APPLICATION OF VARIOUS ANTIMICROBIALS AGAINST *CAMPYLOBACTER JEJUNI* ON POULTRY CARCASSES

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Abstract –Regulatory guidelines to control poultry-borne pathogens, more specifically *Campylobacter jejuni*, necessitate the need for novel applications of antimicrobials during poultry processing. The objective of this study was to evaluate and compare multiple commercially available antimicrobials to reduce *C. jejuni* on fresh poultry using two separate application methods (spray and immersion). Broiler carcasses were inoculated with *C. jejuni* (ca. 7 $\log_{10}$ CFU/mL) and allowed 30 min for bacterial attachment, followed by immersion and spray treatment with DBDMH, PAA, and SH. Following treatment, carcasses were rinsed and the rinsate was plated onto *Campylobacter* Cefex agar and incubated microaerobically at 42°C for 48h. Immersion application significantly ($P \leq 0.05$) reduced *C. jejuni* as compared to the spray application. Results also suggested that PAA in an immersion application showed the lowest survival populations ($P \leq 0.05$) of *C. jejuni* irrespective of the application method. This study demonstrated that DBDMH, like other commercially available antimicrobials, can reduce populations of *C. jejuni* on fresh poultry carcasses when used as short exposure applications.

Key Words – Antimicrobials, *Campylobacter*, Poultry Processing

- INTRODUCTION

Campylobacter is a common poultry commensal and a well-known cause of human gastroenteritis worldwide. Estimates for the U.S alone account for over 800,000 episodes of foodborne illnesses annually (Scallan *et al*., 2011). Pathogen-food pairings attribute *Campylobacter* contaminated poultry to more illnesses than any other bacteria-food combination, and contaminated poultry alone has the greatest public health impact among all foods (Batz *et al*., 2011). The U.S. Department of Agriculture-Food Safety Inspection Service (USDA-FSIS) implemented *Campylobacter* performance standards (USDA, 2009) in an effort to reduce more than 5,000 *Campylobacter* infections in the U.S. annually (USDA, 2011).
The most effective method to achieve reduction of poultry-borne pathogens during commercial poultry procurement is a multi-hurdle approach, where many intervention points are used along the processing line to reduce pathogen survival. This is unique in each poultry processing plant and can utilize multiple inside-outside bird washers (IOBW) and or dip tanks before and after chilling. Carcass chilling also represents an opportunity to reduce or eliminate pathogens. Immersion chilling utilizes a counter-current system, whereby water flows in the opposite direction to carcass movement and therefore continuously exposing carcasses to cleaner water. Hence immersion chilling is the primary site for utilization of many antimicrobial treatments, including Sodium Hypochlorite (SH), Peroxyacetic Acid (PAA), Cetylpyridinium Chloride (CPC), TriSodium Phosphate (TSP), and 1,3-dibromo-5, 5-dimethylhydantoin (DBDMH) at varying concentrations (USDA, 2012). While PAA is allowed to be used at levels up to 2,000 ppm for shorter periods in post-chill dip tanks PAA can also be used at lower concentrations (up to 220 ppm) in extended dwell time chillers (USDA, 2012). Use of SH is driven by its low cost and extensive availability although it is only effective at pH levels below 7.0 (Lillard, 1979), and it's regulatory limit for use is 50 ppm in the chiller (USDA, 2012).

Data on the ability of PAA and SH to reduce *Campylobacter* populations on poultry shows that these antimicrobials can be used as effective treatments if managed properly. Using a pilot plant IOBW, Northcutt *et al*. (2007) demonstrated that 1.6 $\log_{10}$ CFU/mL reductions were achieved

using 50 ppm SH. Likewise, incidence of *Campylobacter* on carcasses was reduced by 12.8 and 43.4% using 30 ppm SH and 85 ppm PAA in a commercial chill tank, respectively (Bauermeister *et al*., 2008a). Sensory properties of both these treatments have been studied, with no negative effects reported on poultry carcasses (Bauermeister *et al*., 2008b). Additionally, these products do not pose any environmental concerns and no special precautious need to be taken while application in poultry processing plants.

A novel intervention that has potential to be used in poultry processing is DBDMH, a compound that has less sensitivity to organic matter than SH and is approved as an intervention in both pre- and post-chill spray and immersion applications in poultry processing (USDA, 2012). There is limited data available regarding the use of DBDMH on poultry; however at 75 ppm DBDMH has shown to reduce *Salmonella* and *E. coli* 0157:H7 inoculated beef parts by 0.7 and 1.1 $\log_{10}$ CFU/cm^2, respectively (Kalchayanand *et al.*, 2009). Hence, the current study was undertaken to compare the efficacy of PAA, SH and DBDMH as post-chill intervention strategies to reduce *Campylobacter jejuni* inoculated chicken carcasses.

- MATERIALS AND METHODS

Bacterial Culture: Campylobacter jejuni was cultured in 1L of *Campylobacter* Enrichment Broth and grown microaerobically (5% O_2, 10% CO_2, 85% N_2) for 18h at 42°C. Following this, 20 mL of *Campylobacter* enrichment broth was centrifuged at 1069 x g for 10 min. at 4°C and the supernatant was decanted. The pellet was then suspended in 10 mL of Phosphate Buffered Saline (PBS) for a final bacterial population of approximately 7 $\log_{10}$ CFU/mL.

Chicken Carcasses and Inoculation: Freshly processed and chilled chicken carcasses (n=3 per treatment) which had not been treated with any antimicrobials were obtained from the Auburn University Poultry Research Unit and held at 4°C until further use. Individual carcasses were inoculated with 10 mL of *C. jejuni* (ca. 10^7 $\log_{10}$ CFU/mL) in a laminar flow biosafety cabinet. The carcasses were placed in a sterile carcass rinse bag and the culture was applied to the breast and backside using a serological pipette. Each carcass was thoroughly massaged inside the carcass rinse bag and allowed 30 min for bacterial attachment. Non-inoculated carcasses served as negative control in the study, while inoculated but untreated carcasses served as the positive controls.

Preparation of Treatment Solutions and Application: Each treatment solution was prepared and held at 10°C until application. Treatment solutions consisted of DBDMH at concentrations of 50, 75, 100, 200 and 300 ppm; PAA at levels of 100 and 200 ppm; SH at levels of 25 and 50 ppm (pH 6.0); and a water treatment. The positive control sample was not subjected to any treatment and was rinsed using the USDA-FSIS whole carcass rinse procedure following 30 min bacterial attachment. Concentration of each solution was determined using Hach® Pocket Colorimeter II Test Kit for Hypobromus Acid and Hypochlorous acid and the LaMotte Test Kit for PAA. Inoculated carcasses were treated: spraying with 460 mL (~62 s of continuous spraying) using a 2-gallon garden sprayer at approximately 20-psi of pressure and a static immersion application in 6.05 L of solution in a 5-gallon bucket for 60 s. The garden sprayer was equipped with a fan nozzle, and this application was carried out under a fume hood by suspending carcasses on a shackle within the cabinet. The fan nozzle covered an area approximately 15 cm wide by 3 cm tall and the distance between the carcass and spray wand was held constant at approximately 15 cm.

Microbiological Analysis: Following treatment, carcasses were rinsed with 200 mL of 0.1% sterile Buffered Peptone Water using the USDA-FSIS whole carcass rinse method. The rinsate was recovered in sterile flasks, serial dilutions were prepared, and plated onto Campy Cefex Agar followed by incubation at 42°C for 48h under microaerobic conditions (5% O_2, 10% CO_2, 85% N_2). Typical mucoid *C. jejuni* colonies were counted and reported as $\log_{10}$ CFU/mL of

rinsate. *C. jejuni* was confirmed by examining typical *Campylobacter* colonies using phase-contrast microscopy and corkscrew-like motility.

Statistical Analysis: Results were reported as survival populations of *C. jejuni* (log_{10} CFU/mL of rinsate). Experiments were performed in triplicate with three sub-samples per replication (n= 9 carcasses for each treatment). Means were separated and differences ($P \leq 0.05$) among and between treatments and application methods were analyzed using the GLM procedure in the SAS statistical software (SAS Institute Inc., Cary, NC).

- RESULTS AND DISCUSSION

Survival populations of *C. jejuni* (log_{10} CFU/mL of rinsate) for each antimicrobial treatment applied as an immersion are shown in Table 1. Populations of *C. jejuni* on carcasses treated with 50, 75, 100, 200, and 300 ppm DBDMH were 0.8, 0.8, 0.61, 0.94, and 0.68 log_{10} CFU/mL lower ($P \leq 0.05$) than the positive control carcasses, respectively. Additionally, there were no significant differences ($P > 0.05$) within the five DBDMH treatment groups when used as an immersion application. The SH treatment group showed only a marginal effect ($P > 0.05$) on the survival populations of *C. jejuni* as compared to the positive control. However, using the 50-ppm SH solution as an immersion treatment decreased ($P > 0.05$) survival populations of *C. jejuni* compared to 25-ppm solution. Table 1 shows that survival populations of *C. jejuni* obtained following immersion application of PAA at 100 and 200 ppm were significantly lower ($P \leq 0.05$) as compared to positive control and the 25 and 50 ppm SH treatments. Furthermore, there were no significant differences ($P > 0.05$) between immersion application of PAA and DBDMH at 100 and 200 ppm (Table 1). Survival populations following spray application method of antimicrobials in this study are presented in Table 2. This data illustrates that with the exception of PAA treatments of 100 and 200 ppm, no differences ($P > 0.05$) were observed between any of the treatments and positive control (5.43±0.12-log_{10}CFU/mL). However, Northcutt *et al.* (2007) reported that SH at 50 ppm was able to achieve a 1.5 log_{10} CFU/mL lower count of *Campylobacter* using a pilot-plant carcass washing cabinet. Spray application method in the present study was not able to achieve the reductions reported by Northcutt *et al.* (2007) because the present study did not use the same volume of treatment solution and application method. Spray application method showed that PAA significantly reduced ($P \leq 0.05$) survival populations of *C. jejuni* on chicken carcasses as compared to the positive control and other antimicrobial treatments.

Survival populations of *C. jejuni* for immersion application were lower ($P \leq 0.05$) than spray application within each concentration of an antimicrobial treatment on the chicken carcasses. The SH 25ppm, SH 50ppm, and PAA 100 ppm treatments showed no significant differences ($P > 0.05$) in survival populations of *C. jejuni* between the two application methods. Immersion application method exhibited lower survival populations when compared to spray application irrespective of the antimicrobial treatment applied. Furthermore, the present study indicates an immersion time of 60 s is an efficient method to decrease populations of *C. jejuni* on fresh poultry carcasses. Adding antimicrobial solutions into extended dwell time chill tanks for up to 2h in poultry plants may be reduced considerably while maintaining similar effects on reducing *C. jejuni* numbers on fresh poultry, thus saving production time and expenses. In our study, water treatments alone were more effective ($P \leq 0.05$) than SH immersion application, however, no significant differences ($P > 0.05$) were observed between the water and the SH spray application treatments. Since SH can be difficult to manage due to the pH requirements and organic load sensitivity its effectiveness as an antimicrobial can be limited in chiller applications during poultry processing.

Table 1 Survival populations[¥] (log_{10} CFU/mL of rinsate) of *C. jejuni* on chicken carcasses following immersion application with various antimicrobials

Treatment	Immersion Application

Negative Control	ND
Positive Control	5.57 ± 0.26^{x}
Water Treatment	4.66 ± 0.20^{y}
DBDMH 50 ppm	4.77 ± 0.21^{y}
DBDMH 75 ppm	4.77 ± 0.19^{y}
DBDMH 100 ppm	4.96 ± 0.34^{y}
DBDMH 200 ppm	4.63 ± 0.26^{y}
DBDMH 300 ppm	4.89 ± 0.21^{y}
SH 25 ppm (pH 6.0)	5.38 ± 0.16^{x}
SH 50 ppm (pH 6.0)	5.22 ± 0.60^{x}
PAA 100 ppm	4.86 ± 0.48^{y}
PAA 200 ppm	4.15 ± 0.45^{z}

[¥]Mean ± Standard deviation; x, y, and z superscripts indicates significant differences ($P \leq 0.05$) between treatment; ND = Not Detectable

Results from our study showing a 1.42 $\log_{10}$ CFU/mL reduction in the *C. jejuni* on chicken carcasses post-immersion application of 200 ppm PAA are in agreement with Bauermeister *et al.* (2008a). Furthermore, use of DBDMH in post-chill applications has the ability to reduce survival populations of *C. jejuni* on fresh poultry carcasses by up to 0.91 $\log_{10}$ CFU/mL when compared to positive control carcasses. Previous studies have not evaluated the impact of DBDMH on *C. jejuni*, but reported reduction of *Salmonella* by 0.7 to 2.3 $\log_{10}$ CFU/cm^2 on inoculated beef (Kalchayanand *et al*., 2009). Efficacy of DBDMH against *C. jejuni* reported in our study is in agreement with Kalchayanand *et al*. (2009), although differences can be due to difference in the meat matrix as well as the pathogen studied. Hence, in commercial poultry processing plants the use of DBDMH will lead to a reliable pathogen reduction system.

Table 2: Survival populations[¥] ($\log_{10}$ CFU/mL of rinsate) of *C. jejuni* on chicken carcasses following spray application with various antimicrobials

Treatment	**Spray Application**
Negative Control	ND
Positive Control	5.43 ± 0.12^{x}
Water Treatment	5.43 ± 0.12^{x}
DBDMH 50 ppm	5.46 ± 0.11^{x}
DBDMH 75 ppm	5.67 ± 0.17^{x}
DBDMH 100 ppm	5.48 ± 0.16^{x}
DBDMH 200 ppm	5.49 ± 0.16^{x}
DBDMH 300 ppm	5.38 ± 0.02^{x}
SH 25 ppm (pH 6.0)	5.45 ± 0.06^{x}
SH 50 ppm (pH 6.0)	5.41 ± 0.14^{x}
PAA 100 ppm	4.97 ± 0.05^{y}
PAA 200 ppm	4.82 ± 0.06^{y}

[¥]Mean ± Standard deviation; x, y, and z superscripts indicates significant differences ($P \leq 0.05$) between treatment; ND = Not Detectable

- CONCLUSION

Decreasing survival populations of *C. jejuni* on fresh poultry is a food safety goal for poultry processors that can be achieved by using antimicrobials accessible to processors. Present data shows that completely eliminating *C. jejuni* is challenging and a multi-hurdle approach in poultry processing plants will prove to be effective in achieving the USDA-FSIS performance standards.

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