PRE-CHILL ANTIMICROBIAL TREATMENT TO ENHANCE THE SAFETY OF CHICKEN PARTS

by

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(Under the Direction of Mark A. Harrison)

ABSTRACT

Increased numbers of pathogenic microorganisms on chicken parts when compared to whole broilers has led to the hypothesis that water retained during the pre-chill stage of processing harbors bacteria which contaminates parts upon cut-up. The effectiveness of antimicrobial treatments (chlorine, peracetic acid, chlorine stabilizer) was evaluated when applied pre-chill in ability to reduce *E. coli*, coliforms, aerobic bacteria, *Campylobacter*, and *Salmonella* prevalence on chicken parts. Samples were collected post evisceration, post pre-chill, post chill, and post cut-up. Due to difficulty in detection of *Campylobacter* spp. from carcass rinsates, a comparative analysis of selective media was conducted using *Campylobacter* enrichment broth (Bolton’s) with and without supplementation with triclosan, Campy-Cefex Agar (CCA), and *Campylobacter* R&F Chromogenic Agar (RFA). Treatments containing chlorine stabilizer resulted in a significant reduction (p<0.05) at all points throughout processing for aerobic bacteria, *E. coli*, and coliforms. A combination of T-Bolton broth and RFA provided the best *Campylobacter* spp. recovery.

INDEX WORDS: pre-chill, T-128, triclosan, chlorine, peracetic acid, *Campylobacter*, *Salmonella*
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DEDICATION

I would like to dedicate this work to those have supported me throughout my entire education, leading me to this point. My loving family for providing me with support in all ways possible, my friends who understood and were considerate of my time and dedication and who kept me sane, my labmates who offered advice and a helping-hand, Dr. Mark Harrison and Dr. Mark Berrang who were always light-hearted and encouraging, and my dog Sophie who was always around when I needed puppy love. To all of you, this would not have been possible without your help. I thank you deeply.
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CHAPTER 1
INTRODUCTION

Foodborne illness has an enormous public health and economic impact on the poultry industry. In 2011, the Centers for Disease Control and Prevention (CDC) estimated 48 million cases of foodborne illness leading to 128,000 hospitalizations and 3,000 deaths (3) which translates to a $51.0 billion annual cost in the United States (28). Poultry was implicated as the leading cause of death attributing to 19% of deaths caused by foodborne illness. Salmonella spp. and Campylobacter spp., pathogens commonly associated with poultry, are responsible for 35.1% and 72% of illnesses, respectively (1).

Typically, foodborne illness related to poultry occurs as a result of the consumption of raw or undercooked food or may result from to a cross-contamination event. The risk of contamination begins pre-harvest, with vertical transmission, or transfer of pathogens from hen to chick during reproduction. As the chick matures broilers can be colonization by either Salmonella spp. or Campylobacter spp. Processing colonized broilers can spread contamination throughout processing to the distribution of the final product (5-8, 13, 31). Intervention strategies aimed at prevention of cross-contamination and targeting bacterial reduction have been implemented from farm to table. Regulations enforced by the United States Department of Agriculture’s (USDA) Food Safety Inspection Service (FSIS) for broiler processing facilities provide initiative for processors to employ new or additional strategies to control the prevalence of Salmonella and Campylobacter. Performance standards issued in 2011 as a response to national baseline studies for young chickens require that the percentage of Salmonella-positive samples be below 9.8% (5 positive samples out of 51 samples) and Campylobacter-positive
samples be less than 15.7% (8 positive samples out of 51 samples) \(12\). With these more rigorous standards, Healthy People 2020 is predicting a 25% reduction in *Salmonella* infections to 11.4 cases per 100,000 people and a 33% reduction in *Campylobacter* infections to 8.5 cases per 100,000 people \(22\).

Currently, trends in food safety include the use of various antimicrobial interventions capable of enduring high organic loads in poultry chillers and optimizing cost while achieving sufficient bacterial reductions. This has caused many U.S. broiler processing facilities to seek alternatives to the traditional use of chlorine as a primary disinfectant in poultry processing operations. Specifically, peracetic acid has gained popularity in the poultry industry. In 2010, results of an industry survey indicated that 52.2% of processing facilities used peracetic acid, 40.3% used chlorine, 4.5% used acidified sodium chlorite, and 3% used acids at a pH of 2 in immersion chiller applications \(19\). A novel chemical blend, T-128 (New Leaf Safety Solutions, LLC, Salinas, California), was developed as a chlorine stabilizer to enhance the disinfection capability of free chlorine in the presence of high concentrations of organic matter \(30\). Use of T-128 has been successful in the produce industry \(18, 21, 29\) and has shown some success in poultry applications, as well \(27\). Along with the use of alternative antimicrobials, advances in technology have led to significant experimentation involving where and how antimicrobials are applied throughout processing. In recent years, post chill decontamination tanks have been incorporated into commercial processing operations immediately following immersion chill tanks. These post chill tanks utilize high antimicrobial concentrations, smaller water volumes, and a short dwell time to achieve significant bacterial reduction \(24\).

Following evisceration and prior to immersion chilling, carcasses are subjected to a pre-chiller consisting of ambient temperature water where the carcasses are soaked and agitated for
10-15 minutes. This allows for water absorption into carcass tissue to increase yield and to reduce the heat load before entering the chiller. At this point in processing, the body temperature of the carcass is, on average, 30-35°C and feather follicles are still open as a result of scalding and picking. Entrapment of bacterial cells, specifically *Salmonella*, within the muscle, skin, and adipose tissues of chicken carcasses during processing is a widely studied phenomenon (2, 14-16, 26). Feather follicles begin to shrink in response to changes in temperature following scalding allowing for any cells that have entered the follicles to remain there, shielded from effective treatment by antimicrobials (14, 26). Entrapment of cells may also occur as a result of the swelling and shrinking of muscle fibers during processing that allows *Salmonella* to approach the hydrophobic membranes of carcasses and bind to connective tissue fibers upon changes in volume (2). Swelling and shrinking of muscle tissue may occur upon exposure to solutions of varying tonicity throughout processing, including antimicrobials that may alter the pH of the water used to chill carcasses (15, 16). Entrapment may occur during immersion chilling due to rapid decrease in temperature. Because antimicrobials are not typically applied in pre-chillers due to cost limitations and short dwell time, a high potential for cross-contamination exists. In addition to surface contamination, it is possible that retained water in carcass tissue may harbor bacterial contamination, as well, which is subsequently shielded from the bactericidal effects of antimicrobials in further processing steps. As a result, there is potential for processors to benefit from application of an antimicrobial treatment in pre-chill tanks. This work seeks to investigate the efficacy of 50 ppm chlorine, 50 ppm chlorine in combination with 0.5% T-128, 20 ppm peracetic acid, 20 ppm peracetic acid in combination with 0.5% T-128, and 0.5% T-128 as antimicrobial additives in pre-chill tanks.
National microbiological baseline data collected from 2007-2008 indicates an estimated prevalence of 10.66% and 5.19% for *Campylobacter* and *Salmonella* on post chill broiler carcasses, respectively (9). Similar data collected for chicken parts reported that the prevalence of *Salmonella* is four times higher and the prevalence of *Campylobacter* is doubled on chicken parts when compared to whole carcasses (11). This increase in prevalence may be a result of a single positive carcass producing multiple contaminated chicken parts, ultimately increasing the risk of cross-contamination during processing and handling. It is also a possibility that water retained within the tissues of the carcass during the pre-chill step of processing may be released upon cut-up, subsequently leading to contamination of chicken parts. The objective of this study was to determine the effectiveness of the application of an antimicrobial treatment in the pre-chill tank on reducing the numbers and prevalence of generic *E. coli*, *Campylobacter* spp., and *Salmonella* spp. on chicken parts.

Enumeration and detection of naturally occurring *Campylobacter* spp. originating from carcass rinses has proven to be extremely difficult on many selective media due to interference by background microflora. In one study, 14 different non-*Campylobacter* spp. contaminants grew on Campy-Cefex agar (CCA) (17). FSIS, the governing body responsible for confirming compliance with performance standards in the poultry industry, currently tests for *Campylobacter* spp. on whole broiler carcasses using the following procedures: sample preparation in 400 mL of buffered peptone water (BPW), direct plating by distribution of 1 mL of rinsate onto 4 CCA plates, enrichment in double strength Bolton broth (30 mL:30 mL), isolation on CCA, with confirmation via microscopic examination and a latex agglutination immunoassay (10). Alternative media for the selective isolation of *Campylobacter* spp. such as supplementation of Bolton broth with triclosan, Campy-Line agar (CLA), modified-charcoal
cefoperazone-deoxycholate agar (mCCDA), and *Campylobacter* R&F chromogenic agar (RFA) have shown success in elimination of background microflora (4, 17, 20, 23, 25). Due to the desire to accurately detect *Campylobacter* spp. at various points throughout processing where contamination by competing indigenous microflora may be present, this study was also designed to compare the effectiveness of various combinations of enrichment broths and plating media for detection of naturally occurring *Campylobacter* spp. in broiler carcasses rinsates. Specifically, RFA and triclosan-supplemented Bolton broth (T-Bolton) were evaluated as alternatives for the current FSIS media types, CCA and Bolton broth.
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CHAPTER 2
LITERATURE REVIEW
Impact of Poultry on Public Health

Poultry has commonly been associated with foodborne illness in the United States. Based on Centers for Disease Control and Prevention (CDC) estimation, approximately 48 million people become sick from foodborne illness, 128,000 are hospitalized, and 3,000 die, annually (29). *Salmonella*, a microorganism prevalent on poultry, can be attributed to 11% of those foodborne illnesses, 35% of hospitalizations, and 28% of deaths. The top 5 serotypes of *Salmonella* isolated from broilers by the United States Department of Agriculture (USDA) are Kentucky, Enteritidis, Typhimurium, Thompson, and Infantis (57). Of these, Enteritidis, Typhimurium, and Infantis are listed by the CDC as three of the top ten *Salmonella* serotypes found to cause foodborne illness (31). *Campylobacter*, another bacterial pathogen prevalent on poultry, is responsible for 9% of foodborne illnesses, 15% of hospitalizations, and 6% of deaths (29). A report has recently ranked poultry as the primary source for *Campylobacter* illness (11). *Campylobacter jejuni* is linked to the majority of foodborne infection related to *Campylobacter* worldwide (60). From retail market products, Willis and Murray (1997) isolated *C. jejuni* from 229 out of 330 raw poultry carcasses for sale in North Carolina in 1997 (149), Jorgensen et al. (2002) isolated *C. jejuni* from 98% of isolates taken from 241 raw poultry samples (79), and Zhao et al. (2001) identified 58% of isolates collected from 595 raw poultry carcasses as *C. jejuni* in Washington, D. C. (157). Foodborne illness results in an estimated cost of $51.0 billion, annually, with *Campylobacter* spp. representing $1.56 billion and *Salmonella* spp. representing
$4.44 billion (133). Based on this data, it is clear that preventative measures need to be taken in the reduction or elimination of these foodborne pathogens on poultry.

*Campylobacter* is one of the primary causes of gastrointestinal illness in the United States and the resulting illness, campylobacteriosis, is characterized by cramping, abdominal pain, fever, and diarrhea that may be accompanied by nausea and vomiting. Symptoms typically occur two to five days following exposure to as few as 500 cells and can last for up to a week (32). One in every 1,000 cases of campylobacteriosis may develop into an autoimmune disease such as Guillian-Barrè syndrome or Miller Fisher syndrome, both of which result in temporarily paralysis. Infection caused by exposure to *Salmonella*, salmonellosis, results in symptoms that include diarrhea, fever, and abdominal cramps that may continue for 4 to 7 days (32). Onset of illness is typically 6-72 hours following exposure. In some cases involving elderly, infant, or immunodeficient individuals, the infection may spread from the intestine into the blood stream and result in more severe illness (30).

In 2009, the USDA’s Food Safety and Inspection Service (FSIS) was presented the challenge of reducing *Salmonella* risk in poultry products by the President’s Food Safety Working Group (FSWG). As opposed to a focus on effectively responding to food-related outbreaks, performance standards were implemented with the intention of providing a more proactive approach to food safety by attempting to prevent these outbreaks. In 2010, FSIS released performance standards for the control of *Salmonella* and *Campylobacter* on post chill young chicken and turkey carcasses. These performance standards included a 9.8% tolerance for *Salmonella* positive samples (5/51) and a 15.7% tolerance for *Campylobacter* positive samples (8/51) in broiler processing plants (58). Retesting for plants that fail initial inspection is mandatory and the identity of these plants is made available to the public (58). Healthy People
2010 targeted 12.3 cases per 100,000 people for *Campylobacter* infections and 6.8 cases per 100,000 people for *Salmonella* infections (115); however, based on FoodNet data released by the CDC, 14.31 cases per 100,000 people were reported for *Campylobacter* and 16.5 cases per 100,000 people were reported for *Salmonella* (29). With stricter regulation in the poultry industry, Healthy People 2020 are estimating a 25% reduction in salmonellosis or 11.4 cases per 100,000 people and a 33% reduction in campylobacteriosis or 8.5 cases per 100,000 people (115). FSIS estimates that with implementation of performance standards, 39,000 illnesses due to *Campylobacter* and 26,000 illnesses due to *Salmonella* will be eliminated (58).

The risk of contamination of poultry meat begins on farms during live production, continues throughout processing, and remains a risk during distribution of the final product. Equipment and carcasses can become contaminated postmortem by transfer of pathogens from skin, feathers, or intestinal content during processing (140). Following processing, it is estimated that between 5.18% (54) and 35% (88) of ready-to-market broilers may be positive for *Salmonella*, while only 3–4% typically test positive for *Salmonella* upon entering the plant. While many may argue that the consumer is liable for cross-contamination during food preparation, a study that simulated a worst case scenario where the food handler used the same knife, cutting board, and gloves to prepare the raw product as they did to prepare the cooked product, only one event of cross-contamination was observed out of 57 meal preparations (incidence of 1.8%) (118). Contamination can be a result of pre-harvest colonization or cross-contamination during processing and preparation, therefore, prevention should be a continuous goal from the farm to the table.
Microorganisms of Concern

*Campylobacter* spp.

*Campylobacter* is a Gram-negative, non-sporeforming microorganism with spiral rod morphology and darting motility. The thermophilic cells have an optimal growth temperature of 42°C (the internal temperature of a chicken) and thrive at a near neutral pH (32). *Campylobacter* requires a microaerobic environment for growth consisting of 5% O₂, 10% CO₂, and 85% N₂ (32). Susceptibility to stressors such as drying, low pH, heat, freezing, and prolonged storage may result in the formation of a viable-but-nonculturable cell (VBNC) (137). *Campylobacter* cells in the VBNC state become coccoidal, losing their characteristic shape and motility. They cannot be detected by standard culture methods, but still possess the ability to remain pathogenic under exposure to favorable conditions (137). While *Campylobacter* may be considered fragile due to its susceptibility to drying and oxygen, it exhibits high prevalence throughout the environment and is resilient both inside and outside of a host (32, 104, 107). This resilience may be attributed to highly mutable sites within the genome of *C. jejuni* that allow for rapid adaptation (77). Survival of *Campylobacter* cells has been recorded for up to 3 months in slurries and water containing high organic loads (110), and for up to 10 months in manure compost (73). FSIS along with CDC recommend a minimum internal temperature of 165°F (73.9°C) for safe food preparation to eliminate *Campylobacter* on poultry.

Chickens are known to be a natural reservoir for *Campylobacter* spp., and the pathogen can colonize a flock via vertical transmission. The organism has been detected in chicken reproductive tracts (44), semen, and in hatchery samples from egg shells, tray liners, and feather fluff (44, 47, 139). Horizontal transmission, or contamination by contact, however, is the most likely route of initial colonization of *Campylobacter* in broiler flocks. Resilient clones that
persist in the environment are capable of infecting multiple broiler flock rotations (121). 

*Campylobacter* is often found in surface waters with a heightened risk during summer months due to increased rainfall and higher temperatures (1, 107, 155). Multispecies farms may pose a risk for infection as *Campylobacter* is not only a part of the natural intestinal microbiota of chickens, but is also carried by a wide range of wild and domestic animals including cattle, pigs, and laying hens (4, 49, 125, 145, 159). Other common vehicles that may introduce *Campylobacter* into a farm include rodents, flies (17, 62, 63, 109), and contamination of personnel or farm equipment (122).

Most flocks typically become colonized at an age of 2-4 weeks (17, 66). Prior to this time, it is thought that maternal antibodies offer protection against *Campylobacter* colonization in young chickens (128). The transmission rate of *Campylobacter* was determined to be 2.37 new cases per colonized chick per day (146), resulting in a 5-7 day colonization period where 95-100% of birds become infected regardless of age (138). Studies have shown that there are several factors that may contribute to a higher probability of flock colonization including an increased risk during rearing (66, 123) and increased flock size (17).

Results from the FSIS National Microbiological Baseline Data Collection Program for Young Chickens from July 2007-July 2008 indicated a prevalence of *Campylobacter* on post chill broilers to be 10.66% (54). The European Food Safety Authority (EFSA) reported an average of 60-80% of analyzed broiler flocks to be positive for *Campylobacter* at slaughter age worldwide (50). While colonization of the cecum is primarily a risk pre-harvest, contamination of food products distributed for consumption often occurs during processing. Several critical control points exist where there is an increased likelihood of the introduction of *Campylobacter* during broiler processing. Berrang and Dickens (2001) determined that between 4-5 logs
CFU/ml of carcass rinsate could be detected early during processing (19). Prior to scalding, carcasses may contain 5.4 log CFU/g of *Campylobacter* (20), 7.5 log CFU/g within the feathers (84), and 3.8 (20)– 6.9 (84) log CFU/g in the breast skin. Scalding typically results in an estimated 3 log reduction with an average of 1.8 log CFU/ml *Campylobacter* remaining on the carcasses (18). The predominant site for *Campylobacter* contamination in broilers is in the mucosal layer of the cecum (14, 101) where bacterial populations are as high as 6-7 log CFU/g (18, 105, 117) and may increase by as much as 1 log due to stressors such as transport (93). Cross-contamination typically occurs during defeathering and evisceration due to the cloaca and visceral rupture of the ceca and release of gut contents from the lower bowel that allows for transfer of fecal matter to the external surface of the carcass (14, 20, 105). *Campylobacter* levels have been shown to increase 1-2 logs following picking (19). While chilling is an effective control for the reduction of *Campylobacter*, 1.5 log CFU/ml have been reported from post-chill carcass rinsates (20). It can be concluded that while *Campylobacter* levels are reduced during processing, they are still detectable following chilling methods and may pose a risk to public health.

Despite numerous statistics that rank chicken as the number one source for *Campylobacter* illness in humans, regulatory agencies, farmers, and processing facilities lack an effective strategy for the reduction of *Campylobacter* prevalence in poultry (67). On the farm level, no vaccines are available for controlling *Campylobacter* colonization in breeding and grow-out flocks (98), although the use of bacteriophages and bacteriocins looks promising (91). In broiler grow-out facilities, biosecurity measures such as litter management (due to the susceptibility of *Campylobacter* to drying and desiccation) and water acidifying treatments have been identified as some of the best methods of control for *Campylobacter* on the farm (98).
Some poultry houses employ an “all in/all out” system where houses are cleaned, disinfected, and dried prior to the arrival of a new flock. This leads to a significant decrease in the probability of carryover from previous flocks (108). The application of fly-screens around broiler house ventilation systems during the summer months has also proven to reduce *Campylobacter* colonization (7). During processing, the primary method of control involves antimicrobial intervention strategies. The growing popularity of alternative antimicrobial solutions such as peracetic acid (PAA) has resulted in greater prevention of *Campylobacter* when compared to a standard chlorine solution. A concentration of 85 ppm PAA was found to reduce *Campylobacter* by 43.4% while a concentration of 30 ppm chlorine resulted in only a 12.8% reduction (13).

Because *Campylobacter* is detected on post-chill carcasses at levels of around 1.5 log CFU/ml (20), post-chill treatments have been incorporated in the hurdle approach which target antimicrobials that are capable of at least a 2 log reduction (120).

Just as *Campylobacter* is difficult to control, regulatory agencies also find it difficult to accurately enumerate and detect naturally occurring *Campylobacter* spp. from poultry carcasses due to interference from background microflora on selective media types. Extended-spectrum-β-lactamase (ESBL) producing *E. coli* have conferred resistance to cephalosporin antibiotics, allowing for growth on cefoperazone-based media such as *Campy*-Cefex agar (CCA), Bolton enrichment broth, and modified-charcoal-cefoperazone-deoxycholate agar (mCCDA), some of which are commonly used in standard USDA-FSIS methods (39). One group isolated up to 14 non-*Campylobacter* contaminants on selective *Campylobacter* media (92), and additional studies have identified between 93% (103) -100% (39) of contaminants to be ESBL-producing *E. coli*. Alternative methods have been investigated to allow for easier enumeration of naturally occurring *Campylobacter* spp. and elimination of background microflora including substitution
of CCA with more selective media such as Campy-Line agar (CLA) (92, 119) or Campylobacter R&F chromogenic agar (RFA) (124) and supplementation of Bolton broth with antibiotics such as triclosan (39). Triclosan is an antimicrobial that is included in selective media for Yersinia and Pseudomonas spp., and in household and personal care products. It primarily exhibits bactericidal activity against Gram-positive species and also some Gram-negatives through disruption of fatty acid synthesis (64). Chon et al. (2014) found that supplementation of Bolton broth with triclosan at concentrations of 1 µg/mL resulted in a 43.8% better recovery of Campylobacter spp. on mCCDA from chicken carcass rinsates (39).

Salmonella spp.

Salmonella is a genus of Gram-negative, non-sporeforming, rod-shaped bacteria in the Enterobacteriaceae family. The mesophilic cells are facultative anaerobes and have an optimal growth temperature between 35 and 37°C at a neutral pH (30). Salmonella cells are susceptible to changes in pH with pH>9 or pH<4 resulting in cell death (71). Studies have proven Salmonella to be very difficult to remove from contaminated hides, feathers, muscle tissue, or equipment surfaces post-slaughter (16, 26, 40, 46, 88, 96, 116, 152). Viable organisms were able to persist on the surface of a carcass following the use of high pressure water sprays with or without chlorine germicides, detergents, or pH alterations in the water used to chill the carcasses (26, 40, 46, 96, 116). Salmonella spp. in high-moisture foods such as poultry are susceptible to heat treatments and are typically killed at temperatures of 70°C or above (95). Cells may confer some resistance to heat if harbored in fatty portions of the carcass, or if at a neutral pH (116). Salmonella spp. are, however, resistant to drying and may persist in dust and dirt for several years (95).
Salmonella spp. are commonly associated with poultry and are capable of infecting a flock through both vertical and horizontal transmission (74). Salmonella has been isolated from breeder flocks, infected ovaries and oviduct tissue, and eggs prior to shell formation, indicating a potential for vertical transmission of the bacteria (43, 68). Cells have also been found in egg shell fragments, chick pads, and chick fluff in poultry hatcheries (42). However, contamination is more likely to occur as a result of horizontal transmission due to interactions with environmental sources such as litter, feed, water, flies (infection rate of 13% on poultry farms), humans, animals, rodents, and fecal matter (6, 69, 78, 139). Salmonella in the environment is often disseminated via water currents, underground springs, and rain runoff (151). Water sources can act as reservoirs for the transfer of bacteria between hosts (38, 53). Cells can survive in sewage waste for up to 15 days and have remained viable in waste slurries of infected fecal matter for up to 21 days (10). Poultry house disinfection has proven to be ineffective in many cases resulting in the documented persistence of Salmonella for over a year (45). Due to isolation of the microorganism from a wide array of animal species, it is thought that Salmonella lacks host adaption specificity (53), allowing for a lifecycle consisting of shedding from an original host, survival in the environment, and transfer to a new host (142).

Salmonella is believed to associate with carcasses in several different ways, resulting in varied recovery by rinsing procedures. Cells may be unattached in the water layer on the surface of the tissues allowing for easy removal by rinsing while others may be attached to collagen fibers of the skin and muscle tissue or may be harbored in crevices created by the skin, muscle fibers, and feather follicles shielding cells from rinsing (51, 82, 129). Once Salmonella cells have become embedded in muscle, skin, or adipose tissues, they often resist removal by routine processing methods and become unavailable to disinfectants (15, 82, 89). Entrapment of cells
may occur as a result of the swelling and shrinking of muscle fibers during processing that allows *Salmonella* to approach the hydrophobic membranes of the carcass and bind to connective tissue fibers upon change in volume (16). This swelling and shrinking of muscle tissue is most likely due to exposure to solutions of varying tonicity throughout processing (88, 90). Scalding opens up feather follicles to allow for easy feather removal down the line, but can often result in entrapment of *Salmonella* (55). Feather follicles begin to shrink in response to changes in temperature following scalding allowing for any cells that have entered the follicles to remain there (82, 127).

Results from the National Microbiological Baseline Data Collection Program for Young Chickens from July 2007-July 2008 indicated a prevalence of *Salmonella* on post-chill broilers to be 5.19% (54). Prevalence decreased from re-hang to post-chill (40.7% to 5.19%) validating the effectiveness of antimicrobial interventions in reduction of pathogens. Carcass picking and evisceration are major sites where bacteria may be transferred from the intestines to the skin or outer surface of the carcass (88, 97). Further spread of *Salmonella* may occur as a result of poor sanitation and hygiene practices by workers and consumers handling the product during cut-up, in markets, and in kitchens. Insufficient thermal processing during cooking can result in survival of bacterial cells, and improper refrigeration allows for multiplication of *Salmonella* in raw and cooked poultry products (25). In order to effectively reduce the impact of *Salmonella* on public health, it is necessary that precautions be taken on the farm, during processing, and during preparation.

The poultry industry has been combating the issue of *Salmonella* contamination in the United States since the late 1900s. As a result, there are many intervention strategies that have been employed to reduce the pathogen’s prevalence on food. Multi-hurdle approaches have
proven to be most effective, including interventions on the farm such as vaccination of breeder flocks, litter management, and feed and water treatments, as well as antimicrobial interventions during processing (98). Supplementation of poultry drinking water with acid reduced *Salmonella* prevalence in broiler crops by up to 80% (28). Antimicrobials are typically applied as a spray application during online reprocessing or inside-outside bird washes (IOBW) (average of 0.5 log reduction), but result in the greatest reduction when applied in an immersion chiller with a 1-2 hour contact time (55). Recently, post-chill antimicrobial application has gained popularity due to the potential to use higher concentrations of antimicrobials, smaller volumes of water, and a shorter contact time (120). McKee states that in order to achieve optimal product quality at the lowest cost, an ideal solution would be application of a lower level of antimicrobial in the chiller followed by an antimicrobial intervention post-chill (97).

**Poultry Processing**

**Critical Control Points and Hazard Analysis**

Various stages throughout broiler processing exist that can be identified as critical control points for prevention of contamination by pathogens such as *Campylobacter* and *Salmonella*. Following arrival at processing plants, birds undergo stunning, exsanguination, scalding, picking, evisceration, and chilling. Stunning serves as a method of immobilization and desensitization to pain reducing struggle and convulsions in an attempt to preserve the quality of the final product. Due to cost and efficiency, electrical stunning is the most popular method used in the United States. Birds are typically subjected to a water bath carrying an electrical current not to exceed 50 volts AC that renders them unconscious for approximately 60 to 90 s (34). Wing flapping and quivering that occur as a result of electric shock may cause a release of fecal material, transferring bacterial pathogens from the inside of the bird to the outside of the bird, other birds,
or to equipment (55). Musgrove et al. (1997) found that *Campylobacter* prevalence detected from carcass rinses increased after stunning (105).

Exsanguination, or bleeding, ensures that poultry have stopped breathing prior to further processing in order to satisfy compliance with 9 CFR 381.65(b) (36). A rotating circular blade severs the jugular veins and carotid arteries, resulting in 40-50% blood loss from the carcass after 1.5-3 min bleed-out time (150). Following bleed-out, carcasses are scalded in single- or multi-staged immersion tanks. High temperatures cause denaturation of proteins that hold feathers in place, as well as open up the feather follicles to prepare for defeathering. To control cross-contamination, processors monitor pH, temperature, and water conditions. FSIS claims that scalding can be used as an intervention if pH is properly maintained in the scald tanks. Humphrey and Lanning (1987) reported that alkaline pH (9.0 ± 2) was best at reducing *Salmonella* and *Campylobacter* in scald water (72), while Okrend et al. (1986) found an acidic pH (3-4) to be most effective in reducing *Salmonella* (116). Organic matter released into the water, specifically uric acid primarily leaked from internal organs such as the kidneys, is capable of reducing the pH from 8.4 to 6.0 in less than 2 hours (71), providing optimal conditions for bacterial reproduction. In an attempt to moderate organic-loads in the scald tanks, a counter current flow with continuous water replacement is typically employed to create a dirty-to-clean water gradient (55). For processing of broilers, two different time-temperature combinations may be used: soft scald or hard scald. A hard scald requires shorter scald time (30-75 s) at higher temperatures (59-64°C) while a soft scald requires longer scald time (90-120 s) at lower temperatures (51-54°C). Hard scald is the most popular method in the United States. *Salmonella* is unable to grow at temperatures higher than 47°C (51) and a scalding temperature of 58°C or above yields a significant reduction in *Campylobacter* on carcasses (75). If temperatures are too
high, however, carcasses may become oily, enhancing attachment by *Salmonella*, or the meat may begin to cook (55). While a high degree of control is necessary, scalding is capable of achieving a 38% decrease of *Salmonella* positive carcasses (61) and a 2-3 log reduction of *Campylobacter* in carcass rinses if the necessary precautions are taken (20).

Feather removal is accomplished by passage of carcasses through rubber picking fingers that mechanically remove feathers from the uppermost layer of skin via agitation and rubbing. This process often results in release of fecal material leading to contamination of rubber picking fingers that massage bacteria into pores and cross-contamination between carcasses (20, 24). It has been reported that a single contaminated carcass is capable of contaminating 200 additional carcasses during defeathering (99, 147). Researchers have widely confirmed that *Campylobacter* and *Salmonella* levels significantly increase following picking (2, 20, 75, 88). Several steps may be taken to reduce the risk of cross-contamination including a post-pick rinse of chlorine, acetic acid, or hydrogen peroxide maintained at 160°F (71.1°C) (55).

Evisceration, or removal of internal organs, can result in rupture of the viscera and leakage of crop contents during processing, largely contributing to cross-contamination of carcasses (25). Berrang and Dickens (2000) found that 100% of 18 broiler crops tested were positive for *Campylobacter* (18). FSIS recommends a 20 ppm chlorine rinse following evisceration to remove any loose material from the carcass surface. Upon incorporation of a rinse, the incidence of *Salmonella*-positive carcasses can decrease by 1/3 (113), and reduction in *Campylobacter* prevalence has also been observed (2, 75). Before carcasses can undergo chilling, they are required to pass an external inspection to detect visible fecal or ingesta contamination that may be present on the surface of the carcass (36). If contamination exists, the carcass may undergo on-line reprocessing (OLR) involving an automated washing system that
uses chemical sprays such as 20-30 ppm chlorine, 10% trisodium phosphate, 5% cetylpyridinium chloride, 2% lactic acid, or 5% sodium bisulfate, all of which have been proven to be effective at reducing *Salmonella*, and in some cases *Campylobacter*, on carcasses (52, 153). Inside-outside bird washers (IOBW) may be employed at this step with spray nozzles that rinse both the abdominal cavity and the exterior of the bird. OLR and IOBW are often unreliable as a significant decontamination step due to inconsistencies in contact time, pressure, and bird coverage (55).

Carcass chilling represents one of the most critical points for controlling microbial growth during processing. As mandated by 9 CFR 381.66, internal carcass temperatures must be reduced to 40°F (4.4°C) within 4-8 hours post-mortem, depending on weight (35). This decrease in temperature is essential for reduction of carcass contamination, inhibition of microbial growth, and extension of product shelf-life. In the United States, two methods of chilling are currently approved for use in industry, immersion chilling and air chilling. Immersion chilling involves immersion of carcasses in chilled water with constant agitation while air chilling involves misting of carcasses with water in a temperature-controlled room. Immersion chilling possesses several advantages over air chilling such as a small economic footprint, the ability to use higher concentrations of antimicrobials, better microbial inactivation than spray applications, good coverage, smaller economic footprint, and shorter dwell times. Immersion chillers have been reported to reduce internal carcass temperatures to 4°C in 45 min compared to 130 min when using air chilling methods (158). Research has shown reductions in *Campylobacter* on poultry carcasses to be lower in air chilling systems when compared to immersion chilling systems (5, 131). While air chilling does not involve a chemical intervention, a reduction in cross-contamination may be observed due to a decrease in physical contact between carcasses (70).
Due to the efficiency of immersion chilling, this method is used by most poultry processing operations to chill carcasses in the United States.

Immersion chilling typically involves multi-stage tanks with counter-current flow in an attempt to reduce cross-contamination and to achieve the same dirty-to-clean gradient desired in scald tanks. The pre-chill tank is the first stage following evisceration. In this tank, carcasses are soaked and agitated in ambient temperature water for 10-15 minutes to allow for water absorption. This water absorption is intended to increase yield and to remove some heat from the carcasses prior to entering the chiller. Carcasses entering the chill tank are on average 30-35°C and are exposed to water temperatures that range from 4°C at the entrance to 1°C upon exit. Agitation in the tanks is typically created by the injection of air from the bottom of the tank into the water, preventing thermal layering and increasing the rate of heat exchange (97). Cross-contamination by pathogens due to physical contact between birds and contaminated water during immersion chilling presents a large risk for higher incidences of carcass contamination (8), but may be combated by the utilization of antimicrobials and water reuse systems, as well as strict monitoring of pH and organic-loads. Without these controls in place, an increase in Salmonella-positive carcasses may occur (141).

**Water Retention**

FSIS mandates that water retained by carcasses and parts of carcasses as a result of post-evisceration washing and chilling be an unavoidable consequence of ensuring product safety and quality. Poultry products that retain water must be appropriately labeled to inform consumers of the maximum possible percentage of retained water based on product weight. For whole chickens that measure 4.25 pounds or less, a limit of <8% retained water is allowed, while water retention limitations for chicken that is ice-packed or subsequently cut up is set at <12% (35).
a study evaluating water retention during a 30 minute immersion chill, carcasses retained, on average, 11.7% moisture (156). Young and Northcutt reported that larger carcasses older than 44 days at slaughter absorbed more water than smaller carcasses (112). Moisture is typically desirable in poultry meat products in moderation due to increased quality for the consumer and increased yield for processors, but requires moderation. Water-holding capacity is highly correlated with broiler pH and decreases as pH drops below 5.8-6.0 leading to adverse effects such as pale, soft, exudative (PSE) meat (154).

**Contamination of Chicken Broilers vs. Chicken Parts**

Analysis of microbiological profiles of whole chicken carcasses and chicken parts revealed the prevalence of *Salmonella* is four times higher and the prevalence of *Campylobacter* is doubled on chicken parts when compared to whole carcasses (56). It is hypothesized that this increase in prevalence may be a result of a single positive carcass producing multiple contaminated chicken parts, ultimately increasing the risk of cross-contamination during processing and handling (56). FSIS compliance guidelines for controlling *Salmonella* and *Campylobacter* indicate a higher possible incidence of *Salmonella* because of possible cross-contamination between *Salmonella*-negative and *Salmonella*-positive parts (55). The estimated national prevalence of *Salmonella* and *Campylobacter* on post-chill young chickens predicted from baseline data collected from 2007-2008 is 5.19% and 10.66%, respectively, whereas results from baseline data from raw chicken parts collected in 2012 suggest *Salmonella* and *Campylobacter* prevalence of 26.3% and 21.4%, respectively (54, 56). Chicken part contamination by *Salmonella* can be ranked neck>giblets>wing>breast>leg>half carcass>quarter carcass with percent positives ranging from 20.61%-54.55% (56). Chicken part contamination by *Campylobacter* can be ranked neck>giblets>quarter carcass>wing>leg>half carcass>breast with
percent positives ranging from 16.11%-54.55% (56). According to FSIS, breasts, legs, and wings are the most frequently produced chicken parts in the United States (>90%), leading to increased concern of contamination (59).

A survey conducted from samples taken in Hawaii for the presence of *C. jejuni* reported percent positives of 83.3% for whole carcasses and 91.7% for chicken parts (80). Oscar (2013) found that upon enrichment of *Salmonella* from inoculated chicken parts (wing, breast, thigh, and drumstick), the type of chicken part did not affect the growth of *Salmonella*, despite any differences in size, shape, and chemical/microbial composition (118). FSIS estimates that of foodborne illnesses resulting from consumption of poultry, 81% can be attributed to chicken parts, 13% to whole carcasses, and 6% to comminuted product. As a response to consistent reports of increased prevalence on chicken parts, FSIS has released a notice indicating revisions to current performance standards regarding chicken parts to be implemented in March 2015 (59). Revisions include a pathogen reduction performance standard for *Salmonella* of eight positives out of 52 samples for raw chicken parts. The 2015 Risk Assessment performed by FSIS estimated that a pathogen reduction performance standard for *Campylobacter* on chicken parts of four positives out of 52 samples would be sufficient (59). With implementation of these revised performance standards, FSIS is proposing a 30% reduction in salmonellosis from raw chicken parts and a 32% reduction in campylobacteriosis with elimination of 29,000 and 14,300 illnesses, respectively (59).

**Antimicrobial Interventions**

Currently, approved antimicrobials for poultry application include acidified sodium chlorite, bromine, chlorine dioxide, cetyl pyridium chloride, organic acids, peracetic acid (PAA), trisodium phosphate, sodium metasilicate, monochloramine, electrolyzed water, and
hypoiodous acid (chlorine) (33). An industry survey conducted in 2010 by McKee including a total of 67 plants reported that the majority of processing facilities used peracetic acid as an intervention in chiller applications, followed by chlorine, acidified sodium chlorite, cetylpyridium chloride, and acids at a pH of 2 (97).

**Chlorine**

Chlorine has historically been used as an antimicrobial in the poultry industry since the 1950s as a preventative for carcass cross-contamination primarily in immersion chillers, but also as spray applications during OLR and IOBW (97)(90). Low cost and availability are the key reasons for the widespread use of chlorine, as well as minimal impact to end-product quality (114). Currently, FSIS allows for the use of 50 ppm free available chlorine as an indirect food additive in poultry processing (33). The efficacy of chlorine as an antimicrobial for bacterial reduction is largely decreased by increasing pH and organic load (27). The active ingredient of chlorine, hypochlorous acid (HClO), is most abundant in solution around pH 4.0-7.0 with disinfection increasing with decreasing temperatures (65, 148). In commercial broiler chilling operations, a pH of 6.0 is desired to maximize disinfection activity of chlorine while still maintaining water-holding capacity of the carcass (132). The pH often has to be lowered by the addition of an organic acid, most commonly citric acid, converting the active chemical to acidified sodium chlorite (65).

It is hypothesized that uncharged chlorous acid is capable of penetrating bacterial cell walls, disrupting protein synthesis via interactions with sulfhydryl-, sulfide-, and disulfide-containing amino acids and nucleotides, and ultimately causing rupture of the cells (81). In the presence of high concentrations of organic matter, chlorine combines with ammonia and other nitrogenous compounds, rendering it inactive as a disinfectant (48). In a study conducted by Tsai
et al. (1992), simulated poultry chiller water with 35% solids composed of lipids, ash, and total nitrogen was used to determine the consumption of chlorine and the necessary dosage required for disinfection (143). It was found that chiller water had a greater chlorine demand than the highest dosage of chlorine tested, 400 ppm, and no free chlorine was detected in water following a 30 minute exposure time (143). Due to the high rate of chlorine consumption, 100-150 ppm chlorine was required to reduce the number of bacteria by 99% within 3-5 minutes (143). Replenishment of chlorine periodically throughout processing is a common practice in industry; however, continuous addition of sodium hypochlorite to chiller water of high organic loads may result in the formation of toxic chlorine by-products such as chloramines and chlorine off-gas into the environment (114). USDA-FSIS requires that 2.3-2.6 L of fresh chill water be circulated per carcass (111). Despite circulation of fresh water, high organic loads in chill water have been shown to reduce the antimicrobial efficacy of chlorine (87). For chlorine to serve as an effective antimicrobial in the poultry industry, processors must overcome the hurdle of organic matter.

When evaluating contact time of various antimicrobials necessary for bacterial reduction, it was concluded that, on average, chlorine requires a 1-1.5 hour contact time in chiller water (90). Tamblyn et al. showed a 2.3 and 2.5 log reduction of Salmonella on broiler skin when chlorine levels of 400 and 800 ppm were applied, respectively (97). Yang et al. (2001) proposed that the susceptibility of bacteria attached to chicken skin to chlorine and other antimicrobials is reduced as a result of an oil layer that inhibits contact (152). Many studies have found the use of chlorine in chiller water to be effective in significantly reducing bacterial populations in chiller water (22, 100), leading to the suggestion that chlorine is more suitable for the prevention of cross-contamination of pathogens in solution rather than elimination from poultry carcasses (76, 99, 143).
**Chlorine Stabilizer T-128**

In 2008, as a response to the interference of organic matter with chlorine in the produce industry, an antimicrobial solution, T-128 (New Leaf Safety Solutions, LLC, Salinas, CA), was developed with the intention of enhancing the stability of free chlorine in wash solutions (135). This chemical blend is classified as generally recognized as safe (GRAS) and is composed primarily of ortho-phosphoric acid and propylene glycol. A synergistic effect with chlorine has been widely observed upon implementation in produce applications and also holds merit when used as a chemical additive in poultry processing, as well (94, 114, 132, 134).

Nou et al. (2011) found that T-128 successfully decreased the rate of free chlorine depletion in the presence of soil and lettuce extracts during fresh-cut lettuce processing, as well as significantly reduced bacterial pathogens in wash solutions with high organic loads (114). Similarly, the use of T-128 in produce wash water was found to significantly reduce the survival of *E. coli* O157:H7 and was effective in preventing cross-contamination of un-inoculated shredded iceberg lettuce in the presence of inoculated spinach leaves (94). Luo et al. (2012) suggests that bactericidal activity of chlorine and T-128 against *E. coli* O157:H7 decreases at high concentrations of T-128 (>2.5%) and extended contact times (94). When evaluated for use against *Salmonella* and *Pseudomonas* biofilms on stainless steel contact surfaces in produce processing, application of T-128 decreased the rate of free chlorine depletion due to organic matter and significantly enhanced inactivation of both *Salmonella* and *Pseudomonas* biofilms when compared to treatments not including T-128 (134).

T-128 was evaluated as an immersion chill additive in poultry processing to control cross-contamination by *Salmonella* and *Campylobacter* on chicken carcasses. When used in combination, chlorine and T-128 showed significantly better control of cross-contamination by
both microorganisms than chlorine alone during pilot-scale chilling of drummettes and whole broilers (132). In post-chill applications with a contact time not exceeding 20 seconds, the efficacy of T-128 and chlorine, as well as T-128 and peracetic acid, was assessed for control of Salmonella spp., Campylobacter spp., and Listeria monocytogenes. A synergistic effect between peracetic acid and T-128 was not observed, but performance of PAA did not significantly differ from PAA+T-128 (120). This data indicates the potential of T-128 for use as a commercial processing aid to reduce the risk of cross-contamination by pathogens in both immersion- and post-chill operations.

**Peracetic Acid**

Peracetic acid (PAA) is the peroxide of acetic acid formed by the reaction of acetic acid and hydrogen peroxide (3).

\[
\text{CH}_3\text{CO}_2\text{H} + \text{H}_2\text{O}_2 \rightarrow \text{CH}_3\text{CO}_3\text{H} + \text{H}_2\text{O}
\]

PAA has an acidic pH of less than 2 (pKₐ=8.2) and is soluble in water and polar organic solvents (83). The germicidal properties of peracetic acid were first identified in 1902, but did not become generally available for use until around 1990 (21). Peracetic acid was approved for use as an indirect food additive (33) and was indicated to be ideal as a non-rinse applicant for clean in place systems where byproducts in high dilution proved to be negligible in regards to taste, odor, or toxicity (21, 48). In industry, a quaternary equilibrium mixture of peracetic acid, hydrogen peroxide, acetic acid, and water is used (3), generally with PAA concentrations of around 10-15% PAA (w/w%) (83). Hydrogen peroxide (HP) is commonly used as a disinfectant, as well, but requires much larger doses to achieve the same level of disinfection when compared to PAA. Combination of PAA and HP results in a synergistic effect that subsequently reduces the required concentrations of both chemicals, as well as reduces cost (90). As designated by federal
regulations for food additives, the maximum allowable concentrations of the chemical mixture are 220 ppm for PAA and 120 ppm for HP in chill applications (33).

Disinfection by PAA is based on the release of active oxygen resulting in the oxidation of proteins and enzymes (86) further disrupting the integrity of bacterial cell walls. The undissociated acid is thought to be the biocidal form and is present at highest concentrations at low-neutral pH (41). A pH range of 5.5-8.2 results in decomposition to acetic acid and oxygen with fairly consistent disinfection activity (3). Under alkaline conditions (pH>9), the dissociated acid is present in the majority largely decreasing disinfection efficiency (9, 130, 144). PAA has the highest disinfection efficiency towards bacteria, followed by viruses, spores, and protozoan cysts (86, 126). PAA disinfection is active over a broad range of temperatures; however, microbial reduction shows increased efficacy at higher temperatures (136). When evaluating contact time at minimal bactericidal concentrations, peracetic acid resulted in a 5 log reduction, while rapidly decreasing from 12 to 3 ppm in 30 minutes, and then remaining stable for 7 hours (3). Unlike chlorine, the PAA/HP mixture (PAHP) resists consumption by organic matter. At a concentration of 0.1% with a 10 minute contact time, PAHP decreased Staphylococcus spp., Listeria spp., and E. coli by greater than 5 logs, regardless of organic matrices (23). An advantage of PAA use in chilling systems is the absence of any known toxic or mutagenic residual breakdown products (9) with byproducts consisting primarily of carboxylic acids formed through oxidation of organic matter (102). Peracetic acid, along with organic acids, are advertised to be two of the most environmentally friendly antimicrobials used in industry (97). A disadvantage, however, may be the risk of microbial regrowth upon buildup of acetic acid in effluent, increasing the concentration of organic matter (85). As previously mentioned, PAA
readily decomposes to acetic acid (AA) and oxygen at pH 5.5-8.2 (3), with 5 ppm of PAA being equivalent to the production of 13 ppm AA (85).

Studies have shown PAA to be effective against *Salmonella* (13) over a broad spectrum of concentrations. When comparing the efficiency of PAA for *Salmonella* Typhimurium reduction, less than a 1 log difference was observed at 25 ppm and 200 ppm. Overall, PAA concentrations as low as 25 ppm were effective in decreasing *Salmonella* spp., while PAA concentrations of 200 ppm were required to reduce *Campylobacter* spp. levels. When compared to 30 ppm chlorine, 85 ppm PAA resulted in a 91.8% reduction of *Salmonella* and a 43.4% reduction of *Campylobacter* from pre-chill to post-chill, while chlorine was only able to achieve a 56.8% and 12.8% reduction, respectively (12). When studying reduction of *C. jejuni* on chicken skin, the use of both 100 ppm chlorine and 100 ppm PAA with a 15 minute contact time produced approximately a 1 log reduction (33) (37) alluding to the conclusion that at lower concentrations, PAA and chlorine exhibit similar antimicrobial efficiency against *Campylobacter* spp.. In post-chill applications, PAA is approved for use up to 2,000 ppm (33). When comparing the effectiveness of various antimicrobial solutions in finishing chillers, PAA at concentrations of 400 and 1,000 ppm resulted in 2.02-2.14 log CFU/ml reductions of *Salmonella* Typhimurium, while 40 ppm chlorine and 1,000 ppm-5,000 ppm lysozyme resulted in less than 1 log CFU/ml CFU/ml reduction by 400 ppm and 1,000 ppm PAA, and less than 1 log CFU/ml reduction with chlorine and lysozyme solutions (106).
References


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CHAPTER 3

PRE-CHILL ANTIMICROBIAL TREATMENT TO ENHANCE THE SAFETY OF
CHICKEN PARTS

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1Steininger, C. G., Harrison, and M. A., Berrang, M. E. To be submitted to Journal of Food Protection
Abstract

Pathogen numbers typically increase as a chicken carcass transitions from a whole broiler to cut-up parts. One hypothesis to explain this occurrence is that bacteria in water retained during the pre-chill processing step is released upon cut-up, leading to detection on chicken parts. The objective of this study was to determine the effectiveness of applying an antimicrobial treatment in the pre-chill tank to reduce the prevalence or numbers of *E. coli*, *Campylobacter*, and *Salmonella* on chicken parts. Commercially eviscerated broiler carcasses were collected and subjected to sequential pre-chill, chill, and cut-up procedures. Selective enrichment and isolation of *Campylobacter* spp. and *Salmonella* spp., as well as enumeration of total aerobic bacteria and *E. coli*/coliforms, was performed using carcass rinses at the following points: post evisceration, after 15 min pre-chill (22-25°C water), after 30 min immersion chill (0-4°C water), and following cut-up into parts. Six pre-chill, antimicrobial treatments were evaluated: 50 ppm chlorine, 50 ppm chlorine + 0.5% T-128 (a chlorine stabilizer), 20 ppm peracetic acid, 20 ppm peracetic acid + 0.5% T-128, 0.5% T-128, and water. Addition of 0.5% T-128 to water, 20 ppm peracetic acid, and 50 ppm chlorine during pre-chill resulted in a significant decrease by ~1.5-2.0 log (p<0.05) in total aerobic bacteria and *E. coli*/coliforms following pre-chill. There was some variation in *E. coli* levels on parts that received these treatments following cut-up to levels similar to post evisceration levels, but they were not significantly different from previous levels throughout processing (p<0.05). No trends were observed for *Salmonella* or *Campylobacter* for any treatment.

INDEX WORDS: chicken, pre-chill, chlorine, peracetic acid, T-128, *Campylobacter, Salmonella*
Introduction

In the pre-chill tank, the first stage of chilling following evisceration, carcasses are soaked and agitated in ambient temperature water for 10-15 min allowing for water absorption. Water absorption is intended to increase yield and to remove some heat from the carcasses prior to entering the chiller. Typically, antimicrobials are not applied at this point in processing. Recently, it has been proven that application of antimicrobials as an alternative to or in addition to application in immersion chillers results in a reduction in microbial numbers on chicken carcasses (29). Specifically, this has been shown in post-chill tanks, or finishing chillers, that have been incorporated into chilling operations where carcass contact time is less than 1 min, minimal volumes of water are used, and higher concentrations of antimicrobials may be used.

Advances in technology have led to various shifts in the poultry industry including a shift away from the historically favored antimicrobial, chlorine, in poultry chillers. Chlorine originally gained popularity due to low cost and wide availability; however, the efficacy of chlorine as an antimicrobial is heavily influenced by organic load, pH, and temperature. The active ingredient of chlorine disinfection, hypochlorous acid (HClO), is most abundant at pH between 4.0-7.0 with bactericidal effects decreasing with increased temperatures and organic loads (15, 39). In order to maintain chlorine activity in poultry chiller water, the pH may be lowered by the addition of organic acids and water must consistently be replaced in an attempt to reduce deletion of free chlorine by organic matter. While chlorine has shown success in the prevention of cross-contamination and bacterial reduction on broiler carcasses, processors must combat issues such as pH stability and interference by organic matter in order to decrease prevalence of harmful pathogens such as *Salmonella* spp. and *Campylobacter* spp. commonly found on chicken. The use of chlorine stabilizers such as T-128, a chemical blend of ortho-phosphoric acid and
propylene glycol, has been investigated in the poultry industry as a means to enhance antimicrobial effectiveness of chlorine (32). It is thought that the T-128 encourages the formation of chlorine diatoms and hypochlorous acid while simultaneously limiting the formation of hypochlorite (34), ultimately allowing for greater efficacy.

Peracetic acid (PAA), the current leading antimicrobial used in commercial broiler chilling applications (21), combines an organic acid (acetic acid) with an oxidant (hydrogen peroxide) to produce optimal disinfection. PAA provides a broad spectrum of activity in the presence of organic matter and exhibits high efficacy at low pH and high temperatures (18). As an antimicrobial in the poultry industry, PAA has proven to be effective against both Salmonella spp. and Campylobacter spp. on broilers at relatively low concentrations and shorter contact times (1). A maximum concentration of 220 ppm has been approved for use as an additive to poultry chiller water (7). Because PAA was applied as a pre-chill additive in this study, a lower concentration, approximately 20 ppm, was used in order to maintain the water-holding capacity of the chicken. Despite limitations in concentration, pre-chill application of PAA could still be considered advantageous due to short contact time (15 min) that would otherwise inhibit chlorine performance and higher temperatures than would typically be seen in chill tanks.

Salmonella prevalence is four times higher and Campylobacter prevalence is twice as high on chicken parts than on whole chilled carcasses (14). The estimated national prevalence of Salmonella and Campylobacter on post-chill young chickens predicted from baseline data collected from 2007-2008 is 5.19% and 10.66%, respectively, whereas results from baseline data from raw chicken parts collected in 2012 revealed Salmonella and Campylobacter prevalence of 26.3% and 21.4%, respectively (13, 14). Due to the potential of up to 12% (w/w%) water retention in chicken carcasses during the pre-chill stage of processing (8), there is a concern that
bacteria harbored in the water used to chill carcasses that may be taken up in the muscle tissue may be shielded from disinfectants during further processing steps. Upon cut-up, release of this retained water could lead to increased levels of contamination detected on chicken parts. The objective of this study was to determine the effectiveness of applying antimicrobial treatments, including the use of a chlorine stabilizer, in the pre-chill tank to reduce the prevalence and numbers of *E. coli*, *Campylobacter* spp., and *Salmonella* spp. on chicken parts.

**Materials and Methods**

**Pilot Scale Immersion Chiller**

The internal temperature of poultry carcasses were reduced to below 4°C through immersion chilling (8). Immersion chilling was simulated using 37.9 L (10 gal) buckets; an apparatus was constructed to allow for the use of compressed air to generate water agitation. A frame for the apparatus was built out of 5.08 cm (2 in) PVC pipes. A 0.953 cm (3/8 in) ID hose was used to transfer the air from the outlet through a pressure regulator with 0.635 cm (1/4 in) ID tubing used to deliver air at 40 psi into each of the 37.9 L buckets via a manifold. Hollow stainless steel tubes and zip ties were used to affix the tubing to the inside of the bucket thus forcing air flow in an upward direction from the bottom of the bucket allowing for maximum agitation of carcasses. In order to avoid cross-contamination as well as the introduction of any residual antimicrobial effects, tubing was changed between pre-chill and immersion chill applications.

**Antimicrobial Solution Preparation**

Solutions were prepared in 37.9 L buckets (Rubbermaid BRUTE, 39.69 cm ID) with lids to prevent potential contamination from the air. Six buckets, one for each treatment, were designated for pre-chill application with a final volume of 14 L. Buckets were filled with 14 L of
tap water and were set out overnight to equilibrate to room temperature (~25°C); chemical
additives were added immediately prior to processing. Treatments included tap water, 0.5% T-
128 (v/v%) (SmartWash Solutions, Salinas, CA), 50 ppm chlorine (Clorox bleach, Oakland, CA;
8.25% sodium hypochlorite), a mixture of 50 ppm chlorine and 0.5% T-128, 20 ppm peracetic
acid (PAA; Persan MP-2; Envirotech, Modesto, CA), and a mixture of 20 ppm peracetic acid and
0.5% T-128. In order to simulate industry conditions which indicate that 72% of commercial
broiler processing facilities process birds with city water, municipal tap water was used for
solution preparation (26). The volume of solutions was determined to fulfill the requirement of a
minimum of 1.9 L per bird in immersion chill tanks (25). Before and after samples were
immersed in pre-chill solutions, pH (pHTestr Eutech Instruments; Oakton, Vernon Hills, IL),
temperature (pHTestr Eutech Instruments, Vernon Hills, IL), PAA concentration and total and
free chlorine concentrations via a colorimetric assay, and chemical oxygen demand (V-2000
Multi-Analyte Photometer; CHEMetrics, Midland, VA) were measured. Six additional buckets
were designated for chill immersion application, filled with 14 L of tap water, and set in a 4°C
cool room overnight to equilibrate to refrigeration temperatures. Prior to processing, 3 gallons of
crushed ice were added to each bucket in order to lower the water temperature <4°C, as required
by FSIS.

Handling of Carcasses

In order to standardize initial microbial loads, chicken carcasses were stored in the same
cooler when collected from the line at a commercial processing plant. Initial measurements were
taken using normal handling practices that would be expected without sanitizing equipment
between each carcass. Following pre-chill treatments, carcasses within treatments were
considered to be the same, but aseptic technique was practiced when handling carcasses from
different treatments (changing gloves, changing aluminum foil on scale, sterilization of pH and temperature probes, etc.) and carcasses from each treatment were hung on separate sanitized shackles to drip. Similar precautions were followed when handling post-chill carcasses as were taken with post pre-chill carcasses. When broilers were cut up, the saw was sprayed and wiped with 70% ethanol to remove any residual byproduct and to kill any microorganisms that may have been transferred to the blade. Gloves were changed between each carcass.

**Multiple Rinse Study**

A preliminary study was conducted to determine the effect of successive 100 mL rinses on the microbial numbers on broiler carcass. For each of 6 replications, 6 carcasses were collected post evisceration from a commercial broiler processing and were transported to the lab in insulated coolers to maintain their temperature at approximately 34.6°C. Each carcass was then transferred to a plastic bag, rinsed with 100 mL of water, and shook using a mechanical shaker for 1 min. The rinsate was collected in a sterile specimen cup and kept on ice until analyzed for total aerobic and *E. coli* coliiform counts. Each carcass was rinsed a total of 6 times in succession. Microbial counts obtained were transformed to log_{10} values and reported as log colony forming units per ml. For each carcass, the data obtained for the initial 6 rinses was compared to the data from the final 6 rinses to determine if successive rinsing of the same carcass resulted in successively reduced recovery.

**Processing and Sample Collection**

For each of 6 replications, 24 carcasses were collected post evisceration from a commercial broiler processing line in Athens, GA and transported as described previously. Four sampling points throughout processing were established: post evisceration, post pre-chill, post-chill, and post cut-up. Each sampling point was represented by 6 different carcasses, 1 per
treatment. Prior to collection of any samples, each carcass was weighed, the internal muscle temperature was taken, and the pH of the breast tissue was measured (HI 99163; Hanna Instruments, Carrollton, TX).

Samples for microbiological analysis were collected by rinsing individual carcasses in plastic bags with 100 mL of BPW (BP; Acumedia, Lansing, MI) mixed with 1 mL of 5% sodium thiosulfate (w/v%) and shaking using a mechanical shaker for 1 min. Rinses were collected in sterile specimen cups and kept on ice until analyzed. Post evisceration samples were collected by removing 6 carcasses directly from the cooler after carcass weight, temperature, and pH measurements were taken. The remaining 18 carcasses per replication were identified with plastic ID tags and placed in respective pre-chill treatment buckets for a duration of 15 min. Following the 15 min dwell time, all carcasses were removed from the buckets, hung on shackles, and allowed to drip for 1-2 min. Weight, pH, and temperature measurements were repeated for all 18 carcasses employing aseptic techniques to prevent mixing of treatments. To collect post pre-chill samples, 6 carcasses, 1 from each treatment, were removed from the shackles and sampled. The remaining 12 carcasses were transferred to chill buckets containing only tap water and ice for 30 min of air-agitated chilling. The temperature of randomly selected carcasses was taken during this time interval to ensure an internal temperature below 4°C was reached. After 30 min, all carcasses were removed from the buckets, hung on sanitized shackles, and allowed to drip for 1-2 min. Weight, pH, and temperature measurements were repeated for all 12 carcasses employing aseptic technique, once again, to avoid mixing treatments. To collect post-chill samples, 6 carcasses, 1 from each treatment, were removed from the shackles and sampled. The remaining 6 carcasses were then cut into parts (breast, thigh, drumstick, wing).
aseptically using a mechanical circular saw, sanitizing the blade between each carcass. Parts were then all placed together in one bag and sampled.

**Enumeration of Aerobic Bacteria and E. coli/Coliforms**

Serial dilutions were prepared for all samples and were plated in duplicate on plate count agar (PCA) (Oxoid, Basingstoke Hampshire, England) and E. coli/Coliform Count Plates (3M™Petrifilm™, St. Paul, MN) using spread plate methods and manufacturer instructions, respectively. Plates were incubated at 35°C for 24 h. Colonies were counted and the values transformed to log_{10} colony forming units.

**Enumeration/Enrichment of Campylobacter spp.**

For enumeration of Campylobacter spp., serial dilutions were prepared using post evisceration, post pre-chill, post-chill, and post cut-up samples and plated on R&F Campylobacter chromogenic agar (RFA) (R & F Products, Downers Grove, IL) and Campy-Cefex agar (Acumedia) using the spread plate method in duplicate. Campy-Cefex agar was prepared with 5% (v/v) lysed horse blood (Acumedia), 33 ppm cefoperazone (Sigma, St. Louis, MO), and 200 ppm cyclohexamide (Sigma). One g of cefoperazone was dissolved in 10 mL of deionized water, filter sterilized, and added to the solution. Two g of cyclohexamide was dissolved in 5 mL of sterile water and 5 mL of 100% methanol, filter sterilized, and added to the solution (23). R&F Campylobacter chromogenic agar was made based on manufacturer’s instruction. Plates were incubated at 42°C for 48 h in resealable bags flushed with microaerobic gas mixture of 5% O₂, 10% CO₂, and 85% N₂ (Airgas, Athens, GA). Colonies typical of Campylobacter spp. were enumerated.

For Campylobacter spp. enrichment, 1 mL of each sample was transferred to 9 mL of Campylobacter enrichment (Bolton’s) broth (Acumedia) and 9 mL of triclosan-supplemented
Bolton broth (T-Bolton). T-Bolton was prepared by adding a concentration of 1 µg/ml of triclosan (Sigma Aldrich, Laramie, WY) to prepared Bolton broth (0.1 mL of a 10 µg/ml stock solution per 100 mL Bolton broth). The samples were incubated at 42°C for 24 h in resealable bags flushed with a microaerobic gas mixture of 5% O₂, 10% CO₂, and 85% N₂. Following incubation, colonies from each sample were struck for isolation on both CCA and RFA using a three-quadrant streaking method. Plates were incubated at 42°C for 48 h in the microaerophilic environment and then examined for colonies typical of Campylobacter spp.

Campylobacter confirmation was performed on all suspect colony types isolated by both enumeration and enrichment procedures. Microscopic examination under a phase contrast microscope (100x mag/1.25 oil) was used to visualize the spiral rod morphology and darting motility typical of Campylobacter spp. A latex agglutination kit (Microgen Bioproducts, Surrey, England) was used as a secondary confirmation.

Enrichment of Salmonella spp.

Because Salmonella spp. are typically present in low levels on chicken carcasses, qualitative data was collected via enrichment procedures. The remaining volume of each carcass rinsate following all transfers for enumeration and enrichment procedures (~70-85 ml) was incubated at 35°C for 24 h. Following incubation, 1 mL of each sample was transferred to 10 mL of Rappaport-Vassiliadis (RV) Salmonella enrichment broth (BD; Difco, Sparks, MD) and incubated at 42°C for 24 h. Samples were then struck for isolation using a three-quadrant method on xylose-lysine-tergitol-4 (XLT-4) (BD; Difco) and incubated at 35°C for 24 h. Plates were then examined for colonies typical of Salmonella spp. Suspected colonies were stabbed in triple sugar iron (TSI; BD; Difco) and lysine iron agar (LIA; BD; Difco) slants and incubated at 35°C for 24-48 h. If TSI and LIA slants showed a positive result for presence of Salmonella spp., an A-O
latex agglutination kit (Microgen Bioproducts, Surrey, England) was used for secondary confirmation.

**Statistical Analysis**

For the preliminary rinse study, bacterial counts were transformed to $\log_{10}$ colony-forming units per milliliter. A Student’s T-test was used to determine if there was a significant difference between initial rinsate counts and counts following 6 successive rinses. Significance was reported at P values of < 0.05.

Six replications were conducted for the experiment comparing antimicrobial treatments. Bacterial counts for the enumeration of total aerobic bacteria, *E. coli*/*coli*forms, and *Campylobacter* were transformed to $\log_{10}$ colony-forming units per milliliter. To determine a representative initial microbial load for all carcasses, a geometric mean of all bacterial counts from post evisceration samples was taken and used for analysis. Due to the variability in microbial loads between flocks, a completely randomized block design was used with replication as a block. Following a GLM analysis, means were separated by a Tukey’s HSD test using STATISTICA 12 (Statsoft, Inc., Tulsa, OK). Significance was reported by P values of < 0.05.

**Results and Discussion**

**Aerobic Bacteria, *E. coli*, and Total Coliforms**

Total aerobic bacterial numbers at post pre-chill, post-chill and post cut-up were significantly different (p<0.05) than post evisceration counts on carcasses treated with the chlorine stabilizer, T-128, and resulted in a 1.1-1.4 log CFU/mL reduction immediately following pre-chill treatments. Treatments not containing T-128 did not have a significant decrease (p>0.05) of total aerobic bacteria at any point throughout processing (Table 3.1).
E. coli populations on broilers rinsed with treatments containing T-128 significantly decreased (p<0.05) from post evisceration to post pre-chill with a 1.2-1.4 log CFU/mL reduction. There was a some variation in E. coli levels on parts that received these treatments following cut-up to levels similar to post evisceration levels, but they were not significantly different from the post-chill levels (~0.5 log ; p<0.05) (Table 3.2). Treatments not containing T-128 did not result in a significant decrease (p>0.05) of E. coli at any point throughout processing. Total coliform counts followed a similar trend (1.6-1.7 log CFU/mL reduction post pre-chill) with the exception of 20 ppm PAA + 0.5% T-128 which did not result in a significant decrease (p<0.05) in total coliforms until the post-chill step (Table 3.3). There was an overall log CFU/mL reduction of 1.2-1.4 for E. coli and for coliforms with treatments using T-128, which exceeded overall reductions by treatments not containing T-128 of 0.6-0.8 log CFU/mL and 0.7-0.8 log CFU/mL, respectively (Tables 3.2-3.3). This data reflects similar trends noted in chicken parts for Salmonella and Campylobacter prevalence in earlier reports (14).

Campylobacter spp. Recovery

Since Campylobacter has proven to be more difficult to control than Salmonella throughout poultry processing, the newest mentality in industry is that if an intervention strategy is capable of controlling Campylobacter prevalence, it will also be successful in controlling Salmonella (21). In this study, a 15 min contact time with a PAA concentration at 20 ppm and chlorine concentration of 50 ppm resulted in no significant trends in Campylobacter recovery throughout processing from post evisceration to post cut-up and no enhanced performance was observed by T-128 (Table 3.4, Fig. 3.1). Enumeration data suggests no significant decrease (p>0.05) in Campylobacter spp. numbers from post evisceration to post cut-up for any treatment (Table 3.4). National baseline prevalence data indicates average recovery at re-hang to be 2.93
log CFU/mL and 0.96 log CFU/mL post-chill (13). This data is consistent with that of the current study with an average post evisceration recovery of 2.08 log CFU/mL and post-chill recovery of 1.08 log CFU/mL (Table 3.4). Researchers have reported 3.6 CFU/carcass as typical for *Campylobacter* contamination (28). Yang et al. (2001) proposed that chlorine is unable to eliminate *Campylobacter* on chicken skin because oil from the skin prevents the sanitizer from contacting the surface, leading to the conclusion that alternate antimicrobials such as peracetic acid may not be making contact with *Campylobacter* populations, as well (40).

In studies involving the use of higher concentrations of PAA and chlorine and longer exposure times, *Campylobacter* was reduced. The addition of 85 ppm PAA to a poultry chiller resulted in a 43% reduction of *Campylobacter* spp. following a 20 min contact time (4) and concentrations of 200 ppm PAA were found to be effective in decreasing *Campylobacter* spp. following a 1 h contact time producing a 1.5 log reduction (4). The use of 30 ppm chlorine showed no significant reductions in *Campylobacter* spp. when applied as a 20 min chill additive (3). Another study found PAA to be effective in reducing *C. jejuni* on broilers at both 400 and 1,000 ppm with 1.93 and 2.03 log reductions, respectively, while 40 ppm chlorine was unable to achieve a 1 log reduction (22).

*Campylobacter* prevalence was calculated to be 59.0% (n=144) for post evisceration carcasses collected from the commercial processing line. Data collection was somewhat inconsistent for *Campylobacter* spp. because only 6 replications were performed evaluating the contamination of 6 different flocks, 3 of which appeared to be *Campylobacter*-positive flocks. The other 3 flocks tested possessed very low levels. Normand et al. (2008) confirmed that carcasses can become contaminated by cross-contamination of *Campylobacter* between flocks slaughtered successively (24). Because the transmission rate of *Campylobacter* is so high, 2.37
new cases/colonized chick/day (38), with 95-100% colonization of a flock within 7 days following contact with a single seeder bird (35), it is likely that the majority of birds in a Campylobacter-positive flock will show signs of contamination.

**Salmonella spp. Recovery**

Because *Salmonella* levels on chicken carcasses and parts are typically low, samples were only evaluated by enrichment. There was no noticeable difference in the prevalence of *Salmonella* from post evisceration to post cut-up on the samples treated with 20 ppm PAA, 50 ppm chlorine, or 0.5% T-128 (Fig. 3.2). Processors have found various antimicrobials to be effective in controlling *Salmonella* prevalence on chicken carcasses throughout processing. In the current study, the antimicrobial intervention was added in the 15 min pre-chill step. In previous studies, where higher concentrations of PAA and chlorine in combination with longer exposure times were used, the level of *Salmonella* contamination was reduced. Bauermeister et al. (2008) found that concentrations of PAA as low as 25 ppm were effective in decreasing *Salmonella* spp. and observed less than 1 log difference in *Salmonella* Typhimurium recovery when comparing 25 ppm and 200 ppm PAA in poultry chillers with a contact time of 1 hour (4). Another study evaluating a 20 min contact time in a chill tank found that 85 ppm PAA and 30 ppm chlorine were capable of achieving a 91.8% and 56.8% reduction of *Salmonella* spp. on chicken carcasses, respectively, from pre-chill to post-chill (3). Chlorine levels as high as 400-800 ppm were needed to remove *Salmonella* from broiler skin (37) and typically required a 1-1.5 hour contact time (21). Similar to chlorine, King et al. (2005) found that PAA exhibits varying antimicrobial activity based on the extent of bacterial attachment to the broiler surface (17). Because *Salmonella* is known to preferentially attach to broiler skin and tissues (5, 11, 16, 31), this could have affected the efficacy of the antimicrobials used. *Salmonella* prevalence was
calculated to be 52.1% (n=144) for post evisceration carcasses collected from the commercial processing line.

**Breast pH and Water-Holding Capacity**

A common concern when applying antimicrobial treatments to water used to chill carcasses is the potential for negative impact to end-product quality. Both chlorine and peracetic acid are favored for their use in poultry chiller water because at acceptable concentrations, these additives are not objectionable to taste, odor, or toxicity of the final product (6, 10, 27). Water-holding capacity is highly correlated with broiler pH and decreases as pH drops below 5.8-6.0 (41). Two major meat-quality defects exist in the poultry industry related to pH including pale, soft, exudative (PSE-like) meat that is light in color, has a low water-holding capacity, a soft texture, and is characterized by a pH <5.7 (2, 12, 19), and dark, firm, and dry (DFD-like) meat that is dark in color, has a low water-holding capacity, firm in texture, and is characterized by a pH >6.1 (2, 12, 36). A consensus of the literature indicates the average pectoral pH of chicken to be 5.77 (30). In the current study, immersion of chicken carcasses in 50 ppm chlorine (pH=7.74), 50 ppm chlorine + 0.5% T-128 (pH=2.08), 20 ppm PAA (pH=4.72), 20 ppm PAA + 0.5% T-128 (pH=2.19), 0.5% T-128 (pH=2.19), and water (pH=7.17) for 15 min had no significant effect (p>0.05) on chicken breast pH. All treatments resulted in average chicken breast pH values within a range of 5.73-5.92 indicating ‘normal’ chicken quality classified by a pH of 5.7-6.1 (12, 19) (Table 3.5). Thus, no treatment, regardless of pH, had a negative impact on chicken-quality as it relates to pH.

For whole chickens that measure 1.93 kg or less and are destined to be ice-packed or subsequently cut up, a net water-retention limitation is set at 12% (w/w%) (8). In the current study, water retention was evaluated as a net sum of water retained during both pre-chill and
chill processing steps (n=72) with carcasses weighing, on average, 1.55 kg. All treatments successfully retained water satisfying processors’ demand to increase yield, but also remained in compliance with limitations set by FSIS with retention percentages ranging from 6.26-8.83% (Table 3.6). Treatment with 50 ppm chlorine + 0.5% T-128 resulted in a significantly higher (p<0.05) water retention percentage than 50 ppm chlorine alone, and 20 ppm PAA + 0.5% T-128 resulted in a significantly higher (p<0.05) water retention percentage than 20 ppm PAA alone (Table 3.6). This data could indicate an advantage for processors to increase yield by the addition of T-128 to poultry chiller water.

**Relationship between PAA and T-128**

A synergistic relationship between T-128 and chlorine has been confirmed in both produce and poultry applications (20, 27, 32, 33); however, previous work was unsuccessful in confirming a synergistic relationship between T-128 and peracetic acid. When used as a post-chill dip, a complex of 1,100 ppm PAA + 0.5% T-128 showed no significant difference (p>0.05) when compared to 1,200 ppm PAA alone in the reduction of *Campylobacter coli*, *Salmonella Typhimurium*, and *Listeria monocytogenes* on chicken skin (29). This study provides evidence that a relationship may be present between PAA and T-128 based on enhanced reductions seen in total aerobic bacteria and *E. coli* recovered from chicken carcass rinsates. It should also be mentioned that PAA concentrations prior to treatment were, on average, 19.3 ppm for PAA alone and 19.7 ppm for PAA + 0.5% T-128 and decreased to 7.77 ppm and 12.6 ppm, respectively, following a 15 min pre-chill. The PAA + T-128 complex showed a higher retention of active PAA following exposure to carcasses which could ultimately lead to time and money savings in the poultry industry.
References


Table 3.1. Mean number of aerobic bacteria (log$_{10}$CFU/mL of rinsate ± SD) recovered from poultry carcasses and parts treated at the pre-chill stage with chlorine (Cl), peracetic acid (PAA), and a chlorine stabilizer (T-128).

<table>
<thead>
<tr>
<th></th>
<th>Control (Water)</th>
<th>50 ppm Cl</th>
<th>50 ppm Cl + 0.5% T-128</th>
<th>0.5% T-128</th>
<th>20 ppm PAA</th>
<th>20 ppm PAA + 0.5% T-128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post Evisceration</td>
<td>4.85±0.32$^a$</td>
<td>4.85±0.32$^a$</td>
<td>4.85±0.32$^a$</td>
<td>4.85±0.32$^a$</td>
<td>4.85±0.32$^a$</td>
<td>4.85±0.32$^a$</td>
</tr>
<tr>
<td>Post Pre-Chill</td>
<td>4.61±0.43$^a$</td>
<td>4.08±0.29$^a$</td>
<td>3.49±0.76$^b$</td>
<td>3.77±0.57$^b$</td>
<td>3.95±0.34$^a$</td>
<td>3.59±0.31$^b$</td>
</tr>
<tr>
<td>Post-Chill</td>
<td>4.56±0.43$^a$</td>
<td>4.33±0.43$^a$</td>
<td>3.76±0.40$^b$</td>
<td>3.54±0.38$^b$</td>
<td>4.27±0.46$^a$</td>
<td>3.77±0.44$^b$</td>
</tr>
<tr>
<td>Post Cut-Up</td>
<td>4.41±0.37$^a$</td>
<td>4.37±0.55$^a$</td>
<td>3.80±0.53$^b$</td>
<td>3.78±0.39$^b$</td>
<td>4.17±0.52$^a$</td>
<td>3.63±0.37$^b$</td>
</tr>
</tbody>
</table>

$^{a-b}$ Values are used to denote significance (p<0.05) throughout the table. Values with like superscripts in columns are not significantly different.
Table 3.2. Mean number of generic *E. coli* (log$_{10}$CFU/mL of rinsate ± SD) recovered from poultry carcasses and parts treated at the pre-chill stage with chlorine (Cl), peracetic acid (PAA), and a chlorine stabilizer (T-128).

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Control (Water)</th>
<th>50 ppm Cl</th>
<th>50 ppm Cl + 0.5% T-128</th>
<th>0.5% T-128</th>
<th>20 ppm PAA</th>
<th>20 ppm PAA + 0.5% T-128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post Evisceration</td>
<td>3.32±0.75$^a$</td>
<td>3.32±0.75$^a$</td>
<td>3.32±0.75$^a$</td>
<td>3.32±0.75$^a$</td>
<td>3.32±0.75$^a$</td>
<td></td>
</tr>
<tr>
<td>Post Pre-Chill</td>
<td>2.97±0.94$^a$</td>
<td>2.28±0.20$^a$</td>
<td>1.56±0.64$^b$</td>
<td>1.60±0.35$^b$</td>
<td>2.02±0.39$^a$</td>
<td>1.82±0.56$^b$</td>
</tr>
<tr>
<td>Post-Chill</td>
<td>2.42±0.85$^a$</td>
<td>2.07±0.72$^a$</td>
<td>1.61±0.36$^b$</td>
<td>1.47±0.33$^b$</td>
<td>2.77±1.23$^a$</td>
<td>1.67±0.43$^b$</td>
</tr>
<tr>
<td>Post Cut-Up</td>
<td>2.72±0.72$^a$</td>
<td>2.54±0.81$^a$</td>
<td>2.04±0.85$^{ab}$</td>
<td>1.94±0.69$^{ab}$</td>
<td>2.67±1.02$^a$</td>
<td>2.00±0.45$^{ab}$</td>
</tr>
</tbody>
</table>

$^a$-$^b$ Values are used to denote significance (p<0.05) throughout the table. Values with like superscripts in columns are not significantly different.
Table 3.3. Mean number of total coliforms (log_{10} CFU/mL of rinsate ± SD) recovered from poultry carcasses and parts treated at the pre-chill stage with chlorine (Cl), peracetic acid (PAA), and a chlorine stabilizer (T-128).

<table>
<thead>
<tr>
<th></th>
<th>Control (Water)</th>
<th>50 ppm Cl</th>
<th>50 ppm Cl + 0.5% T-128</th>
<th>0.5% T-128</th>
<th>20 ppm PAA</th>
<th>20 ppm PAA + 0.5% T-128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post Evisceration</td>
<td>3.69±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.69±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.69±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.69±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.69±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.69±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post Pre-Chill</td>
<td>3.29±0.91&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.58±0.13&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.09±0.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.97±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.56±0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.30±0.48&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post-Chill</td>
<td>2.70±0.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.49±0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.95±0.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.87±0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.06±1.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.11±0.39&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Post Cut-Up</td>
<td>2.97±0.70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.95±0.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.34±0.71&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.47±0.52&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.92±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.46±0.40&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-b</sup> Values are used to denote significance (p<0.05) throughout the table. Values with like superscripts in columns are not significantly different.
Table 3.4. Mean number of *Campylobacter* spp. (log$_{10}$CFU/mL of rinsate ± SD) recovered from poultry carcasses and parts treated at the pre-chill stage with chlorine (Cl), peracetic acid (PAA), and a chlorine stabilizer (T-128).

<table>
<thead>
<tr>
<th>Sample Point</th>
<th>Control (Water)</th>
<th>50 ppm Cl</th>
<th>50 ppm Cl + 0.5% T-128</th>
<th>0.5% T-128</th>
<th>20 ppm PAA</th>
<th>20 ppm PAA + 0.5% T-128</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post Evisceration</td>
<td>2.30±1.34</td>
<td>2.12±1.38</td>
<td>2.13±1.41</td>
<td>2.07±1.27</td>
<td>1.97±1.26</td>
<td>1.90±1.10</td>
</tr>
<tr>
<td>Post Pre-Chill</td>
<td>1.85±1.76</td>
<td>1.11±1.14</td>
<td>1.51±1.62</td>
<td>1.29±1.25</td>
<td>1.40±1.30</td>
<td>1.00±1.00</td>
</tr>
<tr>
<td>Post-Chill</td>
<td>1.28±1.17</td>
<td>0.85±1.07</td>
<td>0.97±1.21</td>
<td>0.93±1.00</td>
<td>1.52±1.63</td>
<td>0.90±0.95</td>
</tr>
<tr>
<td>Post Cut-Up</td>
<td>1.34±1.23</td>
<td>1.25±1.17</td>
<td>0.77±0.93</td>
<td>0.94±1.04</td>
<td>1.23±1.13</td>
<td>1.07±0.99</td>
</tr>
</tbody>
</table>

No treatment resulted in a significant reduction (p<0.05) in *Campylobacter* spp. from chicken carcass rinsates. Treatments did not significantly differ (p<0.05) from one another.
Table 3.5: Mean breast pH and internal temperature (°C) of chicken carcasses. Samples were analyzed post evisceration, post pre-chill, and post-chill.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>pH (50 ppm Chlorine)</th>
<th>°C (50 ppm Chlorine)</th>
<th>pH (50 ppm Chlorine + 0.5% T-128)</th>
<th>°C (50 ppm Chlorine + 0.5% T-128)</th>
<th>pH (0.5% T-128)</th>
<th>°C (0.5% T-128)</th>
<th>pH (Control (Water))</th>
<th>°C (Control (Water))</th>
<th>pH (20 ppm PAA)</th>
<th>°C (20 ppm PAA)</th>
<th>pH (20 ppm PAA + 0.5% T-128)</th>
<th>°C (20 ppm PAA + 0.5% T-128)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-Evisceration</td>
<td>5.89± 0.20 34.7± 1.38</td>
<td></td>
<td>5.86± 0.14 35.0± 0.98</td>
<td>5.83± 0.18 35.1± 0.88</td>
<td>5.86± 0.19 34.5± 1.38</td>
<td>5.92± 0.19 34.7± 1.06</td>
<td>5.92± 0.19 34.7± 1.42</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post Pre-Chill</td>
<td>5.89± 0.23 25.4± 1.56</td>
<td></td>
<td>5.85± 0.18 25.5± 1.41</td>
<td>5.84± 0.38 25.2± 1.46</td>
<td>5.75± 0.19 25.3± 1.14</td>
<td>5.79± 0.15 24.7± 1.25</td>
<td>5.81± 0.20 25.5± 1.34</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-Chill</td>
<td>5.82± 0.27 6.32± 0.79</td>
<td></td>
<td>5.87± 0.25 6.68± 1.57</td>
<td>5.82± 0.20 6.08± 1.82</td>
<td>5.73± 0.25 6.73± 1.11</td>
<td>5.80± 0.28 6.47± 1.13</td>
<td>5.82± 0.22 6.68± 1.50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No treatment had a significant effect (p<0.05) on breast pH.
**Table 3.6:** Mean net water retention (w/w%) of chicken carcasses measured as a cumulative percentage of water gained in pre-chill and chill stages.

<table>
<thead>
<tr>
<th>Water Retention (w/w%)</th>
<th>Control (Water)</th>
<th>50 ppm Cl</th>
<th>50 ppm Cl + 0.5% T-128</th>
<th>0.5% T-128</th>
<th>20 ppm PAA</th>
<th>20 ppm PAA + 0.5% T-128</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.97±2.18&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>7.37±1.34&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.74±0.72&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8.83±0.78&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.26±0.97&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.46±1.27&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a-c</sup> Values are used to denote significance (p<0.05) throughout the table. Values with like superscripts in columns are not significantly different.
Table 3.7: Mean pH, temperature (°C), free chlorine concentration (ppm), and peracetic acid concentration (ppm) of pre-chill water solutions. Samples were analyzed before (B) and after (A) pre-chilling.

<table>
<thead>
<tr>
<th></th>
<th>Control (Water)</th>
<th>50 ppm Cl</th>
<th>50 ppm Cl + 0.5% T-128</th>
<th>0.5% T-128</th>
<th>20 ppm PAA</th>
<th>20 ppm PAA + 0.5% T-128</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pH</strong></td>
<td><strong>°C</strong></td>
<td><strong>Cl (ppm)</strong></td>
<td><strong>pH</strong></td>
<td><strong>°C</strong></td>
<td><strong>Cl (ppm)</strong></td>
<td><strong>pH</strong></td>
</tr>
<tr>
<td>B 7.17</td>
<td>23.1</td>
<td>0.39</td>
<td>7.74 23.3</td>
<td>43.0</td>
<td>2.08 23.1</td>
<td>44.4</td>
</tr>
<tr>
<td>A 6.83</td>
<td>21.4</td>
<td>0.80</td>
<td>6.96 21.2</td>
<td>2.50</td>
<td>2.59 21.1</td>
<td>1.34</td>
</tr>
</tbody>
</table>
Figure 3.1. Percent positive recovery of *Campylobacter* spp. from chicken carcass rinsates. Samples analyzed post-evisceration, post pre-chill, post-chill, and post cut-up (n=6 per treatment).

1 A: Control (Water); B: 50 ppm Chlorine; C: 50 ppm Chlorine + 0.5% T-128; D: 20 ppm PAA; E: 20 ppm PAA + 0.5% T-128; F: 0.5% T-12
**Figure 3.2.** Percent positive recovery of *Salmonella* spp. from chicken carcass rinsates. Samples analyzed post evisceration, post pre-chill, post-chill, and post cut-up (n=6 per treatment).

1 A: Control (Water); B: 50 ppm Chlorine; C: 50 ppm Chlorine + 0.5% T-128; D: 20 ppm PAA; E: 20 ppm PAA + 0.5% T-128; F: 0.5% T-128
CHAPTER 4

COMPARISON OF SELECTIVE MEDIA FOR THE DETECTION AND ENUMERATION OF NATURALLY OCCURRING CAMPYLOBACTER SPP. ON POULTRY

Steininger, C. G., Harrison, M. A., and Berrang, M. E. To be submitted to Journal of Food Protection
Abstract

Enumeration and detection of low numbers of naturally occurring *Campylobacter* spp. on poultry products is very difficult due to the presence of competing microflora that is not eliminated by commonly used selective media. This study compared the effectiveness of various combinations of enrichment broths and plating media for the detection of naturally occurring *Campylobacter* spp. in broiler carcass rinsates. Specifically, the use of *Campylobacter* RF chromogenic agar (RFA) and the addition of 1 μg/mL triclosan to *Campylobacter* enrichment (Bolton’s) broth (T-Bolton) were evaluated based on their ability to eliminate background microflora. Campy-Cefex agar (CCA) and RFA were used for enumeration of *Campylobacter* spp. recovered from whole carcass rinsates using direct plating methods. These two selective media were also used in coordination with Bolton broth and T-Bolton broth for detection after enrichment. No significant difference (p>0.05) was observed between enumeration of *Campylobacter* spp. on RFA compared to CCA, however, RFA showed less contamination by background microflora than CCA. When analyzing *Campylobacter* positive flocks (n=3), T-Bolton broth resulted in an 18.1% and 1.39% better recovery compared to Bolton broth when using CCA and RFA as the isolation media, respectively. T-Bolton and RFA resulted in a 27.8% better recovery than T-Bolton and CCA. Bolton and RFA resulted in a 44.4% better recovery than Bolton and CCA. When enumerating or enriching for naturally occurring *Campylobacter* spp. in carcass rinsates, enrichment in T-Bolton and plating on RFA proved to be most effective in the elimination of background microflora, therefore allowing for more accurate enumeration and enrichment procedures.

INDEX WORDS: *Campylobacter*, chicken, triclosan, bolton, CCA, RFA
**Introduction**

Cultural enrichment techniques used to detect foodborne pathogens such as *Campylobacter* spp. must be able to effectively function in the presence of various food matrices and competing microorganisms. This need for functionality often leads to a compromise between sensitivity and specificity. While *Campylobacter* is a relatively hardy microorganism under ideal conditions, various stressors such as exposure to oxygen, drying, low pH, heat, freezing, and prolonged storage may cause transformation into a viable-but-nonculturable cell (VBNC). *Campylobacter* cells in the VNBC state become coccoidal and immotile, cannot be detected by standard culture methods, but can remain pathogenic upon exposure to favorable conditions (13).

National baseline data indicates *Campylobacter* prevalence on post-chill chicken carcasses to be 10.66% (6). Typically, when *Campylobacter* spp. are present on broiler carcasses, counts per milliliter of carcass rinsate may vary between 1 and 3 log CFU; Berrang and Dickens (2000) reported an average of 1.5 log CFU/mL post-chill (3, 10). Low bacterial cell numbers in addition to the potential of cells to be in a VNBC state make enrichment procedures necessary for accurate detection of *Campylobacter* spp.

Interference by indigenous bacterial contaminants has proven to be a significant obstacle in detection of *Campylobacter* on numerous selective media. Researchers have identified 93 (9)-100% (4) of these contaminants to be extended-spectrum-β-lactamase (ESBL)-producing *E. coli* species. ESBL-producing *E. coli* have conferred a resistance to cephalosporins, resulting in the hydrolysis of cefoperazone in *Campylobacter* media such as CCA, mCCDA, and Bolton broth (4, 9). Other contaminants identified include *Acinetobacter baumannii* on CCA, mCCA, mCCDA, CAMPY, and Karmari, and *Pseudomonas* spp. and *Staphylococcus heminis* on CCA.
and mCCDA (2, 11). One study identified an average of 14 non-Campylobacter colonies per mL of carcass rinsate on CCA (8).

When comparing the performance of three enrichment broths specific for Campylobacter using naturally contaminated food samples, Bolton broth (BB), Preston broth (PB), and Campylobacter enrichment broth (CEB), BB achieved the best balance between Campylobacter recovery and inhibition of competitor microorganisms. CEB supported the growth of the majority of Campylobacter strains but was unable to eliminate competitor microorganisms, while PB inhibited all competing microorganisms but did not support the growth of Campylobacter spp. (2). Potassium clavulanate and clavulanic acid, ESBL inhibitors, have been used as supplements in enrichment broths allowing for an increase in Campylobacter detection in Bolton broth from 41% to 91% (4, 9). Triclosan, an antimicrobial supplement found in personal care and household products as well as in Yersinia and Pseudomonas selective agars, has shown potential to inhibit growth of cefoperazone-resistant flora through disruption of fatty-acid synthesis (4, 7). An innate resistance to triclosan by Campylobacter spp. has been reported (1, 5). Supplementation of Bolton broth with triclosan at concentrations of 1 µg/mL resulted in a 43.8% better recovery of Campylobacter spp. on mCCDA from chicken carcass rinsates (4).

The objective of this study was to compare the effectiveness of various combinations of enrichment broths and plating media for the detection of naturally occurring Campylobacter spp. in broiler carcass rinsates. Specifically, the use of Campylobacter RF chromogenic agar (RFA) and the addition of 1 µg/mL triclosan to Bolton enrichment broth (T-Bolton) were evaluated based on their ability to eliminate background microflora originating from broiler carcass rinsates.
Materials and Methods

Processing and Sample Collection

For each of 6 replications, 24 carcasses were collected post-evisceration from a commercial broiler processing line and were transported to the lab in an insulated cooler to maintain their temperature at approximately 34.6°C. Carcasses were transferred to individual plastic bags and rinsed with 100 mL of BPW (BP; Acumedia, Lansing, MI) containing 1 mL of 5% sodium thiosulfate (w/v%) by shaking in a mechanical shaker for 1 min. Rinsates were transferred to sterile specimen cups and kept on ice until analyzed.

Direct Plating of Campylobacter spp.

Chicken carcass rinsates were serially diluted and plated on Campy-Cefex Agar (CCA) and Campylobacter R&F chromogenic agar (RFA). Campy-Cefex agar (Acumedia) was made with 5% (v/v) lysed horse blood (Acumedia), 33 ppm cefoperazone (Sigma, St. Louis, MO), and 200 ppm cyclohexamide (Sigma). One g of cefoperazone was dissolved in 10 mL of deionized water, filter sterilized, and added to the solution. Two g of cyclohexamide was dissolved in 5 mL of sterile water and 5 mL of 100% methanol, filter sterilized, and added to the solution. R&F Campylobacter chromogenic agar (R & F Products, Downers Grove, IL) was made based on manufacturer’s instruction. Plates were incubated at 42°C for 48 h in resealable bags containing flushed with a microaerobic gas mixture of 5% O₂, 10% CO₂, and 85% N₂ (Airgas, Athens, GA) and then examined for colonies typical of Campylobacter spp.

Enrichment of Campylobacter spp.

One mL of each sample was transferred to 9 mL of Campylobacter enrichment (Bolton) broth (Acumedia) and 9 mL of triclosan-supplemented Bolton broth (T-Bolton) for enrichment.
T-Bolton was prepared by adding triclosan (Sigma Aldrich, Laramie, WY) to prepared Bolton broth (0.1 mL of a 10 µg/ml triclosan stock solution per 100 mL Bolton broth). The samples were incubated at 42°C for 24 h in resealable bags flushed with a microaerobic gas mixture of 5% O₂, 10% CO₂, and 85% N₂. Following incubation, each sample was struck for isolation on both CCA and RFA using a three-quadrant streaking method. Plates were incubated at 42°C for 48 h in the microaerophilic environment and examined for colonies typical of *Campylobacter* spp. Percent-positive was reported as a comparison between *Campylobacter* spp. recovery from various combinations of T-Bolton/Bolton enrichment broths and CCA and RFA plating media.

*Campylobacter* confirmation was performed on selected suspected colonies isolated from both enumeration and enrichment procedures. Microscopic examination under a phase-contrast microscope (100x mag/1.25 oil) was used to visualize the spiral rod morphology and darting motility typical of *Campylobacter* spp. Latex agglutination (Microgen Bioproducts, Surrey, England) was used as a secondary confirmation.

**Statistical Analysis**

Six replications were conducted for the experiment. Bacterial counts for the enumeration of *Campylobacter* were transformed to CFU/ml. Due to the variability in microbial loads between flocks, a completely randomized block design was used with replication as a block. Following a GLM analysis, means were separated by a Tukey’s HSD test using STATISTICA 12 (Statsoft, Inc., Tulsa, OK). Significance was reported by P values of ≤ 0.05.

**Results and Discussion**

In the current study, triclosan-supplemented Bolton broth (T-Bolton) equaled or outperformed Bolton broth (BB) regardless of the media used for isolation by 7.08% and
considerably reduced background contamination (Fig. 4.1). When considering the media used for selective isolation and taking the average for the recovery rates, T-Bolton broth resulted in an 18.1% and 1.39% better recovery over BB when CCA and RFA were used as the isolation media, respectively (Fig. 4.1). For flocks with high levels of contamination (n=3), T-Bolton and RFA resulted in a 27.8% better recovery than T-Bolton and CCA, while BB and RFA resulted in a 44.4% better recovery than BB and CCA (Fig. 4.1). Incorporating flocks where little to no contamination by Campylobacter was detected, T-Bolton broth resulted in a 10.8% and 3.4% better recovery over Bolton broth on CCA and RFA, respectively (Fig. 4.1). In all combinations, T-Bolton achieved a higher recovery of naturally occurring Campylobacter spp. from chicken carcass rinsates and showed the best results when used in combination with RFA. This improved recovery by T-Bolton may have also been a result of considerable reductions in contamination on both CCA and RFA making Campylobacter colonies much easier to detect. While microbial contaminants in this study were not identified, this data suggests that the majority of contaminants were ESBL-producing E. coli since the contamination level was considerably less on triclosan-containing media.

A study by Oyarzbal et al. (2005) compared the effectiveness of Campy-Cefex agar (CCA), modified-Campy-Cefex agar (mCCA), modified-charcoal-cefoperazone-deoxycholate agar (mCCDA), Campy-Line agar (CLA), Karmali agar, and CAMPY agar for direct plating methods. Results indicated that CCA and mCCA produced the best results but were not significantly different than CAMPY, mCCDA, or Karmali (11). CLA, the most selective of the media types, resulted in the lowest counts due to high concentrations of antimicrobials. When comparing direct plating methods for enumeration of Campylobacter spp. on Campy-Cefex agar
and *Campylobacter* R&F chromogenic agar (RFA) in the current study, no significant difference (p<0.05) was observed. CCA and RFA recovered 3.17 log CFU/mL and 3.38 log CFU/mL, respectively, of *Campylobacter* from post evisceration chicken carcasses (Table 4.1). These values are comparable with those reported by Berrang and Dickens (2000) with average *Campylobacter* recovery of 3.4 log CFU/mL on post evisceration carcasses (3). It is not surprising that CCA and RFA resulted in similar counts due to the higher selectivity of RFA leading to the potential elimination of some *Campylobacter* cells and contamination interfering with detection of some cells on CCA. However, ease of detection from a subjective perspective was much greater with RFA due to a decreased presence of contaminants (Fig. 4.2). This is consistent throughout the literature with claims that enumeration on CLA and other more selective agar types was much easier than on CCA and mCCDA which were often overgrown with contaminants (8, 12).

Oyarzbal et al. (2005) found that a combination of two or more selective media increased the likelihood of detecting *Campylobacter* spp. from chicken carcass rinsates (11). Based on the results of this study, *Campylobacter* detection can be improved by using triclosan-supplemented Bolton broth for enrichment followed by isolation on Campy-Cefex agar and *Campylobacter* R&F chromogenic agar. Direct plating methods could also benefit from the addition of a second selective media such as RFA to regulatory testing protocols.
References


Table 4.1: Comparison of the mean number of *Campylobacter* spp. (log$_{10}$CFU/mL of rinsate ± SD) recovered from post evisceration poultry carcasses (n=6 per rep) plated on Campy-Cefex Agar (CCA) and *Campylobacter* R&F Chromogenic Agar (RFA).

<table>
<thead>
<tr>
<th>Media Type</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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</thead>
<tbody>
<tr>
<td>RFA</td>
<td>3.17±0.49</td>
<td>1.00±0.00</td>
<td>1.00±0.27</td>
<td>2.58±0.64</td>
<td>1.00±0.00</td>
<td>3.74±0.54</td>
</tr>
<tr>
<td>CCA</td>
<td>2.47±0.66</td>
<td>1.00±0.00</td>
<td>1.00±0.00</td>
<td>2.55±0.75</td>
<td>1.00±0.00</td>
<td>5.13±0.21</td>
</tr>
</tbody>
</table>

No significant difference (p<0.05) was observed between enumeration of *Campylobacter* spp. on CCA compared to RFA.
Figure 4.1: Comparison of percent positive recovery of *Campylobacter* spp. from chicken carcass rinsates (n=144) enriched in Bolton (B) and T-Bolton (T) broths and plated on Campy-Cefex Agar (CCA) and *Campylobacter* R&F Chromogenic Agar (RFA).
Figure 4.2. Comparison of background microflora originating from a single post-evisceration carcass rinsate enriched in Bolton broth and T-Bolton broth selectively plated for isolation on Campy-Cefex agar (CCA) and Campylobacter R&F chromogenic agar (RFA).

(a) Bolton enrichment broth plated on Campy-Cefex agar; (b) T-Bolton enrichment broth plated on Campy-Cefex agar; (c) Bolton enrichment broth plated on Campylobacter R&F chromogenic agar; (d) T-Bolton enrichment broth plated on Campylobacter R&F chromogenic agar
CHAPTER 5
CONCLUSION

Based on the results of the study, Smartwash T-128 may serve as a viable antimicrobial additive to poultry industry when used in combination with low concentrations of peracetic acid and chlorine. Notable findings: (1) Significant decreases in total aerobic bacteria, *E. coli*, and total coliforms observed following pre-chill processing steps when T-128 was used (Table 3.1-3.3); (2) Significant increases observed in water retention when T-128 was used compared to comparable treatments without (Table 3.6); (3) Potential to retain higher concentrations of active chlorine and peracetic acid in prechill water containing T-128 (Table 3.7). Upon incorporation of T-128 in prechill water, poultry processors may be able to achieve an additional reduction in bacterial counts, an increased yield, and may be able to better maintain antimicrobial chemical concentrations at functional levels. Broilers treated with Smartwash T-128 did, however, in some instances experience an increase in *E. coli* and/or total coliform counts following cut-up that resulted in counts that were statistically the same as the original post evisceration counts. Because cross-contamination as a factor was highly controlled throughout experimentation, it is possible that the water retained during pre-chill for those carcasses treated with T-128 that experienced an increase following cut-up harbored bacteria that were not effectively eliminated, leading to contamination upon cut-up. An inoculation study should be done in order to confirm these conclusions further.

Based on results from the comparative media study, it can be concluded that the majority of the bacterial contaminants present in *Campylobacter* Enrichment (Bolton’s) broth and on Campy-Cefex agar are susceptible to the bactericidal effects of triclosan. Despite the similarities...
seen in enumeration on Campy-Cefex agar (CCA) when compared to *Campylobacter* R&F chromogenic agar (RFA), enumeration on RFA resulted in a substantial decrease in background contamination making enumeration much easier. When suggesting an alternative method for the detection and isolation of naturally occurring *Campylobacter* spp. from broiler carcass rinsates, the results of the current study indicate that enrichment in T-Bolton broth followed by selective isolation on both CCA and RFA would give the most accurate results.