

Efficacy of Antimicrobials Against *Campylobacter jejuni* on Chicken Breast Skin

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Primary Audience: Poultry Processing Researchers, Microbiologists, Quality Assurance Personnel, Laboratory Personnel, Food Safety and Sanitation Managers

SUMMARY

Raw chicken is a significant source of the bacterial pathogen *Campylobacter*. The commercial processing of poultry for consumption presents many opportunities in which carcasses and other raw products can become newly contaminated or cross-contaminated with this microorganism. Increased use of chlorine and higher volumes of water during processing may not be effective for reducing *Campylobacter* populations. This study examined the efficacy of 10% trisodium phosphate (TSP), 0.1% acidified sodium chlorite (ASC), 0.1 and 0.5% cetylpyridinium chloride (CPC), 1% Tween 80, and water (50°C at application) for their ability to inactivate, reverse the attachment of, or inhibit the attachment of *Campylobacter jejuni* applied to chicken breast skin. Statistically significant ($P \leq 0.05$) differences in the reduction of *C. jejuni* populations were observed across chemical treatments and contact times (30 s, 3 min, or 10 min). When bacteria were applied before treatment, a reduction of $>1.0 \log_{10}$ cfu/skin was achieved with 0.5% CPC (2.89), 10% TSP (1.63), 0.1% ASC (1.52), and 0.1% CPC (1.42). When bacteria were applied after chemical treatment, a reduction of $>1.0 \log_{10}$ cfu/skin was achieved with 0.5% CPC (4.67) and 10% TSP (1.28). The commercial use of an effective antimicrobial chemical spray may help to reduce the level of *Campylobacter* on raw poultry carcasses and reduce the volume of rinse water applied for carcass washing.

Key words: antimicrobial, attachment, *Campylobacter*, chicken, acidified sodium chlorite, trisodium phosphate, cetylpyridinium chloride

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DESCRIPTION OF PROBLEM

Raw chicken and turkey are significant sources of the bacterial pathogens *Salmonella* spp. and *Campylobacter* spp. There are many points in rearing and processing of poultry at which carcasses and products can become newly contaminated or cross-contaminated with micro-

organisms. *Campylobacter* infections have been linked to poultry in many foodborne illness outbreaks [1, 2, 3], primarily due to consumption of raw or undercooked chicken. The prevalence of *Campylobacter* on raw poultry products varies from 0 to 100% with an average of 62% [4]. It has also been noted that there is a greater population of bacteria on the breast skin than

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other edible portions of the chicken carcass [5, 6] making this an important site to control the organism and to study bacterial attachment properties. To comply with the Pathogen Reduction and Hazard Analysis Critical Control Point (HACCP) Systems Final Rule [7], many poultry processors have increased their usage of chlorine and rinse water volumes to reduce *Salmonella* and *Escherichia coli* on carcasses. These strategies may have little effect for reducing *Campylobacter* populations [8].

The objective of this research was to determine the ability of several chemical spray solutions to inactivate, reverse the attachment of, or inhibit the attachment of *Campylobacter jejuni* when applied to chicken breast skin. During the past few years, many commercial poultry processors have used trisodium phosphate (TSP), in an 8 to 12% solution, as an antimicrobial rinse or dip for raw carcasses. Also, some commercial poultry processors have recently initiated use of acidified sodium chlorite (ASC) as an antimicrobial intervention. When applied as a spray or dip, the sodium chlorite concentration should be between 500 and 1,200 ppm and have acid levels high enough to produce a pH between 2.3 and 2.9.

In 2002, a food additive petition was filed to permit the safe use of cetylpyridinium chloride (CPC) as an antimicrobial in poultry processing. Cetylpyridinium chloride (1-hexa-decyl pyridinium chloride) is a quaternary ammonium compound with antimicrobial properties against many microorganisms including viruses. The pH of a 1% CPC solution is approximately 7.2. The US Food and Drug Administration permits a maximum concentration of 0.1% in several dental products, and CPC can be found in several commercial products, including mouthwashes. Tween 80 (polysorbate 80) has many approved uses in foods and food processing. This chemical usually functions as a wetting agent, dispersing agent, surfactant, or defoaming agent. The commercial use of an effective antimicrobial chemical spray may help to reduce the level of *Campylobacter* on poultry carcasses and reduce the volume of rinse water per poultry carcass. The effectiveness of these chemicals for inhibiting the attachment of the organism to chicken skin was also studied.

MATERIALS AND METHODS

Skin Sample Preparation

Chicken breast skin samples were obtained from a commercial processor and trimmed into round pieces approximately 28 cm². The samples were vacuum-packaged and frozen in sterile 3 × 5 in. Whirl-Pak retain bags. The packaged skins were shipped frozen, by overnight courier, to Auburn University and sterilized with 12 kGy of ionizing radiation using a cobalt-60 source. Upon return, the skins were frozen to -20°C and maintained at that temperature until testing [9, 10]. The protocol for sample irradiation, inoculation, spray application, and enumeration followed the skin attachment model (SAM) [9, 11, 12].

Chemical Treatment Preparation

Chemical treatments including 10% TSP (wt/vol) [13], 0.1% solution of ASC (vol/vol) [14], 0.1 or 0.5% CPC (wt/vol) [15], 1% Tween 80 (polysorbate 80; vol/vol) [15], and sterile, deionized, hot water (50°C at application) were applied to separately inoculated skin samples. As an experimental control, sterile, deionized water at ambient temperature (21°C at application) was evaluated. ASC was prepared with citric acid and used within 5 min of mixing. Each test chemical was misted onto skins at 2 mL/s for 3 s [16]. A consistent spray pressure (~8 psi) was used throughout the study.

C. jejuni Inoculation Before Chemical Treatment Application

Thawed skins were drip-inoculated with approximately 0.1 mL of a cocktail of four strains of *Campylobacter jejuni* [18] (ATCC 3444, ATCC 29428, ATCC 33291, and a typed strain 61-784 [20]) after being placed into a sterile specimen cup. Skin samples remained undisturbed at approximately 21°C for 10 min to allow for bacterial attachment. One chemical spray was applied to each skin sample. The eluate from the skin spray application was collected and diluted in buffered peptone water (BPW). The samples remained undisturbed at room temperature for 30 s, 3 min, or 10 min prior to analysis (Table 1).

TABLE 1. Treatment summary table

Chemical	Contact time (min)					
	Bacteria applied before chemical			Bacteria applied after chemical		
10% TSP	0.5	3	10	0.5	3	10
0.1% ASC	0.5	3	10	0.5	3	10
0.1% CPC	0.5	3	10	0.5	3	10
0.5% CPC	0.5	3	10	0.5	3	10
1% Tween 80	0.5	3	10	0.5	3	10
Water at 50°C	0.5	3	10	0.5	3	10
Water at 21°C (control)	0.5	3	10	0.5	3	10

Twenty milliliters of sterilized BPW was added to the samples in the cup and shaken by hand for 30 s. The rinse and the collected eluate from the spray application were serially diluted and plated onto Brucella-FBP agar. All plates were placed into anaerobic containers flushed with a microaerobic gas mixture of approximately 5% O₂, 10% CO₂, and 85% N₂ and incubated for 48 h at 42°C. The numbers of cells counted on these plates were representative of loosely attached cells. The cup was then removed from its lid, and the skin sample was aseptically transferred from its location into a small sterile stomacher bag. The sample was blended in a laboratory stomacher for 2 min with 20 mL of fresh BPW. This solution was also serially diluted and plated onto Brucella-FBP agar. These remaining cells were considered the firmly attached cells. The difference between the number of cells inoculated and the loosely attached plus firmly attached counts indicated cell death and nonviable cells [21].

C. jejuni Inoculation After Chemical Treatment Application

The same chemical sprays were also applied before the skin was inoculated to test their abilities to prevent attachment. Sprays were allowed to contact the skin for 10 min prior to inoculation with *C. jejuni*. Skins then remained undisturbed for 30 s, 3 min, or 10 min after inoculation and prior to analysis. All of the chemical concentrations, temperatures, and analytical methods were the same as those used when bacteria were applied before a chemical spray (Table 1) [22].

RESULTS AND DISCUSSION

Bacterial Inocula Application Before Chemical Treatment

In Table 2, the chemical sprays are listed in order of their effectiveness, based on log₁₀ reductions in colony-forming units per skin. This table displays cumulative results for all three contact times and replications (two per treat-

TABLE 2. Means and standard errors of log reductions^A when *Campylobacter jejuni* was applied before chemical treatment spray. Values represent an average of all contact times and replications (n = 6)

Chemical spray ^B	Mean	SE
0.5% CPC	2.89 ^a	0.12
10% TSP	1.63 ^b	0.14
0.1% ASC	1.52 ^b	0.16
0.1% CPC	1.42 ^b	0.14
1% Tween 80	0.56 ^c	0.09
Control	0.15 ^d	0.05
Water (50°C)	-0.28 ^d	0.08

^aMeans followed by a different letter are significantly different ($P \leq 0.05$) by Tukey's HSD.

^AReductions given as log₁₀ cfu/skin.

^BCPC = cetylpyridinium chloride; TSP = trisodium phosphate; ASC = acidified sodium chlorite; Control = water at 21°C.

TABLE 3. Means and standard errors of log reductions^A when *Campylobacter jejuni* was applied after chemical treatment spray. Values represent an average of all contact times and replications (n = 6)

Chemical spray ^B	Mean	SE
0.5% CPC	4.67 ^a	0.30
10% TSP	1.28 ^b	0.41
0.1% ASC	0.93 ^{bc}	0.33
0.1% CPC	0.77 ^{bcd}	0.35
Control	0.12 ^{cd}	0.05
Water (50°C)	-0.11 ^{cd}	0.03
1% Tween 80	-0.21 ^d	0.08

^aMeans followed by a different letter are significantly different ($P \leq 0.05$) by Tukey's HSD.

^AReductions given in log₁₀ cfu/skin.

^BCPC = cetylpyridinium chloride; TSP = trisodium phosphate; ASC = acidified sodium chlorite; Control = water at 21°C.

ment). The population reductions represent the difference between the mean number of organisms applied to and recovered from each skin sample. The main effect of chemical treatment was statistically significant ($P < 0.01$). The 0.5% CPC treatment was the most effective for reducing the population of *C. jejuni* with a log₁₀ reduction of 2.89 cfu/skin. Chemical effectiveness was defined as the degree of bacterial population reduction (inactivation or preventing attachment to skin) caused by the chemical. 10% TSP, 0.1% ASC, and 0.1% CPC followed in effectiveness with log₁₀ reductions of 1.63, 1.52, and 1.42 cfu/skin, respectively. Tween 80 (1%) had little effect in reducing the level of *C. jejuni* on chicken skins. The hot water treatment (50°C at application) led to a slight increase in the *C. jejuni* population at each contact time.

Bacterial Inocula Application After Chemical Treatment

The second phase of the study involved the inoculation of *Campylobacter jejuni* on the chicken skin after the chemical treatment was applied by spray. Table 3 displays the chemical sprays listed in order of the log₁₀ reductions in colony-forming units per skin. This table displays cumulative results for all three contact times and replications (two per treatment). The population reductions represent the difference between the mean number of organisms applied to and recovered from each skin sample. The main effect of chemical treatment ($P < 0.01$) and contact time ($P = 0.01$) were statistically significant. Again, 0.5% CPC was considerably more effective than other chemical treatments with an average log₁₀ reduction of 4.67 cfu/skin.

TSP (10%), 0.1% ASC, and 0.1% CPC followed in chemical effectiveness with mean log₁₀ reductions of 1.28, 0.93, and 0.77 cfu/skin, respectively.

Application of 0.5% CPC to skins before inoculation with bacteria increased inhibitory effect (4.67 vs. 2.89 log reduction) when compared to application after inoculation. But chemical applications of 10% TSP, 0.1% ASC, and 0.1% CPC were more effective if applied after bacterial contamination (Tables 2 and 3). The Tween 80 (1%) and hot water treatments led to a slight increase in the *C. jejuni* population at each contact time.

Contact Time of Bacteria with Chemical

When *C. jejuni* was applied prior to chemical application, the effect of contact time (0.5, 3, or 10 min) was not significant ($P = 0.9$). However, when *C. jejuni* was applied after chemical sprays were used, the main effects of contact time were statistically significant ($P = 0.01$). Furthermore, the mean log reduction after 10 min contact time (1.42 cfu/skin) was significantly different than the log reduction achieved after 0.5 min contact time (0.70 cfu/skin) for all treatments combined.

Figures 1 and 2 show the log reductions achieved with each chemical and contact time. A comparison of these figures suggests that the average log reduction of *C. jejuni* when bacterial inocula are applied before chemical treatment (Figure 1) is less influenced by chemical contact time than when the bacteria are applied after chemical treatment (Figure 2), especially for 0.1% ASC and 10% TSP. Additionally, the log reduction was considerably higher when *C. jejuni* were applied after 0.5% CPC, 0.1% CPC,

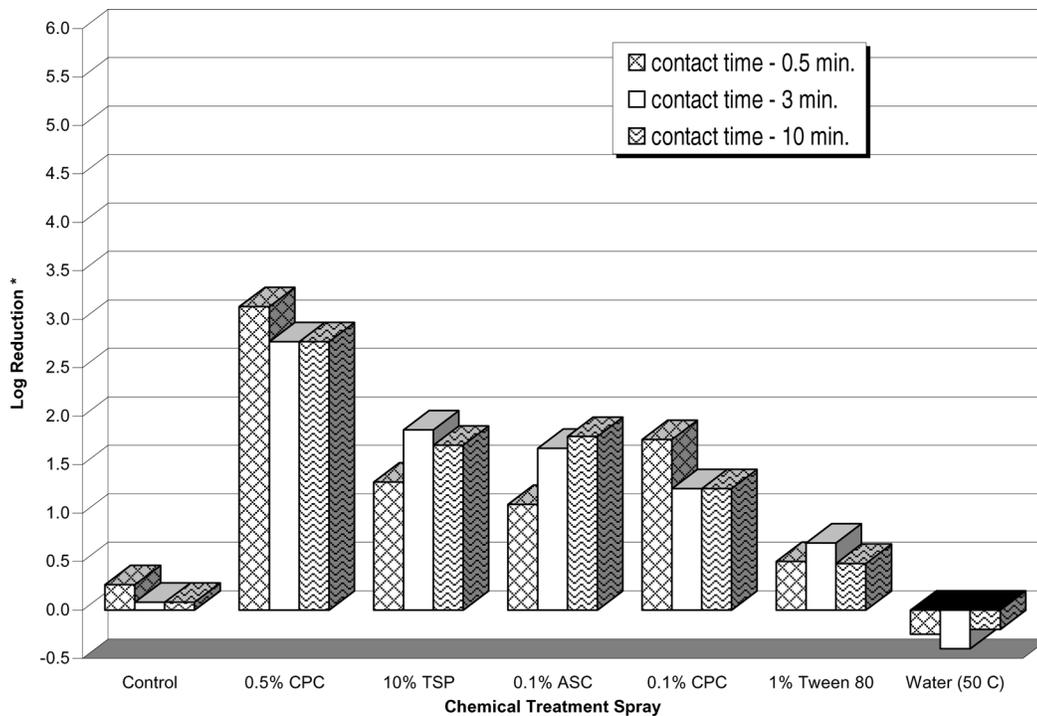


FIGURE 1. Average log reduction of *Campylobacter jejuni* when bacterial inocula applied before chemical treatment (n = 2 for each contact time-chemical combination). TSP = trisodium phosphate; CPC = cetylpyridinium chloride; ASC = acidified sodium chloride; * reductions in log (base 10) cfu/skin.

and 10% TSP treatments and permitted 10 min of contact (Figure 2).

Antimicrobial Effectiveness of TSP, ASC, and CPC

TSP. Bacterial inoculation before chemical treatment spray produced \log_{10} reductions of 1.63 \log_{10} cfu/skin, whereas bacterial inoculation after chemical treatment spray resulted in a 1.28 \log_{10} reduction across all contact times. The greatest bacterial population reduction (2.20 \log_{10} cfu/skin) was achieved when the bacteria were applied after chemical treatment with 10 min contact (Figure 2). The log reduction was only 1.20 \log_{10} cfu/skin after 3 min contact. Therefore, TSP was more effective at inhibiting bacterial attachment when longer (10 min) contact was studied.

Most research on the antibacterial properties of TSP has been done with *Salmonella*. But, Slavik et al. [24] studied TSP usage for reducing *Campylobacter* in postchill chicken carcasses, dipped into a 10% solution of TSP at 50°C for

15 s. Reductions of 1.5 and 1.2 \log_{10} cfu were observed for TSP-treated carcasses stored at 4°C for 1 and 6 d, respectively. And, Whyte et al. [25] reported a mean *Campylobacter* reduction of 1.71 \log_{10} cfu/g of broiler neck skins that were dipped in a 10% TSP solution for 15 s.

ASC. Bacterial inoculation before chemical treatment spray produced \log_{10} reductions of 1.52 \log_{10} cfu/skin, whereas bacterial inoculation after chemical treatment spray resulted in a 0.93 \log_{10} reduction across all contact times. The greatest bacterial population reductions (1.79 and 1.44 \log_{10} cfu/skin) were achieved with 10 min of contact. The Alcide Corporation performed a study on whole carcasses sampled from five commercial plants and reported a reduction of 2.6 \log_{10} cfu/mL for *Campylobacter* spp. [26]. In a study with naturally contaminated raw broiler carcasses, Kemp et al. [27] reported that maximum antimicrobial activity of ASC was achieved when carcasses were prewashed and then exposed to a 5-s dip of phosphoric acid or citric acid activated ASC (1,200 ppm). Only

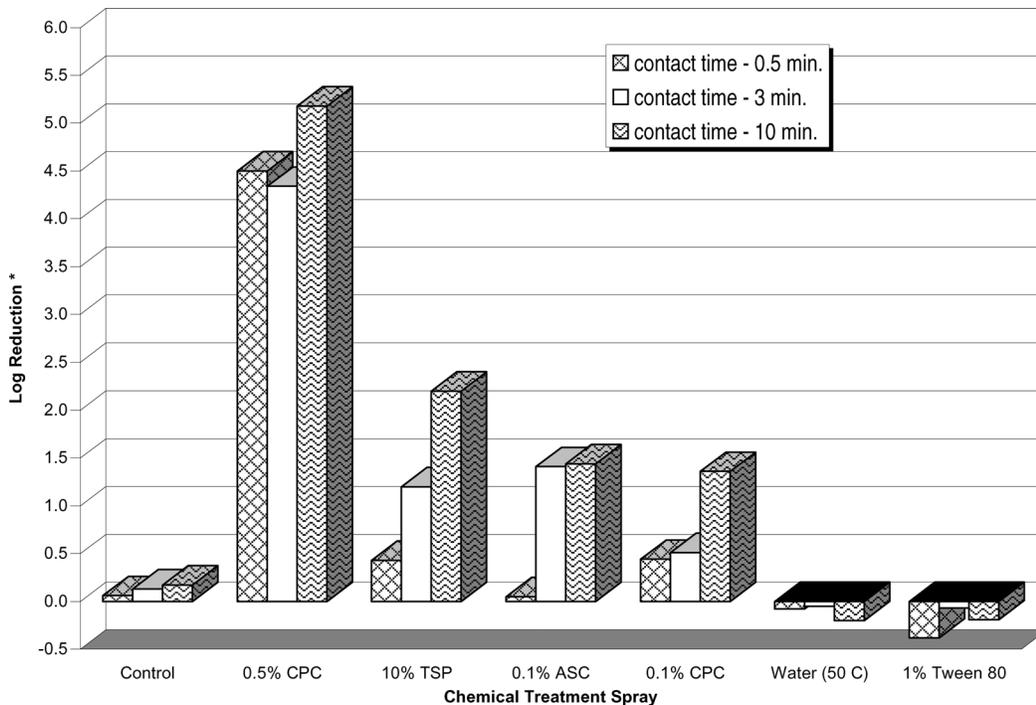


FIGURE 2. Average log reduction of *C. jejuni* when bacterial inocula applied after chemical treatment ($n = 2$ for each contact time – chemical combination). TSP = trisodium phosphate; CPC = cetylpyridinium chloride; ASC = acidified sodium chloride; * reductions in log (base 10) cfu/skin.

aerobic bacteria, *E. coli*, and total coliform counts were determined. In another study with visibly fecal-contaminated chicken carcasses, Kemp et al. [28] applied a solution of 1,100 ppm acidified sodium chlorite for 15 s to carcasses routed through a spray cabinet. The mean *Campylobacter* count was 1.14 log₁₀ cfu/mL of rinse from carcasses that received continuous online processing (ASC spray). Carcasses that were diverted to offline reprocessing (no ASC treatment) had a mean *Campylobacter* count of 2.89 log₁₀ cfu/mL of carcass rinse.

CPC. Skins inoculated before treatment with 0.1% CPC showed a reduction of *C. jejuni* by 1.42 log₁₀ cfu/skin. When skins were inoculated after treatment with 0.1% CPC, reductions of 0.77 log₁₀ cfu/skin were observed across all contact times. In this study, the greatest bacterial population reductions were achieved with the 0.5% CPC treatment when applied before or after inoculation with *C. jejuni*.

Several studies have demonstrated the effectiveness of CPC as an antimicrobial against *Salmonella*. For example, Wang et al. [29] reduced

Salmonella with 0.1% CPC by 1.5 to 2.5 log₁₀ cfu under varied conditions of temperature (10, 35, or 60°C), spray pressure (0 to 1,034.2 kPa), and spray duration (30 s). Li et al. [30] used a 0.1% CPC treatment on prechill chicken carcasses by spraying for 30 or 90 s. They reported a reduction of 0.59 to 0.85 log₁₀ cfu and 1.20 to 1.63 log₁₀ cfu, respectively. Yang et al. [31] applied a 0.5% CPC solution to prechill chicken carcasses and reduced *Salmonella* by 3.62 log₁₀ cfu/mL. Breen et al. [32] compared four levels of CPC (0.1, 0.2, 0.4, and 0.8%) and three reaction (contact) times of 1, 3, and 10 min. *Salmonella* reductions of 0.59 to 4.91 log₁₀ cfu occurred when the bacteria were applied before the treatment.

Microbial Attachment

The attachment of pathogenic bacteria, including *Campylobacter* spp., to poultry skin should be of concern to processors. Bacterial cells may need only one to a few minutes to attach to a surface. Once attached, they can multiply and require a great effort to remove. The

TABLE 4. Effect of test chemicals on *Campylobacter jejuni* attachment reversal.^A Values represent an average of all contact times and replications (n = 6)

Chemical spray	Log ₁₀ cfu/skin	Reduction from control (%)
Control	6.66	—
Water (50°C)	6.41	44
1% Tween 80	6.17	68
0.1% CPC	5.76	87
0.1% ASC	5.74	88
10% TSP	5.67	90
0.5% CPC	5.48	93

^ABacteria applied before chemical.

^BCPC = cetylpyridinium chloride; TSP = trisodium phosphate; ASC = acidified sodium chlorite; Control = water at 21°C.

ability and rate for bacterial attachment and detachment varies widely under different environmental conditions and for different attachment surfaces. Antimicrobial chemical applications in commercial poultry processing can include prevention of bacterial attachment to carcasses or food contact surfaces in addition to bacterial inactivation. In the present study, we compared several chemicals for their abilities to inhibit or reverse bacterial attachment.

Nayak et al. [33] described a method to estimate the ability of zinc chloride solutions to inhibit or reverse bacterial attachment to chicken skin. By using a skin attachment model, these authors demonstrated that the bactericidal activity and bacterial cell detachment ability of zinc chloride could reduce *Salmonella* Typhimurium populations. In the present study, *Campylobacter* cells enumerated from stomach skins were considered firmly attached cells. When compared to the control treatment, all sprays caused a reduction in the number of firmly attached cells recovered when *C. jejuni* was ap-

plied prior to the spray. Because a lesser quantity of firmly attached cells was enumerated, we can conclude that all chemical sprays were able to reverse cell attachment to some extent (Table 4). This reduction, ranged from 44 to 93%, across all contact times, compared to the control group. Additionally, some sprays caused a reduction, from the control group, in the number of firmly attached cells recovered when *C. jejuni* was applied after the spray. When a lesser quantity of firmly attached cells were enumerated, we concluded that these chemical sprays (TSP and CPC only) were able to inhibit cell attachment to some extent (Table 5). The reduction ranged from 39.7 to 99.7%, across all contact times, compared to the control group. The 0.5% CPC spray was especially inhibitory of the attachment of *C. jejuni* to chicken skin (Table 5). And, overall, the 10% TSP and 0.5% CPC treatments were most effective for reversing bacterial attachment and inhibiting bacterial attachment. These chemicals may demonstrate greater antimicrobial activity in a poultry slaughter plant if

TABLE 5. Effect of test chemicals on *Campylobacter jejuni* attachment inhibition.^A Values represent an average of all contact times and replications (n = 6)

Chemical spray	Log ₁₀ cfu/skin	Reduction from control (%)
Control	6.04	—
1% Tween 80	6.49	None
Water (50°C)	6.34	None
0.1% ASC	6.19	None
0.1% CPC	5.82	39.7
10% TSP	5.47	73.1
0.5% CPC	3.56	99.7

^ABacteria applied after chemical.

^BCPC = cetylpyridinium chloride; TSP = trisodium phosphate; ASC = acidified sodium chlorite; Control = water at 21°C.

they could be applied as a spray as early as possible prior to the chilling (tank) process. In this case, the chemicals will have a longer period

to be in contact with potentially hazardous bacteria and greater opportunity to prevent bacterial attachment.

CONCLUSIONS AND APPLICATIONS

1. Cetylpyridinium chloride (0.5%) was an effective antimicrobial agent for inactivating, reversing attachment, and inhibiting attachment of *Campylobacter jejuni* to chicken skin.
2. Trisodium phosphate (10%) and acidified sodium chlorite (0.1%) were similarly effective for reducing *Campylobacter jejuni* populations on chicken skin.
3. Trisodium phosphate (10%) and acidified sodium chlorite (0.1%) were more effective as antimicrobials when permitted a longer chicken skin contact time (3 or 10 min vs. 0.5 min), especially when they were used prior to *Campylobacter jejuni* application.
4. Cetylpyridinium chloride (0.5%) was the most effective antimicrobial agent in this study, but 0.1% cetylpyridinium chloride was generally less effective than trisodium phosphate (10%) or acidified sodium chlorite (0.1%) for inactivating, reversing, and inhibiting attachment of *Campylobacter jejuni* to chicken skin.
5. The commercial use of an effective antimicrobial chemical spray may help to reduce the level of *Campylobacter* on raw poultry carcasses and reduce the volume of rinse water applied for carcass washing.
6. Additional studies are needed that consider the effect of chemical concentration, spray pressure, spray duration, contact time, solution recycling, and point of application in commercial processing to ascertain the effectiveness of chemical applications against *Campylobacter* spp.

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18. *Campylobacter jejuni* strains were maintained on separate Brucella agar (Difco, Detroit, MI) FBP (ferrous sulfate, sodium metabisulfate, and pyruvic acid) slants [19]. Inoculated slants were incu-

bated under microaerobic conditions (5% O₂, 10% CO₂, 85% N₂) at 42°C for 48 h. Following incubation, each slant was washed with sterile buffered peptone water (BPW) (Difco, Detroit, MI) and fresh cells were scraped off of the medium into the liquid. The resulting suspensions were pipetted and combined. Final inoculum concentration was ~10⁸ cells/mL.

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21. Incubation time for all Brucella-FBP plates and slants was for 48 h at 42°C. Typical *C. jejuni* colonies on this particular medium were small (~1 mm), round, convex, and red (due to addition of 200 mg/L triphenyltetrazolium chloride to the media). Confirmation tests included Gram stains (–), wet mounts, catalase (+) tests, agglutination tests (Med-Ox Diagnostics, Ogdensburg, NY), and API *Campylobacter* biochemical test kits (bioMérieux, Hazelwood, MO).

22. When the bacterial inocula were applied before or after the chemical spray, the effects of the spray (seven test chemicals, contact time 0.5, 3.0, or 10 min) and their interactions on log reduction were analyzed by two-way ANOVA of a completely randomized factorial design [23].

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