with high bacterial loads both internally in their viscera and externally on feathers and skin. During processing, such contamination will inevitably be transferred to the processing equipment, carcasses, and final product *(3)*. In 1996, the U.S. Department of Agriculture Food Safety and Inspection Service (USDA-FSIS) published regulations for meat and poultry inspection, applying the concept of hazard analysis and critical control point (HACCP) systems and including microbiological performance criteria for *Escherichia coli* and *Salmonella* for evaluating HACCP compliance at processing facilities *(17)*. In an attempt to comply with zero tolerance requirements for visible contamination on carcasses and to meet microbiological performance criteria under the HACCP final rule *(17)*, many meat and poultry establishments began implementing or further supplementing antimicrobial intervention strategies.

Many of the intervention strategies for meat and poultry involve the use of antimicrobial solutions in rinses and spray washes; however, no individual intervention is adequate to ensure the necessary reduction of microbiological hazards on carcasses throughout the process. Reducing the microbial hazards on finished carcasses requires implementation of multiple-sequential interventions, the multiple-hurdle approach *(1)*. The major categories of interventions used in the poultry industry include (i) a scald tank, which is a common bath containing hot water (42 to 60°C) in which birds are submerged; (ii) rinse and/or spray washes, which are applications of antimicrobial solutions at various places in the processing line (postpicking, postevisceration, prechilling, and postchilling); (iii) on-line reprocessing, in which automated reprocessing systems deluge and/or spray carcasses with antimicrobial solutions to ensure that visible contamination is removed; and (iv) carcass chillers, which chill carcasses by immersion in a common bath containing an antimicrobial solution such as chlorine.

Recently, the USDA-FSIS has become increasingly concerned about the control of microbiological hazards, especially *Salmonella,* on poultry during processing *(21)*. Regulators are requiring that more processing facilities conduct verification and validation of their intervention strategies to provide evidence that their interventions are effective in controlling microbiological hazards.

The objective of this study was to evaluate the effect of individual and multiple-sequential interventions during processing on bacterial populations and *Salmonella* incidence on poultry carcasses and parts and in processing water in three commercial processing facilities in the western United States.

MATERIALS AND METHODS

Antimicrobial interventions. The study was conducted in three commercial processing plants (A, B, and C) in the western

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TABLE 1. *Collection of samples (carcasses or carcass parts) used to evaluate the effects of independent and multiple-sequential interventions on microbial populations during poultry processing*[a]

Intervention evaluation	Poultry processing plants: A			B			C		
	Intervention	No. of samples[b] Pre	Post	Intervention	No. of samples[b] Pre	Post	Intervention	No. of samples[b] Pre	Post
In sequence[c]	New York wash	75	75	New York wash	75	75	TSP wash	75	75
	Postevisceration wash	75	75	IOBW1	75	75	CI_2 chiller	75	75
				IOBW2	75	75			
	IOBW1	75	75	TSP wash	75	75			
	IOBW2	75	75	Cl_2 chiller	75	75			
	ClO_2 wash	75	75						
	ClO_2/Cl_2 chiller	75	75						
	Chiller exit spray	75	75						
	Postchiller wash	75	75						
Independently[d]	Dropped carcass wash	75	75	Dropped carcass wash	75	75	Dropped product wash	75	75
	Dropped product wash	75	75	Red water system	75	75	Red water system	75	75
				Neck tube chiller	75	75	Product dip	75	75
	Neck tube chiller	75	75	Liver tube chiller	75	75	Neck ice chiller	75	75
	Liver tube chiller	75	75	Heart tube chiller	75	75			
	Heart tube chiller	75	75	Gizzard tube chiller	75	75			
	Gizzard tube chiller	75	75						
Total no. of samples		1,050	1,050		825	825		450	450

[a] IOBW, inside-outside bird wash; ClO_2, chlorine dioxide; Cl_2, chlorine; TSP, trisodium phosphate.
[b] Evaluation of antimicrobial interventions was achieved by collecting 75 separate samples before (Pre) and 75 separate samples after (Post) each intervention. All 75 samples in each case were analyzed for *Salmonella*, but only 50 of the 75 samples were analyzed for microbial counts.
[c] Samples collected before and after individual interventions applied sequentially along the evisceration line.
[d] Samples collected before and after individual interventions applied independently rather than sequentially.

United States from January through December 2005. Plants A, B, and C operated with evisceration chain speeds of 140, 275, and 90 carcasses per minute, respectively. Individual interventions evaluated were (i) New York (NY) wash, spray application of 20 to 50 ppm chlorinated water following defeathering; (ii) post-evisceration wash, spray application of 20 to 50 ppm chlorinated water following carcass evisceration; (iii) inside-outside bird wash 1 (IOBW1), spray application of 20 to 50 ppm chlorinated water following neck removal; (iv) IOBW2, spray application of 20 to 50 ppm chlorinated water following IOBW1; (v) chlorine dioxide (ClO_2) wash, spray application immediately before carcass chilling of a solution prepared by acidifying 500 to 1,200 ppm sodium chlorite with citric acid to pH 2.5 to 2.9; (vi) trisodium phosphate (TSP) wash, spray application of 8 to 12% TSP immediately before carcass chilling; (vii) chlorine (Cl_2) chiller, a carcass chilling by immersion in 20 to 50 ppm chlorinated water (these chillers were operated at pH 6.5 to 7.0 according to the facilities' HACCP plans); (viii) ClO_2-Cl_2 chiller, carcass chilling by immersion in a combination of ClO_2 (prepared by acidifying 50 to 150 ppm sodium chlorite with citric acid to pH 2.8 to 3.2) and 20 to 50 ppm chlorinated water (this chiller was operated at pH 6.5 to 7.0 according to the facility's HACCP plan); (ix) chiller exit spray, spray application of 20 to 50 ppm chlorinated water immediately following carcass chilling; (x) postchiller wash, spray application of 20 to 50 ppm chlorinated water immediately following carcass sizing; (xi) dropped carcass wash, spray application of 20 to 50 ppm chlorinated water used to treat product dropped on the floor; (xii) dropped product wash, spray application of 20 to 50 ppm chlorinated water used to treat carcass cuts dropped on the floor; (xiii) red water system, recirculated chiller water with up to 160 ppm chlorinated water; (xiv) product dip, immersion of carcass cuts in a solution prepared by acidifying 500 to 1,200 ppm sodium chlorite with citric acid to pH 2.5 to 2.9; (xv) neck tube chiller, chilling of carcass necks by immersion in 20 to 35 ppm chlorinated water; (xvi) neck ice chilling, chilling of carcass necks by layering in fresh ice; (xvii) liver tube chiller, chilling of carcass liver by immersion in 20 to 35 ppm chlorinated water; (xviii) heart tube chiller, chilling of carcass heart by immersion in 20 to 35 ppm chlorinated water; and (xix) gizzard tube chiller, chilling of carcass gizzard by immersion in 20 to 35 ppm chlorinated water.

Study design. Samples of poultry carcasses, parts, and processing water were collected at 19 stages of poultry processing at three commercial poultry plants located in the western United States. The individual interventions were evaluated as independent treatments or as part of multiple treatments applied in sequence along the evisceration (slaughter) line (Table 1). Some of the interventions were used at all three processing plants, and the effect of these shared interventions are presented as the average of all sample sets. At each intervention, 75 separate samples were randomly collected before (Pre) and after (Post) each intervention by first collecting 15 Pre and then 15 Post samples on five independent days at each plant to avoid resampling the same carcass once it was reintroduced into the process. Sampling at each intervention

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was preceded by verification that each intervention was operating as specified in the plant's HACCP program. For each 75-sample set, all samples were analyzed for the presence of *Salmonella* but only 50 samples were analyzed for bacterial load, based on statistical power of 80%, an anticipated standard deviation (SD) of 0.8 log CFU/ml *(5),* and a desired resolution of 0.5 log CFU/ml.

Sample collection: whole carcass rinse sampling. Carcass rinse samples were collected before and after each intervention according to the guidelines published in the USDA-FSIS *Microbiology Laboratory Guidebook (20).* Carcasses were aseptically removed from the line with individually packaged sterile deli-style gloves (International BioProducts, Bothell, Wash.), and excess residual fluid was drained for 30 s. Each individual carcass was placed in a sterile Stomacher 3500 bag (International BioProducts), and 400 ml of buffered peptone water (BPW; International BioProducts) was poured into the cavity of the carcass. Bags were rocked with a reciprocal motion in a 45 to 60 cm arc for approximately 60 s to assure that all surfaces, both interior and exterior, were rinsed. Sample rinse fluid was subsequently collected in the original BPW containers, stored in an insulated ice chest with frozen ice packs, and transported to the laboratory for further analysis. Neutralization of samples due to dripping and buffering by BPW was confirmed by testing (acid/alkali test papers, Whatman, Florham Park, N.J.) the liquid remaining in the bag following collection of the rinsate in the bottles.

Sample collection: carcass parts sampling. Samples were collected from poultry necks, gizzards, hearts, livers, and other products (carcass cuts including breast filets, tenderloins, drumsticks, wings, thighs, and front halves) using methods adapted from the USDA-FSIS *Microbiology Laboratory Guidebook (20).* Carcass parts were picked from the line using sterile deli-style gloves and allowed to drain for 30 s before being placed into a sterile 18-oz (510.3-g) Whirl-Pak bag (Nasco, Fort Atkinson, Wis.). One hundred milliliters of sterile BPW (Becton, Dickinson, Sparks, Md.) was added to the bag, and a reciprocal rocking motion as described above was used to rinse the carcass parts. Following rinsing, poultry parts were aseptically removed from each bag. Sample rinse fluid was retained in the bag, stored in an insulated ice chest with frozen ice packs, and transported to the laboratory for further analysis. Neutralization of samples due to dripping and buffering by BPW was confirmed by testing (acid/alkali test papers) the liquid remaining in the bag following collection of the rinsate in the bottles.

Sample collection: process water sampling. Reuse water (red water) recirculated through a chilling system and reintroduced into the chiller bath was tested by collecting samples from water immediately before entering the chilling system (Pre) and immediately after chilling (Post) and entry into the chiller bath. Samples were collected in sterile 125-ml Nalgene bottles (Nalge Nunc, Inc., Rochester, N.Y.) containing 0.1% (wt/vol) sodium thiosulfate (ReagentPlus, 99%, Sigma-Aldrich, St. Louis, Mo.).

Microbiological analysis. Samples were analyzed for aerobic plate count (APC), total coliform count (TCC), and biotype I *E. coli* count (ECC) using Petrifilm aerobic count plates and *E. coli*–coliform count plates (3M, St. Paul, Minn.). Serial dilutions were made by initially transferring 0.1 ml of sample into 9.9 ml of sterile 0.1% BPW (Fisher Scientific, Houston, Tex.). One milliliter was aseptically removed from appropriate dilutions and plated onto Petrifilm plates, which were incubated at 37°C for 24 h. Colonies that exhibited the expected phenotypic characteristics were counted according to the manufacturer's instructions.

Sample enrichment for detection of *Salmonella*. Following microbial analysis, samples were enriched for *Salmonella* analysis using prewarmed (42°C) Trypticase soy broth (TSB; Becton Dickinson) added to 30 ml of rinsate for a final concentration of 1× TSB. Sample enrichments were incubated for 18 to 24 h at 37°C.

***Salmonella* analysis.** After incubation, enrichment cultures were screened for *Salmonella* using the Reveal *Salmonella* test (Neogen, Lansing, Mich.). A portion of enrichment culture (120 μl) was placed onto the test device, and results were interpreted after 15 min at room temperature (25°C). A presumptive-positive result was defined as a line in both the control and test zones of the results window according to the manufacturer's instructions. Cultures that produced a line only in the control zone were considered negative for *Salmonella.*

Presumptive-positive enrichment cultures were streaked onto xylose lysine tergitol 4 agar (XLT4 agar; Hardy Diagnostics, Santa Maria, Calif.) for isolation of *Salmonella* and incubated for 48 h at 37°C. Isolated colonies exhibiting fermentation of xylose, lactose, and sucrose and production of hydrogen sulfide on XLT4 agar were considered suspect *Salmonella* and were inoculated onto tryptic soy agar with 5% sheep blood (Hardy Diagnostics). These plates were subsequently incubated for 24 h at 37°C. Colonies were then tested using the S3 PCR assay for *Salmonella* (Molecular Epidemiology, Inc., Seattle, Wash.) *(14).* Samples producing positive banding patterns in the PCR assay were considered confirmed for *Salmonella.*

Data and statistical analysis. Microbial counts (APC, TCC, and ECC) were converted to log CFU per milliliter to accommodate the anticipated wide fluctuation common to microbiological data. Mean values of the log-transformed counts were compared using a two-sample Student's t test to determine whether there was a significant difference in the microbiological populations following the application of various interventions. *Salmonella* incidence rates were transformed into two-way contingency tables for each treatment, and the incidence rates before (Pre) and after (Post) the application of the treatment were compared using the chi-square test of independence. Fisher's exact test was used whenever the expected frequencies fell below 5%. Treatment effects on the microbiological counts distributed longitudinally through the processing line were compared using an analysis of variance employing the general linear model procedure (PROC GLM) accompanied by Duncan's multiple range test. Statistical analyses were performed with SAS version 8.2 *(13).* Differences were considered significant at $\alpha = 0.05$.

RESULTS AND DISCUSSION

To allow poultry processors to comply with regulatory criteria and run cost-effective operations, it is essential to evaluate the performance of their antimicrobial interventions. Processing steps designated as antimicrobial interventions and included in a facility's HACCP program should continuously be validated to determine which steps are control points and which are critical control points and whether these interventions are operating as intended to reduce, control, or eliminate microbiological hazards. These evaluations will allow processors to make informed decisions about whether to change or modify an intervention to operate as intended or remove the intervention from the HACCP program when this intervention does not reduce, control, or eliminate microbiological hazards. The aim of this study was to determine the effect of individual and

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TABLE 2. *Microbial populations and* Salmonella *incidence for carcasses before (Pre) and after (Post) interventions*[a]

Intervention	Stage	Mean (SD) counts (log CFU/ml)			*Salmonella* incidence (%)
		Aerobic plates	Total coliforms	*E. coli*	
New York wash	Pre	4.5 (0.5) A	3.3 (0.8) A	3.1 (0.8) A	34 A
	Post	4.3 (0.4) B	3.1 (0.7) A	2.9 (0.8) A	26 B
Postevisceration wash	Pre	3.9 (0.5) A	2.8 (0.8) A	2.6 (0.8) A	45 A
	Post	4.0 (0.7) A	3.1 (0.9) A	2.8 (1.0) A	36 B
Inside-outside bird wash 1 (IOBW1)	Pre	3.8 (0.5) A	2.8 (0.7) A	2.5 (0.7) A	25 A
	Post	3.7 (0.7) A	2.5 (0.9) B	2.2 (0.9) B	20 A
Inside-outside bird wash 2 (IOBW2)	Pre	3.7 (0.6) A	2.5 (0.9) A	2.3 (0.9) A	16 A
	Post	3.4 (0.7) B	2.2 (0.9) B	2.0 (1.1) B	12 A
Chlorine dioxide wash	Pre	3.2 (0.5) A	2.2 (0.8) A	1.9 (0.9) A	17 A
	Post	3.0 (0.5) B	1.8 (0.9) B	1.5 (1.0) B	9 B
Trisodium phosphate wash	Pre	3.6 (0.5) A	2.3 (0.6) A	2.0 (0.6) A	10 A
	Post	3.1 (0.6) B	1.5 (0.7) B	1.3 (0.7) B	3 B
Chlorine chiller	Pre	3.3 (0.6) A	1.6 (0.6) A	1.3 (0.6) A	5 A
	Post	2.8 (0.6) B	1.2 (0.6) B	1.0 (0.6) B	7 A
Chlorine dioxide plus chlorine chiller	Pre	3.1 (0.5) A	1.7 (0.8) A	1.4 (0.9) A	4 A
	Post	1.9 (0.5) B	0.7 (0.4) B	0.6 (0.4) B	14 B
Chiller exit spray	Pre	2.1 (0.5) A	<0[b]	<0[b]	8 A
	Post	2.3 (0.5) B	<0[b]	<0[b]	8 A
Postchiller wash	Pre	2.3 (0.4) A	1.1 (0.6) A	0.9 (0.6) A	10 A
	Post	2.1 (0.4) B	0.9 (0.6) B	0.6 (0.5) A	4 A
Dropped carcass wash	Pre	3.1 (0.9) A	1.0 (0.6) A	0.5 (0.5) A	16 A
	Post	2.7 (0.6) B	1.0 (0.6) A	0.3 (0.5) B	8 B

[a] Within each intervention, Pre and Post values followed by different letters are significantly different at $\alpha = 0.05$.
[b] Limit of detection.

multiple-sequential interventions during processing on bacterial populations and *Salmonella* incidence on poultry carcasses and parts and in processing water in three commercial poultry operations.

Individual interventions applied to carcasses. The majority of individual interventions effectively if not significantly ($P < 0.05$) reduced bacterial populations (APC, TCC, and ECC) on carcasses (Table 2). The trend in reduction of bacterial populations on poultry carcasses was as follows: ClO_2-Cl_2 chiller (0.8- to 1.2-log reduction) > TSP wash (0.5- to 0.8-log reduction) > Cl_2 chiller (0.3- to 0.5-log reduction) > NY wash, postevisceration wash, IOBW1, IOBW2, ClO_2 wash, chiller exit spray, postchiller wash, and dropped carcass wash (0- to 0.4-log reduction).

Carcass chillers are used in poultry operations to aid in compliance with current regulations specifying reduction of the internal temperature of the carcass to ≤4.4°C (≤40°F) within a specified time from slaughter for each particular class of poultry *(17)* to prevent bacterial outgrowth. The USDA-FSIS requires that processors add 20 to 50 ppm chlorine to the chiller water to prevent cross-contamination of carcasses and has permitted the alternative use of ozone and chlorine dioxide to achieve the same objective. Carcasses reside in chillers for substantial periods (60 to 120 min, depending on the size and speed of the operation), resulting in high contact time with low-temperature water and any antimicrobial present. Thus, it is not surprising that chillers are commonly considered critical control points in processing operations for the reduction of microbiological hazards. In this study, the combined use of chlorine and chlorine dioxide in the chiller was more effective than the use of chlorine alone. The exclusive use of chlorine relies on free available chlorine for bactericidal activity, and effectiveness is dependent on the amount of chlorine present, the organic load present, and the contact time *(16)*. Efficacy of chlorine in the chiller is highly dependent on maintaining the chemistry of the chiller water because the effectiveness of chlorine is readily counteracted by high loads of organic material in the chiller. There are three critical elements in managing the chiller chemistry: (i) fresh water initially used to fill the chiller must have the highest permitted concentration of chlorine (up to 50 ppm available chlorine) *(19)*; (ii) the continuous supply of fresh water to the chiller must be as high as permitted; and (iii) the chiller overflow setup should maximize the removal of foam, which contains organic matter. An increase in the antimicrobial activity within the chiller due to the addition of chlorine dioxide to the chiller water is expected because this oxidizing biocide kills bacteria by direct action on the cellular membrane and through oxidation of cellular constituents *(18)*. The antimicrobial effectiveness of chlorine dioxide is less affected by pH and organic matter, and chlorine dioxide does not readily react to form chloramines *(18)*. Thus, as demonstrated by the results of this study, the combination of chlorine and chlorine dioxide provides a more effective option than the use of chlorine alone as the antimicrobial in chillers.

The antibacterial effect of TSP as a spray wash was slightly higher than that of the chlorine chiller, most likely because of the limitations of chlorine in the presence of the

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high organic load in the chiller. The antibacterial activity of TSP has been well established, especially for concentrations between 10 and 12% (pH 11.5 to 13) *(9, 11)*. The mechanisms of the TSP mode of action include (i) surfactant properties, (ii) destruction due to high pH, (iii) removal of loosely associated bacteria from the skin, (iv) removal of carcass surface fat, invariably resulting in removal of bacteria attached to the fat, and (v) destruction of the bacterial cell wall *(6)*.

The chlorinated spray applications alone also resulted in limited reduction of bacterial load on carcasses. The efficacy of the carcass washers is greatly influenced by the water volume, pressure, and chlorine concentration *(2)*, which are difficult to keep consistent in processing facilities. The inconsistent maintenance of optimal parameters and the already limited antimicrobial effect of chlorine with high organic loads minimize the singular contribution of any chlorinated spray washer. Most processors are optimizing the antimicrobial effect of chlorine in their chillers by acidifying the solution to maintain a pH between 6 and 7, thereby driving conversion of undissociated hypochlorous acid from hypochlorite *(15)*; however, the same chemical treatment is not being performed on the chlorinated water reservoirs serving the spray washers and other faucets in the facility. The industry should modify the protocols of water chlorination in reservoirs to include acidification and maintenance of the pH in the optimum range of 6 to 7, which will consequently improve the effect of their interventions and thus the reduction of microbiological hazards.

With the exception of the carcass chillers and the chiller exit spray, all the individual interventions reduced *Salmonella* incidence on carcasses substantially (Table 2). The relative reduction of *Salmonella* incidence on poultry carcasses was as follows: TSP wash (reduction of 70%) > postchiller wash (reduction of 60%) > dropped carcass wash and ClO_2 wash (reductions of 47 to 50%) > NY wash, postevisceration wash, IOBW1, and IOBW2 (reductions of 20 to 25%).

Salmonella incidence remained unchanged on carcasses passing under the chiller exit spray. Although the chiller exit spray was included in the HACCP plan at plant A for reduction of microbiological hazards, the design of the intervention comprised two horizontal bars suspended above the chiller exit conveyor with running chlorine water rather than a typical spray application targeted at covering the surface of carcasses. Reassessment of the HACCP plan following this study resulted in removal of this intervention from the plan and replacing it with a conveyor treatment used to break up the microbial load; this new intervention was subsequently successfully validated (data not shown).

Salmonella incidence increased by 29 and 57% on carcasses exiting the line compared with those entering the Cl_2 and ClO_2 chillers, respectively, despite the concurrent reduction in bacterial loads. The overall correlation of bacterial loads (APC, TCC, and ECC) with *Salmonella* incidence throughout this study was about 70%; however, correlation of APC, TCC, and ECC with *Salmonella* incidence specifically at the chillers was about 2, 30, and 32%, respectively. Research *(8, 12, 22)* supports that immersion chilling may be a major source of cross-contamination of poultry carcasses. Results from the present study indicate that *Salmonella* incidence was higher after the immersion chiller than before, thereby apparently supporting previous findings that the chiller is a site of cross-contamination. Although there was an increase in *Salmonella* incidence following chiller immersion, that increase may not necessarily have been due to carcass residence in the chiller but rather to transfer of contamination during transport along the chiller exit contact surfaces, such as the chiller exit chute and conveyor. This idea is supported conceptually by the finding of Geornaras et al. *(4)* that the chute leading into the chiller was a site for microbial cross-contamination of carcasses. The chiller exit area is the first ''common'' location following chilling where carcasses are in direct contact with one another following what is generally considered as the major critical control point for *Salmonella* control in the industry. The chiller, although it is a ''common bath,'' can be used to reduce and remove contamination; however, the chiller exit area by nature of the process does not apply recontamination control procedures, and thus contact of any carcasses with *Salmonella*-positive carcasses will result in cross-contamination. Data from the present study indicate that of 300 water samples collected from the chiller bath, none were positive for *Salmonella,* providing evidence that the chiller may not be the primary site of carcass cross-contamination. The increased *Salmonella* incidence on carcasses following the chiller detracts from the efficacy of this intervention (as determined by reductions in bacterial loads) because it only takes one cell from a contaminated carcass or equipment surface during chiller exit to render a carcass positive for *Salmonella* and as such implicating it as a primary source of cross-contamination. Further investigation should be conducted comparing samples collected from carcasses before they are routed down the chiller exit compared with samples collected after the carcasses are routed to determine whether immersion or commingling of carcasses exiting the chiller is responsible for cross-contamination.

Despite recontamination of carcasses with *Salmonella* in the vicinity of the chiller, results of the present study support the increased antimicrobial effect of using a postchiller wash for subsequent reduction of *Salmonella* incidence on carcasses (Table 2). This study made use of chlorinated water in the postchiller wash; however, there are alternative chemicals that may be included, such as acidified sodium chlorite, and have demonstrated a substantial antimicrobial effect *(10)*.

Many chemical compounds have been approved for use in interventions during processing, including acidified sodium chlorite, cetylpyridinium chloride, chlorine (sodium hypochlorite), chlorine dioxide, ozone, peroxyacetic acid, and TSP. Although there is published literature detailing the effectiveness of these compounds, it is critical that each processor validate the technologies being implemented in each facility.

Individual interventions applied to carcass parts. Carcass parts in this study included poultry necks, gizzards,

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TABLE 3. *Microbial populations and* Salmonella *incidence for carcass parts before (Pre) and after (Post) interventions*[a]

Intervention	Stage	Mean (SD) counts (log CFU/ml)			*Salmonella* incidence (%)
		Aerobic plates	Total coliforms	*E. coli*	
Dropped parts wash	Pre	3.0 (0.9) A	1.1 (0.8) A	0.7 (0.8) A	6 A
	Post	2.5 (1.1) B	0.6 (0.7) B	0.4 (0.6) B	3 A
Product dip	Pre	3.1 (0.4) A	1.5 (0.4) A	0.8 (0.5) A	29 A
	Post	2.5 (0.4) B	0.3 (0.4) B	0.1 (0.2) B	1 B
Neck tube chiller	Pre	3.6 (0.7) A	2.0 (0.6) A	1.7 (0.6) A	10 A
	Post	2.5 (1.1) B	1.3 (0.6) B	1.0 (0.6) B	6 A
Neck ice chiller	Pre	3.8 (0.7) A	2.1 (0.7) A	1.7 (0.6) A	0 A
	Post	3.3 (0.7) B	1.5 (0.5) B	1.1 (0.5) B	0 A
Liver tube chiller	Pre	2.2 (0.5) A	1.0 (0.7) A	0.7 (0.6) A	8 A
	Post	1.6 (0.2) B	0.3 (0.5) B	0 (0.1) B	1 B
Heart tube chiller	Pre	0.7 (0.6) A	0.1 (0.3) A	0.1 (0.2) A	1 A
	Post	0.2 (0.4) B	0 (0.1) B	0 (0) B	0 A
Gizzard tube chiller	Pre	0.8 (1.1) A	<0[b]	<0[b]	9 A
	Post	0.8 (1.0) A	<0[b]	<0[b]	2 B

[a] Within each intervention, Pre and Post values followed by different letters are significantly different at $\alpha = 0.05$.
[b] Limit of detection.

hearts, livers, and products (carcass cuts including breast filets, tenderloins, drumsticks, wings, thighs, and front halves).

During the processing of poultry, some carcasses and parts will drop to the floor. To reduce the economic losses due to disposal of such items, the industry has adopted product salvage programs. Such programs involve the collection of dropped product and subsequent knife trimming to remove visible contaminants followed by manual washing with chlorinated water. Results from the present study demonstrate that the dropped parts wash is effective in reducing bacterial loads (0.3- to 0.5-log reduction) and *Salmonella* incidence (by 50%) (Table 3). Similar results also were observed with the dropped carcass wash (Table 3).

One possible conclusion from these data is that comminuted meat such as ground poultry should receive further antimicrobial treatment than that applied during slaughter and carcass cutup because the commingling of different products increases the risk of *Salmonella* contamination. In this study, the incidence of *Salmonella* on carcass products (breast filets, tenderloins, wings, and drumsticks) from commingled batches was as high as 29% (Table 3). Immersion in acidified sodium chlorite (prepared by acidifying 500 to 1,200 ppm sodium chlorite with citric acid to pH 2.5 to 2.9) was used to treat poultry products and significantly reduced ($P < 0.05$) the bacterial load (0.5- to 0.8-log reduction) and incidence of *Salmonella* (by 97%) on such product.

TABLE 4. *Microbial populations and* Salmonella *incidence in red water (reuse water) before (Pre) and after (Post) water recycling*[a]

Stage	Mean (SD) counts (log CFU/ml)			*Salmonella* incidence (%)
	Aerobic plates	Total coliforms	*E. coli*	
Pre	2.0 (0.6) A	0.9 (0.7) A	0.6 (0.5) A	0[b] A
Post	0.8 (0.9) B	0.2 (0.4) B	0.1 (0.3) B	0[b] A

[a] Pre and Post values followed by different letters are significantly different at $\alpha = 0.05$.
[b] Limit of detection.

Harvesting of carcass giblets (liver, heart, and gizzard) and necks typically involves washing such parts with chlorinated water; however, the major intervention for giblets and necks is achieved through chilling in tube chillers filled with 20 to 35 ppm chlorinated water. Some processing plants are not equipped with tube chillers because harvesting of giblets and necks is not a common practice and may be implemented only seasonally. Plant C was not equipped with tube chillers and only harvested necks seasonally; thus, this facility implemented ice chilling. Ice chilling, as conducted in this facility, involved the layering of necks on shaved ice for 90 min. Plants A and B used tube chillers to chill necks. Tube chilling of necks was slightly more effective than ice chilling for reducing the bacterial load; however, the difference was not significant ($P \geq 0.05$) (Table 3). Use of tube chillers for chilling livers, hearts, and gizzards reduced bacterial loads (0.1- to 0.7-log reduction) and the *Salmonella* incidence (by 1 to 7%) (Table 3).

Individual interventions applied to red water. The majority of carcass chillers use a loop system for reusing "red water" (chiller bath water). The red water is continuously cycled though filters and piping into a separate chilling chamber to rapidly lower the temperature before the water is reintroduced into the chiller bath. This process helps maintain the low temperature of the chiller bath water for consequential reduction of carcass temperatures. Water reused in the chiller may receive additional chlorination while in the loop system not to exceed the regulated 5 ppm free available chlorine measured at the influent into the prechiller *(19)*. Concern has been expressed by regulators about the potential of water reuse to increase the microbiological profile of carcasses when this water is reintroduced into the chiller. Results of the present study provide evidence that the microbiological profile of red water (which

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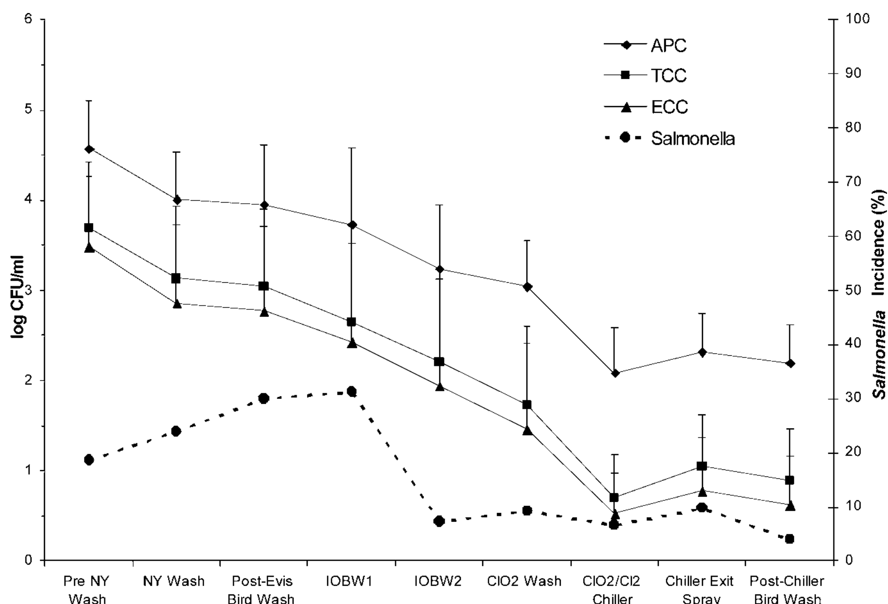

FIGURE 1. *Microbial populations (log CFU per milliliter, mean ± SD) and* Salmonella *incidence (%) of carcasses following multiple interventions applied in sequence along the evisceration (slaughter) line at poultry plant A. The first point represents the stage before the first intervention, and every point thereafter represents the populations after the specific intervention. NY Wash, New York wash; Post-Evis, postevisceration wash; IOBW1, inside-outside bird wash 1; IOBW2, inside-outside bird wash 2; ClO2, chlorine dioxide wash; ClO2-Cl2, chlorine dioxide wash plus chlorine chiller.*

is essentially chiller bath water) is significantly reduced ($P < 0.05$) during reuse (Table 4). The absence of *Salmonella* in red water supports the idea that *Salmonella* recontamination of carcasses following chilling is not due to persistence of the pathogen in the chiller but rather to cross-contamination from other carcasses or surfaces during commingling after exiting the chiller.

Multiple-sequential interventions applied to carcasses. The application of multiple-sequential interventions for decontamination of poultry carcasses during processing is a concept mimicking the original hurdle approach *(7)* and reinforces HACCP principles, i.e., the use of sequential treatments imparting cumulative microbial reductions that are continuously monitored to ensure these treatments are operating as intended. In the present study, the effect of multiple-sequential interventions was assessed at three processing plants. Multiple-sequential interventions resulted in significant reductions ($P < 0.05$) of APC, TCC, ECC, and *Salmonella* incidence of 2.4, 2.8, and 2.9 log CFU/ml and 79%, respectively, at plant A (Fig. 1), of 1.8, 1.7, and 1.6 log CFU/ml and 91%, respectively, at plant B (Fig. 2), and of 0.8, 1.1, and 0.9 log CFU/ml and 40%, respectively, at plant C (Fig. 3).

Although many of the individual interventions studied, especially the chlorinated spray washes, resulted in only slight reductions of bacterial loads and *Salmonella* incidence on carcasses, when taken together and analyzed as part of a multiple-sequential intervention system there was a cumulative decontaminating effect of significant reductions ($P < 0.05$) in microbial populations.

Results from the this study suggest that common individual interventions applied in poultry processing facilities effectively, if not always significantly ($P < 0.05$), reduce bacterial loads and *Salmonella* incidence on carcasses and carcass parts and in processing water. The major interventions responsible for reduction of microbial loads on

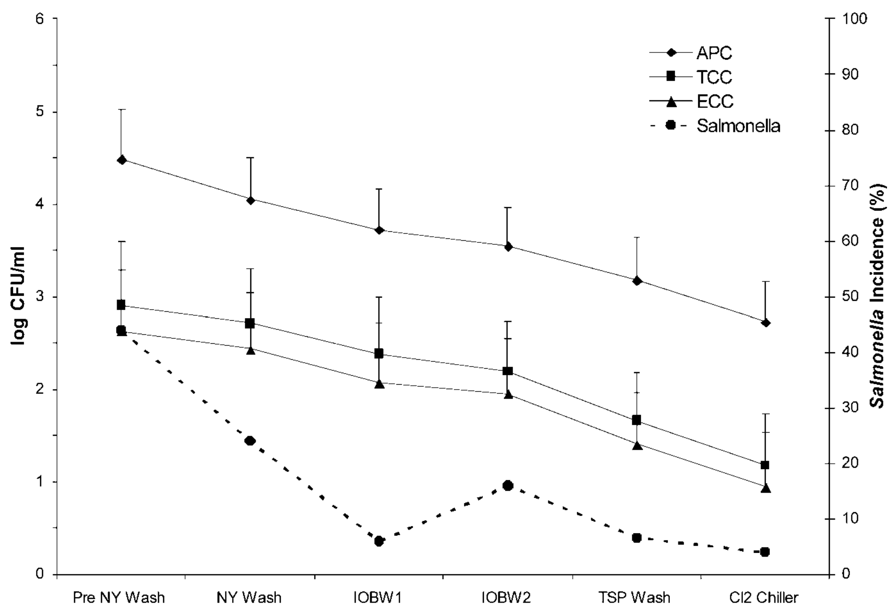

FIGURE 2. *Microbial populations (log CFU per milliliter, mean ± SD) and* Salmonella *incidence (%) of carcasses following multiple interventions applied in sequence along the evisceration (slaughter) line at poultry plant B. The first point represents the stage before the first intervention, and every point thereafter represents the populations after the specific intervention. NY Wash, New York wash; IOBW1, inside-outside bird wash 1; IOBW2, inside-outside bird wash 2; TSP Wash, trisodium phosphate wash; Cl2, chlorine chiller.*

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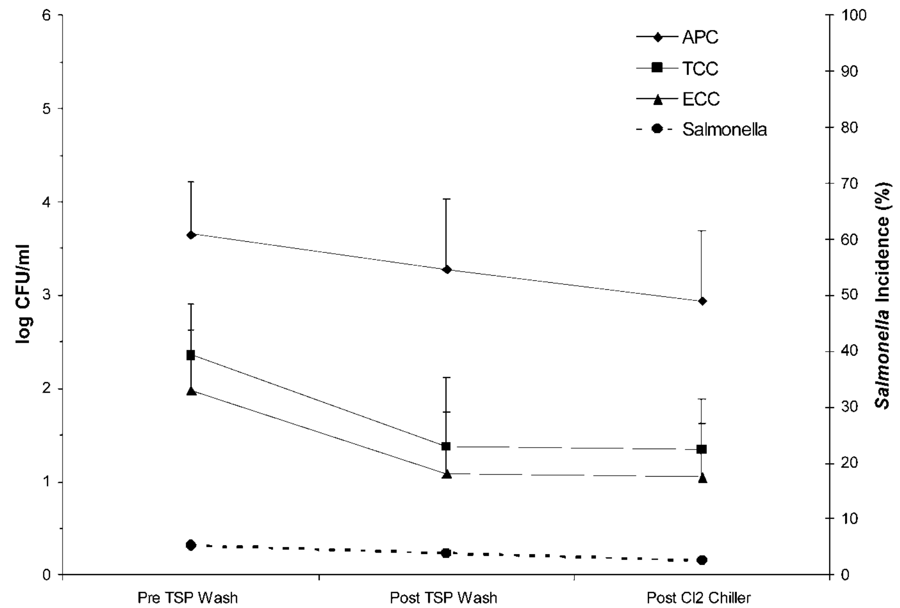

FIGURE 3. *Microbial populations (log CFU per milliliter, mean ± SD) and* Salmonella *incidence (%) of carcasses following multiple interventions applied in sequence along the evisceration (slaughter) line at poultry plant C. The first point represents the stage before the first intervention, and every point thereafter represents the populations after the specific intervention. TSP Wash, trisodium phosphate wash; Cl2, chlorine chiller.*

carcasses are the carcass chillers and TSP spray wash. The use of chlorine dioxide in combination with chlorine in the carcass chiller enhances the antimicrobial activity of the chiller. *Salmonella* was absent in chiller bath water (red water); thus, the carcass chiller may not necessarily be the major site for carcass recontamination as previously thought. The reuse of chiller bath water (red water) improved the microbiological profile of the water and may consequently assist in reducing the microbiological profile of carcasses. Use of a postchiller spray wash assisted in reducing bacterial loads and *Salmonella* incidence of carcasses that became recontaminated in the chiller area and should be considered an option for facilities not meeting the *Salmonella* performance standards following chilling. Multiple-sequential interventions resulted in a cumulative decontaminating effect causing significant reductions ($P < 0.05$) in bacterial loads and *Salmonella* incidence of carcasses and as such allowed the facilities to meet microbiological criteria for *E. coli* and *Salmonella* performance standards.

Carcass products such as wings, breasts, and drumsticks that are mixed for grinding may have a high incidence of *Salmonella* due to commingling of contaminated and clean products, but contamination can be controlled effectively with an immersion treatment in acidified sodium chlorite. Chilling of giblets and necks in tube chillers with chlorinated water is effective in reducing the microbiological hazards associated with these parts. Processing plants that harvest carcass parts such as necks on a seasonal basis but do not have tube chillers may attain the same hazard reduction by using a chlorinated wash plus immersion treatment with subsequent layering on or in ice for adequate chilling. Operations that wish to save product that drops to the floor may implement a salvage program consisting of knife trimming of visible contamination followed by manual washing with chlorinated water to reduce microbiological hazards.

These results support the validation of in-plant processing interventions and provide a source of information for the industry in decision making regarding selection of antimicrobial strategies. The poultry industry has many options for interventions that could be applied at various stages of processing, and producers should be encouraged to implement such interventions both to satisfy regulatory requirements and to reduce *Salmonella* incidence on carcasses in their operations. By so doing, they become eligible for incentive programs offered by the USDA-FSIS *(21)*. The results of this study will assist producers in selection of interventions for their facilities and serve as a guide for effective validation and reassessment of HACCP plans.

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