The Efficacy of Delmopinol in Preventing the Attachment of *Campylobacter jejuni* to Chicken, Stainless Steel and High-Density Polyethylene

Calvin Waldron

Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science in Life Sciences
In
Food Science and Technology

Joseph D. Eifert, Committee Chair
Sean F. O'Keefe
Robert C. Williams

May 23, 2013
Blacksburg, Virginia

Keywords: *Campylobacter*, delmopinol, chicken, steel, HDPE

Efficacy of Delmopinol Against *Campylobacter jejuni* to Chicken, Stainless Steel and High-Density Polyethylene

Calvin Waldron

ABSTRACT

Campylobacter spp. are the second leading bacterial cause of food borne illness in the U.S. New antimicrobials that prevent bacterial attachment may be effective for reducing *Campylobacter*. Delmopinol hydrochloride (delmopinol) is a cationic surfactant that is effective for treating and preventing gingivitis and periodontitis. This study evaluated the effectiveness of delmopinol for reducing attachment of *Campylobacter jejuni* to chicken, stainless steel and high-density polyethylene.

Chicken pieces, steel and HDPE coupons were spot-inoculated with 0.1 mL of a *Campylobacter jejuni* culture. After 10 min, samples were sprayed with 0.5% or 1.0% delmopinol, 0.01% sodium hypochlorite, or distilled water. Contact times were 1, 10, or 20 min prior to rinsing with buffered peptone water. Rinses were serially diluted onto Campy Cefex Agar for enumeration. For additional samples, solutions were applied first, followed by inoculation with *C. jejuni* after 10 min. Cultures remained undisturbed for 1, 10, or 20 min. Then samples were rinsed and plated as above.

When *C. jejuni* was inoculated before treatments, 1% delmopinol application led to mean log reductions of 1.26, 3.70, and 3.72 log CFU/mL, greater than distilled water, for chicken, steel and HDPE respectively. When *C. jejuni* was inoculated after spray treatments, 1% delmopinol reduced *C. jejuni* by 2.72, 3.20, and 3.99 mean log CFU/mL more than distilled water for chicken, steel and HDPE respectively. Application of 1% delmopinol, either before or after bacteria inoculation, resulted in a significantly ($p<0.05$) greater log reduction than 0.01% sodium hypochlorite or distilled water. Delmopinol may be a promising antimicrobial treatment.

ACKNOWLEDGEMENTS

I would like to first acknowledge my family and God, for any of this would not be possible if it wasn't for them. I would like to thank my parents Calvin and Susan for instilling in me great values and the desire to work hard and to give my best at everything I do. I hope to become as great of people as you are and I love you with all my heart. I would like to thank my sister Susan and my brother Michael for always being there for me and keeping a smile on my face when the work became difficult. You both inspire me to challenge myself and to continue to push forward to hopefully be a good role model for you guys. Also, a special thanks to all of my friends for their great support. You kept me sane throughout this entire process, whether if it was through words of wisdom and encouragement or thorough our daily workouts and pickup basketball games at McComas Hall.

I would especially like to thank Dr. Joseph Eifert for being a mentor and committed advisor, for without him none of this project would be possible. He was able to see the potential in this project from the start. I would also like to thank my committee members, Dr. Rob Williams and Dr. Sean O'Keefe, who helped me discover new ways to approach my research as well as giving me their support. Also to Govind Raj who helped me find my way around lab and was always there for me if I needed anything, I appreciate everything you did to help me get started. To the faculty and staff of the Food Science and Technology Department, thank you for welcoming me into the program and for your continued support. I am truly blessed to have such a great support system, and there are not enough words to express how thankful I am for everything.

TABLE OF CONTENTS

LIST OF TABLES

INTRODUCTION

Presently, *Campylobacter* spp. are the second leading cause of confirmed human food borne illness in the U.S. *C. jejuni* is also the most commonly reported species associated with foodborne illness. It is one of the most common causes of human gastroenteritis in the world, and has been linked with the subsequent development of Guillain Barre Syndrome (an autoimmune disorder affecting the peripheral nervous system) (Silvia et al., 2011). Each year, over 10,000 cases of campylobacteriosis are reported to the Centers for Disease Control and Prevention (CDC); however, many more cases go undiagnosed or unreported. Estimates are that *Campylobacter* causes more than 2 million illnesses, 13,000 hospitalizations, and over 100 deaths each year in the United States (Silvia et al., 2011).

Campylobacter studies have led to the development of numerous methods to combat this bacteria. New food safety measures and techniques have been put in place in processing facilities across the world to kill the organism and to limit antimicrobial resistance. The bacterium is sensitive to heat and other common disinfection procedures, and many companies are using updated prevention methods such as pasteurization, chlorination, ozonation, and adequate cooking techniques to kill off the organism (Levin, 2007). Various antimicrobial compounds are also being tested to see how effective they are against *Campylobacter*.

Antimicrobial solutions have been used in the food industry for many years. From a food safety aspect, antimicrobials are known as substances that kill or inhibit the

growth of microorganisms such as bacteria, fungi, or protozoa. There are also antimicrobial drugs and agents that either kill microbes (microbiocidal) or prevent the growth of microbes (microbiostatic) (Marsh, 2010). Some of these include metal ions, phenols, and quaternary ammonium compounds, which are also known to be affective against plaque biofilms (Marsh, 2010). For many companies, these antimicrobial solutions are used on the processing equipment, industrial surfaces, or packaging material to prevent contamination. Examples include trisodium phosphate, acidified sodium chlorite, lactic/acetic acids, and sodium hypochlorite. They are used in the forms of gels, sprays, and dip solutions. Food companies and research facilities are constantly looking for new and better antimicrobials to use for their products.

One area that is beginning to draw more attention for more effective antimicrobials is dentistry. Numerous antimicrobials used in dental applications may be able to be used in the food industry. One such antimicrobial is delmopinol hydrochloride (a morpholinoethanol derivative). This chemical solution is a tertiary amine surfactant, and it is an antiplaque agent intended to be marketed under the proprietary name of Decapinol® (Amebrant, 2010). The main use of delmopinol is in oral hygiene products to prevent and disrupt biofilms. It is currently marketed in several products including mouth rinse/wash, toothpaste or gel, a spray, and even a gum application. It has low antimicrobial activity compared with other solutions such as chlorhexidine, but is effective in inhibiting plaque formation and attachment, which is associated with gingivitis. Gingivitis, the earliest stage of gum disease, is an inflammation of the gums caused by a buildup of bacteria that grow in the coating that forms on teeth between brushings (Amebrant, 2010). When this chemical is applied orally, it changes the

surface properties of the salivary layer which coat the teeth so that bacteria cannot easily adhere and colonize as dental plaque (Amebrant, 2010*)*. The chemical also destabilizes pre-existing plaque, making it easier to remove by brushing.

Delmopinol hydrochloride is very effective as a dental application. The antimicrobial effect of delmopinol has been compared to that of chlorhexidine, and it is less bacteriostatic than chlorhexidine (Simonsson et al., 1992). With that in mind, it would seem promising that the chemical could be used as an effective antimicrobial in the food industry. In my research project, we will be studying its ability to inhibit the attachment of *Campylobacter jejuni* to chicken skin, stainless steel, and polyethylene plastic. To simulate the possible "real life" applications of decapinol, I selected food-contact surfaces (HDPE and stainless steel) found in an industrial food environment that could be treated with a delmopinol solution. Ultimately, the proposed project can provide valuable information on how to combat the threat of *Campylobacter* in poultry processing. By being able to prevent microbial attachment to industrial surfaces or the food itself, we will be able to limit or even negate the threat of food outbreaks; issues such as cross contamination and bacterial colonization become non-existent.

LITERATURE REVIEW

Campylobacter spp. and foodborne campylobacteriosis

In general, the bacteria is a curved, gram-negative, microaerophilic, thermophilic rod that grows best at 42°C (107°F) and low oxygen concentrations (Altekruse et al., 1999). These characteristics are adaptations for growth in its normal habitat – the intestines of warm-blooded birds and mammals. With this being said, *Campylobacter* is found commonly in a wide variety of healthy domestic and wild animals including cattle, sheep, goats, pigs, chickens, ducks, geese, wild birds, dogs, cats, rodents, and marine mammals. The bacterium has been known to also survive in dairy lagoons, livestock water troughs, stock ponds, lakes, creeks, streams, and mud. In the food processing industry, the main sources of contamination come from raw chicken and turkey products. The bacterium is sensitive to heat and other common disinfection procedures such as pasteurization, chlorination, ozonation, and adequate thermal processes or cooking techniques to kill off the organism (Levin, 2007).

Campylobacter association with processed chicken

In the food industry, raw chicken is a significant source of *Campylobacter jejuni*. Additionally, there are many points in the processing of poultry where carcasses and products can become cross-contaminated with *Campylobacter* and other microorganisms. In the US, campylobacteriosis is the third most important bacterial foodborne disease, with an incidence of 12 cases per 100,000 population. It is found at high prevalence in retail broiler carcasses and in retail broiler meat. For example, an

Alabama study conducted from 2005-2011 found that 308 out of 755 (41%) boneless retail broiler meat samples contained the *Campylobacter* spp (204 identified as *C. jejuni*) (Williams and Oyarzabul, 2012). In the EU, the prevalence of *Campylobacter* spp. in broiler carcasses identified at the retail level vary from 3.1% to 58.8% (Wieczorek et al., 2012). Since researchers have reported that a greater amount of the bacteria is present on the chicken breast skin than other edible portions of the chicken carcass, this area is an important site to control and study due to its higher affinity for bacterial attachment. For example, a study with retail meat samples (*n* = 24,566) from 10 U.S. states were collected and analyzed between 2002 and 2007, consisting of 6,138 chicken breast, 6,109 ground turkey, 6,171 ground beef, and 6,148 pork chop samples (Zhao et al., 2010). A total of 2,258 *Campylobacter jejuni*, 925 *Campylobacter coli*, and 7 *Campylobacter lari* isolates were identified. Chicken breast samples showed the highest contamination rate (49.9%), followed by ground turkey (1.6%), whereas both pork chops and ground beef had <0.5% contamination (Zhao et al., 2010). The higher contamination rate in chicken breast samples is most likely due to the breast piece creating a better anaerobic environment. Chicken breasts usually have a denser texture in comparison with other meat products, and its greater ability to hold moisture within its skin creates the perfect environment for *Campylobacter* to grow and persist.

Sources of contamination during processing

In the processing of poultry products, cross contamination of *Campylobacter jejuni* can occur at various steps. Upon arrival to the processing plant, poultry are placed in the live bird loading/receiving area. This area is considered to contain the

highest concentrations of bacterial contamination during processing and can override processing preventative measures, allowing further movement to other areas of the plant (Bolder, 1998; FSIS, 2010). FSIS has stated that movement of bacterial contaminants beyond the receiving area may be due to employee movement between areas and air-contamination (FSIS, 2010).

Load-in/receiving is followed by the stunning and bleeding of poultry during processing. This can be done in three ways: electrical, mechanical and CO_2 exposure (chemical) (FSIS, 2010). The main problem in this area that contributes to contamination is the release of feces onto other carcasses, which can be transferred to other processing steps such as scalding and defeathering. A study by Abu-Ruwaida, et al. (1994) found that other areas of high bacterial contamination were scalding, defeathering and evisceration.

Scalding is used primarily for loosening feather follicles and can also eliminate bacteria due to the elevated water temperatures used (49 - 60° C). Many factors can influence the effectiveness of the process however; including the pH of the scald water, scald temperature, and build-up of organic matter in the scalder (FSIS, 2010). If the pH of scald water remains at 7, *Campylobacter* was found to become heat resistant and can continue its growth and development (FSIS, 2010). The temperature used ranges from 50-60°C, and this does not completely rid the carcass of bacteria (Bolder, 1998; Jacobs-Reitsma et al., 2008). The bacterial load from feces carried on carcass skin or shed through intestinal leakage can enter the scald water, making it another significant risk factor in cross contamination (Jacobs-Reitsma et al., 2008).

The next processing step is generally referred to as evisceration, where birds from the scalding/defeathering area are re-hung on shackles and a series of physical cuts and manipulations result in intestine removal. During evisceration, there is the threat for intestinal rupture or leakage, further exposing carcasses to contaminated feces and intestines. Multiple water sprays of the carcasses can remove most of the visible contaminants. Studies acknowledge the benefit from the use of inside-outside washers, which are responsible for spray washing the carcasses clean (Bashor et al., 2004; Cox and Pavic, 2009). Important factors such as time and temperature of washers, spray speed and pressure, along with chlorine sanitizer concentration have an effect on how well bacteria are eliminated. In some cases the level of *Campylobacter* could be decreased by 1 log cycle using spray washers, but the end product may still contain a significant amount of bacteria (Bashor et al., 2004; Cox and Pavic, 2009). To a certain level, decreased handling in the evisceration area is believed to cut down on contamination by workers (FSIS, 2010). However, machine contamination is still possible (Bolder, 1998). Abu-Ruwaida reported that evidence in his study showed that no change during evisceration occurred, but similar studies pointed to an increase up to one log cycle (Abu-Ruwaida et al., 1994).

Generally, the next processing step is carcass chilling. The chilling process reduces poultry carcass temperature and inhibits microbial growth. In the U.S., water immersion chilling is the predominant method used, while air chilling is used by a small percentage of processors. In air chillers, there is less physical contact between carcasses, reducing the potential for cross-contamination. Immersion chilling may have the advantage that antimicrobials can be used which can reduce biological hazards

further. Therefore, sanitary practices are very important in plants using an air chilling system because this step does not use a chemical intervention (FSIS, 2010).

***Campylobacter* attachment to food and food contact surfaces**

Campylobacter can attach to various surfaces such as plastic and stainless steel, in addition to the chicken carcass, skin and feathers during processing. Cross contamination can occur with any of the previously mentioned sources. In general, bacterial cells may need only one to a few minutes to attach to a surface and spread infection (Arritt et al., 2002). A chicken carcass on a conveyor hanger can contain *Campylobacter*, and contaminate all materials and surfaces throughout processing. An example could be cross contamination between carcasses on a steel hanger, which is dropped onto a plastic conveyor system.

Polyethylene is an inexpensive plastic material that is chemically resistant and can be very durable for use in food industries. Among polyethylene, low density polyethylene has the most excessive branching which causes a less compact molecular structure and lowered density. High density polyethylene has minimal branching of its polymer chains. Due to it being denser, it is more rigid and less permeable then the LDPE. It has a density of 0.941-0.965 g/cm^3 (U.S. Plastic Corp., 2008).

Bacteria can attach to plastic food contact surfaces such as conveyor belts, storage containers, and cutting boards, especially if the surfaces have scratches or pits. Scored lines in the surface should not harbor bacteria as long as the surface is well washed. If there is no food residue on a plastic surface, there is no food source for bacteria to utilize for growth. Unlike wood, plastic boards do allow rinsing with harsher

cleaning chemicals such as bleach and other disinfectants without damage to the board or retention of the chemicals to later contaminate food.

Many studies have shown that the material of the surface has little or no effect on biofilm development. Stainless steel may be just as susceptible to bacterial contamination as plastic. Most stainless steel containers, pipework and food contact equipment are manufactured from either 304 or 316 type austenitic stainless steels (BSSA, 2013). The 17% chromium ferritic stainless steel (430 type) is also used widely for such applications as splashbacks, housings and equipment enclosures, where corrosion resistance requirements are not so demanding (BSSA, 2013). To reiterate the point of surface susceptibility, Mayette (1992) noted that a "piping material that microorganisms cannot adhere to has yet to be discovered. Studies have shown that microbes will adhere to stainless steel, Teflon, PVC and PVDF (Kynar) with nearly equal enthusiasm" (Edstrom, 2013). The finish of a steel material can also effect the bacteria formation on its surface and should be accounted for when applying a certain antimicrobial. In one particular study, three common finishing treatments of stainless steel that are used for equipment during poultry processing were tested for resistance to bacterial contamination. The treatments included sand-blasted, sanded, and electropolished; and each of these treatments were exposed to natural bacterial populations from chicken carcass rinses to allow growth of bacteria and development of biofilms on the surfaces. The sandblasted surface was a darker gray and uniformly pitted. Parallel striations could be seen on the mechanically sanded or ground surface, while the electropolished surface was mirror-like, very smooth, and shiny. The visible differences in the surface finishes were confirmed and extended by SEM (scanning

electron micrographs) (Arnold and Bailey, 2000). Sandblasting pitted the surface, and the pit-marks observed visually with SEM appeared as “craters,” but fewer bacterial cells were present compared to the untreated control. Sanding removed the mill finish and showed scratches and microscopic metal debris embedded on the surface, and even fewer bacterial cells were present. The electropolished surface was difficult to image with SEM because the surface was so smooth and featureless, and few bacterial cells were present (Arnold and Bailey, 2000*).* To summarize, biofilm formation significantly decreased with each finishing treatment. Sandblasting had the greatest amount of dispersed cells (1,534), then sanded (1,217), and lastly electropolished (118). This demonstrates that the finish of a particular stainless steel surface can affect biofilm formation by bacteria.

Even though some studies report that chicken breasts are more prone to carrying *Campylobacter*, that does not mean to say that other parts of the chicken carcass aren’t significantly affected (Zhao et al., 2010). One particular study looked at cross contamination during handling of contaminated fresh chicken parts in kitchens. The numbers of *Campylobacter* present on the surfaces of the chicken parts (excluding breasts), hands, utensils, and ready-to-eat foods were detected by using Preston enrichment and colony counting after surface plating on Karmali agar (Luber et al., 2006). The mean transfer rates from legs and filets to hands were 2.9 and 3.8%. The transfer from legs to the plate (0.3%) was significantly lower ($P < 0.01$) than the transfer from filets to the cutting board and knife (1.1%). Average transfer rates from hands or kitchen utensils to ready-to-eat foods ranged from 2.9 to 27.5%. Transfer rates from chicken breast filets and legs to hands, from filets and legs to kitchen utensils, and from

hands and utensils to ready-to-eat foods were analyzed for significant differences by means of the Mann-Whitney-test (Luber et al., 2006). In summary, any part of the chicken carcass can harbor *Campylobacter* cells if the environmental conditions are right. Cross contamination can further increase spreading as well.

Antimicrobial solutions used in poultry processing

One important effort in poultry processing is to monitor the effectiveness of the antimicrobial solutions that can be used on a daily basis to prevent or reduce contamination. Depending on the environment and how the poultry is prepared, processing plants will choose an antimicrobial that will fit a specific need. The primary antimicrobial chemicals used in poultry processing include cetylpyridinium chloride (CPC), trisodium phosphate (TSP), acidified sodium chlorite (ASC), and chlorine. It should also be noted that household cleaners and sanitizers are not acceptable for USDA-FSIS inspected food plants unless accepted by USDA (Russell, 2009).

Cetylpyridinium chloride (1-hex-decyl pyridinium chloride) is a quaternary ammonium compound with antimicrobial properties against many microorganisms including viruses (FDA, 1998). It has a pH of 7.2 at 1% solution and is permitted by the US Food and Drug Administration to be used in various dental products (FDA, 1998). The chemical can also be found in several commercial products, including mouthwashes and antimicrobial solution sprays. The antimicrobial activity of this solution is due to an interaction of basic cetylpyridinium ions with acidic molecules on bacteria, which subsequently inhibits bacterial metabolism by forming weak ionic compounds that interfere with bacterial respiration. One research study showed that

cetylpyridinium chloride provided a small but significant benefit in control of plaque and gingivitis. In this study, it was shown that gingivitis reduction was around 13.4%, while plaque reduction was about 15% (Gunsolley, 2010).

The chemical has also been effective in removing bacteria (such as *Salmonella*) from poultry. In 2002, a food additive petition was filed to permit the safe use of this antimicrobial in poultry processing (FDA, 1998). One study showed that CPC spraying reduced *Salmonella* by 0.9 to 1.7 log units (87 to 98%) (Kim et al., 1996). For commercial products, the maximum concentration of cetylpyridinium chloride allowed as a spray is usually 0.1% w/v. One new commercial product that contains cetylpridnium chloride is sore throat lozenges. Since cetylpyridinium chloride is a mild antiseptic, it is active against a wide range of microorganisms that might infect sore or broken skin in the mouth and throat. The action of sucking the lozenge allows the active ingredient to work in the area of the discomfort and also helps lubricate and soothe the painful area. This helps relieve the soreness and discomfort of mouth and throat infections (Netdoctor, 2012). Medicated throat lozenges have the added advantage of being slow releasing as they react with saliva in the oral cavity. Furthermore, the portability and convenience of taking lozenges may help to facilitate good adherence/compliance to medication.

Trisodium phosphate is another antimicrobial that has been studied and tested in the food industry. It had been historically used as a household cleanser, but was eventually approved by the Food and Drug Administration as a GRAS (generally recognized as safe) substance for use in food processing in August 1992. Ever since then, the chemical has been used as an ingredient in food processing contact surface

cleaners. TSP has also been used in breakfast cereals, snack foods, artificially sweetened fruit jellies, and processed cheese spreads as antioxidants and emulsifiers. In October of 1992, it was approved by the United States Department of Agriculture for post-chill use in poultry processing plants (Bender and Brotsky, 1992). TSP has been approved for pre-chill, post-chill, and in air-chilling operations in Canada (Canadian Food Inspection Agency, 2004). In 1996, pre-chill carcass TSP application was approved by the USDA (USDA, 1996). Many commercial poultry processors have used this chemical in an 8 to 12% solution as an antimicrobial rinse or dip for raw carcasses (Arritt et al., 2002). TSP's antimicrobial activity works in several ways; 1) the high pH (10-11) causes cell membrane disruption (Mendonca et al., 1994), 2) detachment of bacteria from the carcass surface (Lee et al., 1994), and 3) lipid removal or detergent-like activity (Bender and Brotsky, 1992; Giese, 1992; Kim and Slavik, 1994). TSP is a white, granular or crystalline solid and is highly soluble in water producing an alkaline solution. Numerous scientific studies have shown TSP's effectiveness as an antimicrobial. In one study, dipping chickens in 8, 10 and 12% solutions of TSP reduced *E. coli* by 0.5, 1.2 and 1.6 log CFU/cm^2, respectively, whereas 0.5, 1.0, and 1.7 log CFU/cm^2 reduction was observed in aerobic total counts, respectively. The effectiveness of the TSP was hypothesized to be due to its high pH (Bin Jasass, 2008).

Acidified sodium chlorite is also an effective antimicrobial used in the poultry processing industry. The chemical is being used in many countries, including Australia and the USA, as an antimicrobial treatment in the food industry, for water purification, and for sterilizing hospital and clinic rooms and equipment. In processing applications such as with poultry, the sodium chlorite concentration is between 500 and 1,200 ppm

and the acid levels used are high enough to produce a pH between 2.3 and 2.9 (Arritt et al., 2002). It is usually applied as a spray or dip for incoming or outgoing products. With this solution, some poultry manufacturers are concerned with how the chemical will interact with either organic matter in solution or protein and fat compounds on the carcasses. But in most studies, no detectable changes were observed in the fatty acid profiles even in polyunsaturated fatty acids, which are more sensitive to oxidation (EFSA, 2005). ASC is very effective as an antimicrobial. This particular study demonstrates the effect of acidified sodium chlorite washing on *Campylobacter jejuni* on poultry legs stored at 4°C for 8 days. Fresh chicken legs were inoculated with the bacteria, and afterwards the legs were dipped into a 0.8 g/l, 1.0 g/l, or 1.2 g/l acidified sodium chlorite solution or into distilled water (control). *C. jejuni* concentration was approximately 1.2 log units lower than the water washed chicken legs after the treatment with 1.2 g/l ASC ($p<0.05$) (Naiara et al., 2010).

Chlorine compounds are widely used in the food industry to kill bacteria and disinfect. Examples include treating pasteurizer cooling water, washing fruit and vegetables, and disinfecting food contact surfaces. The chemicals are usually combined with inorganic compounds, such as sodium or calcium, to produce hypochlorites, which are effective disinfectants (Eifert and Sanglay, 2002). The USDA Food Safety and Inspection Service (FSIS) allows for addition of chlorine to processing waters at levels up to 50 ppm in carcass wash applications and chiller make-up water. The FSIS also requires that chlorinated water containing a minimum of 20 ppm available chlorine be applied to all surfaces of carcasses when the inner surfaces have been reprocessed (due to carcass contamination) other than solely by trimming

(Russell, 2009). At recommended levels, hypochlorite- (chlorine derivative) based sanitizers reduce enveloped and non-enveloped viruses. Hypochlorite is also effective against fungi, bacteria and algae. However, under traditional conditions of use, chlorine does not have an effect on bacterial spores (Russell, 2009). The three main types of chlorine used in the poultry industry include chlorine gas, calcium hypochlorite, and sodium chlorite. Chlorine in its elemental gas state is highly toxic and corrosive. So because of this, many food processing facilities have changed to either calcium hypochlorite or sodium hypochlorite for water treatment. Calcium hypochlorite is available in granular or pellet form and is usually more expensive to use compared to other hypochlorite forms. In general, food processing companies use calcium hypochlorite because the concentration can be controlled more effectively than other forms of chlorine. Various studies have reported on the effectiveness of chlorine compounds against foodborne pathogens. In one study, the susceptibility of three *C. jejuni* strains and *Escherichia coli* ATCC 11229 were compared with standard procedures used to disinfect water. Inactivation of bacterial preparations with 0.1 mg of chlorine and 1.0 mg per liter of monochloramine was determined at pH 6 and pH 8 and at 4°C and 25 °C. Under virtually every condition tested, each of the three *C. jejuni* strains was more susceptible than the *E. coli* control strain, with greater than 99% inactivation after 15 min of contact with 1.0 mg per liter of monochloramine or 5 min of contact with 0.1 mg of free chlorine per liter (Blaser et al., 1986). These results suggest that disinfection procedures commonly used for treatment of drinking water to remove coliform bacteria are adequate to eliminate *C. jejuni*. This may also help to explain the absence of *Campylobacter* outbreaks associated with properly treated drinking water.

Surfactant use in food processing

Surface-active agents or surfactants can be used in numerous ways in society. Basically surfactants act as foaming agents, emulsifiers and dispersants, suspending gases, immiscible liquids, or solids, respectively, in water or some other liquid. In general, surfactants are compounds that are amphiphilic in nature – part hydrophilic (has affinity for water or aqueous phases), part lipophilic (has affinity for oily or organic phases) (ROSS, 2013). The combination of these opposing affinities in the same molecule dictates the surfactant's ability to reduce surface and interfacial tensions (ROSS, 2013). Surfactants can be used in the removal of dirt particles, bacteria, and other solids from surfaces such as the human skin, textiles, or industrial surfaces. There are three classifications for surfactants: anionic, nonionic, and cationic.

Anionic surfactants dissociate in water into a hydrophilic anion, and a cation; the cation is usually an alkaline metal (Na+, K+) or a quaternary ammonium compound (Salager, 2002). In the industry, anionic compounds are the most commonly used surfactants. They include sulfonates (detergents), (fatty acid) soaps, lauryl sulfate (foaming agent), di-alkyl sulfosuccinate (wetting agent), lignosulfonates (dispersants) and others. These surfactants account for 50% of the global surfactant usage (Salager, 2002).

Nonionic surfactants come second in volume usage, making up 45% of the industrial solutions (Salager, 2002). These surfactants do not ionize in aqueous solution, because their hydrophilic group is of a non-dissociable type, such as alcohol, phenol, ether, ester, or amide. A large proportion of these nonionic surfactants are

made hydrophilic by the presence of a polyethylene glycol chain, obtained by the polycondensation of ethylene oxide (Salager, 2002); they are called polyethoxylated nonionics.

Lastly, cationic surfactants dissociate in water into a cation and an anion, most often of the halogen type (Cl^-, Br^-). Most of this class corresponds to nitrogen compounds such as fatty amine salts and quaternary ammoniums, with one or several long chain of the alkyl type, often coming from natural fatty acids (Salager, 2002). These surfactants are usually more expensive then anionic surfactants. They are either used as a bactericide, or as a positively charged substance which is able to absorb on negatively charged substrates to produce antistatic and hydrophobic effects, often of great commercial importance such as in corrosion inhibition (Salager, 2002). A commonly used cationic surfactant that is used in oral hygiene products and poultry processing is cetylpyridinium chloride (CPC). The compound is used in some types of mouthwashes, toothpastes, lozenges, throat sprays, breath sprays, and nasal sprays. CPC is an antiseptic that kills bacteria and has been shown to be effective in preventing dental plaque and reducing gingivitis. It is suggested that its interaction with bacteria occurs by the disruption of membrane function, leakage of cytoplasmic material, and ultimately the collapse of the intra-cellular equilibrium (Haps et al., 2008).

Delmopinol: a surfactant to disrupt dental plaque formation

Delmopinol is a tertiary amine surfactant with a pKa of 7.1 and in aqueous solutions a balance exists between the protonated and non-protonated forms. In this respect, aqueous solutions of delmopinol can be regarded as a system containing two

surface active components where the ratio is influenced by the pH of the solution (Svensson, et al., 2010). This compound has significant use in the treatment of the oral cavity, specifically for the prevention and treatment of gingivitis and for the removal or inhibition of dental plaque. Its surfactant mechanism of action inhibits bacterial adhesion to tooth and mucosal surfaces, and also inhibits cohesion between the bacterial cells themselves. The antimicrobial effect of delmopinol, in an oral rinse, has been compared to that of chlorhexidine. Delmopinol was less bacteriostatic, with minimum inhibitory concentrations 5-125 times higher than those of chlorhexidine against various oral and non-oral species (Simonsson et al., 2009). Mouthrinses containing delmopinol prevent bacteria from synthesizing the sticky glucan polysaccharide compounds that cause the adhesion to tooth and gum surfaces and to the other bacterial cells nearby, and disrupt existing dental plaque biofilm colonies (Nagelberg, 2013). When there are existing plaque colonies, the cohesive forces between the bacteria are reduced by delmopinol, which makes removal by mechanical means much easier. One research study further explored the in vitro interaction of delmopinol hydrochloride with salivary films adsorbed at solid/liquid interfaces. It was determined that pure delmopinol adsorbs on both hydrophilic and hydrophobic surfaces. With that being said, adsorption was greater on hydrophilic surfaces. The adsorbed mass quickly reached a plateau value of 0.19 ug/cm^2 on the hydrophilic surfaces and 0.11 ug/cm^2 on the hydrophobic ones. Greater adsorption on hydrophilic surfaces may be due to the hydrophilic interactions yielding the exposure of hydrophobic tails, resulting in the subsequent formation of a second layer of delmopinol/bacteria interaction on the top of the first interaction (leading to more adsorption). The results

indicated that the presence of delmopinol molecules within salivary films cause an increase in the desorbable fraction of these films (Vassilakos, 1993).

Early clinical studies showed the effectiveness of delmopinol as an oral mouth rinse. In 1992, a study was done to investigate a possible dose-response effect of delmopinol hydrochloride, on the development of plaque and on the healing of gingivitis. After experiment preparations were completed, 64 male volunteers rinsed 2x daily for 1 min with 10 ml of 0.05% (15 subjects), 0.1% (17) or 0.2% (16) delmopinol for 2 weeks, respectively. 16 subjects rinsed with 0.2% chlorhexidine. The results showed that mean plaque extension was reduced by 23% for 0.05%, 39% for 0.1% and 55% for 0.2% delmopinol; which was significant (Collaert et al., 1992). A similar study (Lang, 1998) tracked plaque formation and gingivitis after supervised mouthrinsing with 0.2% delmopinol hydrochloride, 0.2% chlorhexidine digluconate and placebo for 6 months. The results were significant, for the plaque index scores with delmopinol were reduced from baseline by 47% after 3 months and 52% after 6 months. Bleeding on probing values (BOP) with delmopinol were reduced from baseline by 37% after 3 months and 36% after 6 months (Lang, 1998). Limited research has been done in between these particular studies that give positive results for delmopinol as well.

Decapinol® is the trade name of the first oral hygiene products that contain delmopinol and made commercially available by Sinclair Pharmaceutical Limited which has been renamed as Sinclair IS Pharma (London, United Kingdom). Decapinol® was first marketed in some countries of the European Union and has been approved as a Class II medical device in both the European Union and the United States. The solutions' novel mode of action prevents the attachment of plaque bacteria to both the

tooth surfaces and the gums, providing a barrier-like effect. It also prevents plaque bacteria from sticking to each, which makes them less cohesive. The solution (along with similar delmopinol rinses) is a cationic surfactant and has a net positive charge. In contrast, the acquired pellicle on tooth surfaces/gums has a net negative charge and this allows for Decapinol® to adhere to the toothsurface/gums easily (Sinclair Pharma, 2008). In addition to the mouth rinse, Decapinol can be found in other oral hygiene products including toothpaste, gel, mouth spray, and gum applicator.

Another mouth rinse product called GUM® PerioShield™ Oral Health Rinse containing 0.2% delmopinol is available in the United States from Sunstar Americas, Inc. This formulation has a very low alcohol content (1.5%) and is indicated for all patients—especially those prone to significant plaque accumulation and chronic gingivitis. It is utilized as an adjunct to normal brushing, flossing, and other mechanical means of dental plaque biofilm removal. However, delmopinol HCl is highly soluble in water and when used in oral formulations, the delmopinol is removed from the oral surfaces (where it exerts its action) by flow of saliva. The compound is so soluble that even in areas of the mouth with low saliva flow, it is only present for a relatively short period of time. The consumer products currently available on the marketplace that contain delmopinol usually recommend that they be held in the mouth for up to one minute, so that maximum efficiency is achieved.

A very positive attribute of delmopinol is that it has minimal to no side effects in commercial use. Some tooth and tongue staining was reported with the use of 0.2% delmopinol, yet the stain was typically not strongly adherent to tooth structure and was easily removed by mechanical brushing. Also, transient anesthesia of the tongue and

mild taste disturbances have been reported in some clinical studies but nothing significant (Bruhn, 2011). This may be due to the mild acidic pH of the delmopinol rinses. This may be due to the mild acidic pH of the delmopinol rinses. For example, the pH of GUM® PerioShield™ Oral Health Rinse containing 0.2% delmopinol is 5.41.

In general, delmopinol hydrochloride can be obtained by three different methods. The first involves the reaction of 2-(benzylamino)-6propylnonan-1-ol (I) with ethylene oxide (II) in ethanol at 100 C yields the corresponding N-(2-hydroxyethyl) derivative (III), which is cyclized with H2SO4 at 140-150 C to 4-benzyl-3-(4propylheptyl) morpholine (IV). The debenzylation of (IV) by hydrogenolysis with H2 over Pd/C affords the free morpholine (V), which is finally condensed with 2-chloroethanol (VI) by means of KI and KOH in refluxing ethanol. The second way involves the Grignard condensation of 4-heptanone (VII) with allyl bromide (VIII) in ethyl ether gives 4-propyl-1-hepten-4-ol (IX), which is cyclocondensed with morphiline (X) by means of H202 and Na2WO4 in methanol to yield 4-(perhydroisoxazol[3,2-c][1,4]oxazin-2-ylmethyl)-4-heptanol (XI). The reductive ring opening of (XI) by hydrogenation with H2 over Pd/C and p-toluenesulfonic acid in isopropanol affordes a mixture of the morpholine (V) and the hydroxymorpholine (XII). This mixture, without separation, is treated first with SOCl2 in chloroform to perform CI-OH interchange, then with NaOH to obtain the corresponding double bond, and finally, the mixture is hydrogenated with H2 over Ra-nickel and triethylamine in dioxane in order to obtain pure (V), already obtained. The last method is the acylation of the alkylmorpholine (V) with oxalic acid monomethyl ester (XIII) by means of triethylamine in refluxing benzene gives the corresponding condensation compound (XIV), which is then reduced with LiAlH4 in refluxing ethyl ester (Hernestam et al.,

2013). The most recent patent for this solution preparation occurred on March 28, 2012 (8,143,463). The primary inventor was Artus Surroca and it was assigned to Sinclair Pharmaceutical Limited (USPTO, 2012).

The Efficacy of Delmopinol in Preventing the Attachment of *Campylobacter jejuni* to Chicken, Stainless Steel and High-Density Polyethylene

by

Calvin Waldron

Abstract

Campylobacter spp. are the second leading bacterial cause of food borne illness in the U.S. New antimicrobials that prevent bacterial attachment may be effective for reducing *Campylobacter*. Delmopinol hydrochloride (delmopinol) is a cationic surfactant that is effective for treating and preventing gingivitis and periodontitis. This study evaluated the effectiveness of delmopinol for reducing attachment of *Campylobacter jejuni* to chicken, stainless steel and high-density polyethylene.

Chicken pieces, steel and HDPE coupons were spot-inoculated with 0.1 mL of a *Campylobacter jejuni* culture. After 10 min, samples were sprayed with 0.5% or 1.0% delmopinol, 0.01% sodium hypochlorite, or distilled water. Contact times were 1, 10, or 20 min prior to rinsing with buffered peptone water. Rinses were serially diluted onto Campy Cefex Agar for enumeration. For additional samples, solutions were applied first, followed by inoculation with *C. jejuni* after 10 min. Cultures remained undisturbed for 1, 10, or 20 min. Then samples were rinsed and plated as above.

When *C. jejuni* was inoculated before treatments, 1% delmopinol application led to mean log reductions of 1.26, 3.70, and 3.72 log CFU/mL greater than distilled water, for chicken, steel and HDPE respectively. When *C. jejuni* was inoculated after spray

treatments, 1% delmopinol reduced *C. jejuni* by 2.72, 3.20, and 3.99 mean log CFU/mL more than distilled water for chicken, steel and HDPE respectively. Application of 1% delmopinol, either before or after bacteria inoculation, resulted in a significantly ($p<0.05$) greater log reduction than 0.01% sodium hypochlorite or distilled water. Delmopinol may be a promising antimicrobial treatment.

MATERIALS AND METHODS

Facility and Equipment

1. Biological Safety Level 2 (BSL-2) laboratory with a laminar flow biological safety cabinet.
2. Petri dishes, plastic
3. 1mL, 5mL, 10mL pipette dispensers and tubes were used.
4. Anaero-Pak rectangular anaerobic jar (2.5L or 7.0L), Mitsubishi Gas Chemical Company Inc. New York, NY.
5. Pack-MicroAero gas packs (Mitsubishi Gas Chemical Company Inc. New York, NY), which created micro-aerophilic conditions (5% O_2, 10% CO_2, 85% N_2).
6. Sterile bent glass rods, flexible plastic hockey sticks, or equivalent.
7. Microscope (phase contrast capable).
8. Incubators used to maintain samples and cultures at 41-42 °C and at 2-8 °C.
9. HDPE spray bottle, 240 mL (#S413505P, Fisher Scientific).

Test microorganism

1. *Campylobacter jejuni* ATCC 33291 (MicroBiologics, St. Cloud, MN)

Microbiological Media

1. Buffered Peptone Water (Edge Biological Inc., Lot #186)
2. Bolton's Broth, 500g container (Oxoid, Lot #1075738)

3. Brucella Agar, 500g container (BBL™; Becton, Dickson and Company; Ref # 211088; Lot # 0153583)
4. Campy-Cefex Agar, 500g container (Acumedia, subsidiary of Neogen Corp; Lot # 104716 A) Dry plates were critical for preventing spreading of colonies. Plates were covered with a plastic container and cloth, and left untouched for about a week. This minimized light exposure and wetness.
5. Campy-Cefex Supplement, 5mL/500mL containers (Acumedia, subsidiary of Neogen Corp; Lot # 104195)
6. Latex agglutination test kit (Oxoid Dryspot *Campylobacter* Test, Cambridge, UK).

Antimicrobial Sprays

1. Delmopinol hydrochloride (hereafter referred to as delmopinol) was obtained from Sinclair Pharma PLC (Godalming, Surrey, United Kingdom) and diluted with sterile, deionized water to concentrations of 0.5% and 1.0%. The solutions were mixed well and stored in 500mL-1000mL glass containers at 20-25 °C. Technical data:
    - Chemical name – 4-(2-Hydroxethyl)-3-(4-propyl-heptyl)-morpholine hydrochloride
    - CAS registry number – [98092-92-03]
    - Molecular weight and formula – 302.90, $C_{16}H_{33}NO_2HCl$
    - Solubility in water and Water content/KF – Dissolves quickly in water, NMT 0.5 w/w
    - Physical description- White or practically white powder

- pH- for a 1% w/v solution in water, pH is 4.0 - 5.0

2. Sodium hypochlorite, 100 ppm or 0.01% - Household bleach (6% NaOCl) was diluted by adding 3.5mL to 1L of distilled water for a 100ppm solution of sodium hypochlorite. For each batch made, the solution was stored in 500mL – 1000mL glass containers at 20-25 °C.
3. As an experimental control, sterile, deionized water at ambient temperature (20-25°C) was evaluated.

Test materials

1. Chicken breast meat samples were obtained from commercially processed chicken sold in the refrigerated meat section at a local retail grocery store. Raw, boneless, skinless, thin-sliced chicken breast were labeled as 98% fat free. Breast portions were aseptically removed from their tray package and cut into square pieces approximately 3cm x 3cm using a sterile scalpel.
2. Stainless steel coupons (Speedy Metals GB LLC; Little Chute, WI) were type 304 hr annealed and were pre-cut to approximately 2.5 cm x 2.5 cm squares. This type of steel is commonly used in food processing and preparation equipment.
3. High density polyethylene (HDPE) sheet (InterstatePlastics.com) coupons were pre-cut into approximately 2.5cm x 2.5cm squares as well. High density polyethylene (0.910-0.925 g/cm^3) is more rigid and less permeable than low density polyethylene (LDPE) (U.S. Plastic Corp., 2013).
4. The chicken skins and industrial surfaces used in this project were sterilized prior to use by acetone (except chicken) and UV radiation. Each industrial piece was

submerged in 400mL of acetone for 20min, and then dabbed with paper towels and air dried for 10 minutes. Next, the chicken skins or industrial surfaces were placed on a sterilized plastic surface under a longwave UV lamp for 30 min. After that time period, the skins or surfaces would be flipped and radiated on the other side for another 30 minutes.

Procedures

Test organism: One strain of *Campylobacter jejuni* ATCC 33291 (obtained from MicroBiologics, St. Cloud, MN) was revived in Bolton's broth and plated onto Brucella agar (BD, Sparks, MD) for visual inspection of purity. These media were incubated under micro-aerophilic conditions (5% O_2, 10% CO_2, 85% N_2) at 42°C for 48 hrs. Micro-aerobic conditions were obtained with Pack-MicroAero gas packs (Mitsubishi Gas Chemical Company Inc. New York, NY). Culture was re-inoculated into fresh Bolton's broth and incubated as above.

Campy-Cefex Agar (Acumedia, subsidiary of Neogen Corp) and Brucella agar plates (BBL ™ vendor; Becton, Dickson and Company) were prepared by manufacturer's directions. Dry plates were critical for preventing spreading of colonies, so the plates were covered with a plastic container and cloth and left untouched for up to 7 days.

Experimental Design: The research study included three test surface materials: chicken meat, steel, and polyethylene. Four solutions were separately tested with each material surface. The solutions included 0.5% delmopinol, 1% delmopinol, 0.01%

sodium hypochlorite, and distilled water (control). The chemical solutions were further evaluated through their application to test surfaces either before or after inoculation with *C. jejuni*. Additionally, the chemical contact times (1min, 10min, or 20min) for each application method were studied. Two replications of the microbiological recovery analyses were performed for each combination of treatment variables (Appendix A).

Inoculation of test materials and chemical spray application:

Bacteria applied before chemical: Chicken meat samples, HDPE coupons and steel coupons were spot-inoculated with approximately 0.1 mL of one strain of *Campylobacter jejuni* broth culture after being placed into a sterile specimen cup. These samples remained undisturbed at approximately 21 C for 10 min. One chemical spray (~10 ml) was applied to the surface of each sample. A plastic (HDPE) spray bottle was used to mist the chemicals or control onto the surfaces. A consistent spray pressure and quantity (11 sprays equivalent to 10 mL) was used. The distance from the spray nozzle to the surface was approximately 6 cm.

Bacteria applied after chemical: For additional samples, *C. jejuni* was inoculated onto the test materials after a spray application of the test solutions. The same chemical spray applications were applied (~10 mL) to chicken, plastic and steel surfaces and these remained undisturbed for 10 min. Then, approximately 0.1 mL of *C. jejuni* broth culture was applied. The application of the sprays prior to the bacterial inoculation provided an opportunity to test their abilities to inhibit bacterial attachment.

Recovery and confirmation of *Campylobacter*: After a contact time of either 1, 10, or 20 min, 20 ml of sterilie buffered peptone water was immediately added to the samples in the cups and shaken by hand for 30 sec. These eluates from the spray applications were further diluted in buffered peptone water. Buffered peptone water aided in the removal of *Campylobacter* cells for plating.

The rinse and the collected eluate from the spray application were serially diluted and plated onto Campy Cefex Agar (Acumedia (Neogen) Lansing, MI). All plates were incubated microaerobically (5% O_2, 10% CO_2, 85% N_2) at 42°C for 48±2 hrs. Micro-aerobic conditions were obtained with Pack-MicroAero gas packs. The number of cells counted on these plates represented loosely attached cells. The concentration of recovered cells was subtracted from the concentration of the inoculum to determine the log reduction of bacteria.

Confirmation tests were also performed regularly to confirm the identification of *Campylobacter jejuni*. Colonies recovered from inoculated test surfaces on to Campy-Cefex agar plates were confirmed with a latex agglutination test kit (Oxoid Dryspot *Campylobacter* Test, Cambridge, UK). Additionally, selected colonies from Campy-Cefex plates were examined microscopically for typical corkscrew morphology and darting motility.

Statistical analyses: All *Campylobacter* populations were converted to $\log_{10}$ CFU/ml for analysis. Analysis of statistical tests was performed using JMP Pro Statistical Software (Version 10.0.0 Copyright 2012, SAS Institute Inc., Cary, NC, USA).

Comparisons of log reduction values in chemical/material combinations for the two inoculation treatment methods (before and after) as well as comparisons of log reduction values in contact times for chemical/material combinations were determined using ANOVA with Tukey's HSD at $\alpha = 0.05$. In cases where there was a zero count on duplicate plates, a count of 1.0 log CFU/mL was assigned to allow for count conversion to $\log_{10}$ CFU/mL.

RESULTS

In this research study, the effectiveness of the chemical delmopinol was evaluated for inhibiting or preventing the attachment of *Campylobacter jejuni* to chicken breast meat, stainless steel and polyethylene. In general, a delmopinol spray reduced *C. jejuni* by 2 to 4 log CFU/mL when compared to 0.01% NaOCl or distilled water. Also, the recovery of *C. jejuni* was lowest (log reduction highest) from stainless steel and chicken breast meat when the bacteria were applied after a chemical spray. Conversely, the log reduction observed on HDPE plastic was highest when the bacteria were applied before the chemical spray.

Application of bacteria before the test chemicals

When *Campylobacter jejuni* was inoculated before the test chemicals were applied, the 0.5% and 1.0% delmopinol solutions produced significantly ($p<0.05$) greater log reductions than 0.01% NaOCl or distilled water among all surfaces tested (Table 1). When comparing the three test materials, the highest log reductions of *C. jejuni* occurred on the HDPE plastic surface when delmopinol (0.5 or 1.0%) or 0.01% NaOcl was applied. For the 1.0% delmopinol solution, the average log reduction was approximately 2 logs greater on chicken breast and HDPE plastic when compared to 0.01% NaOCl on the same surface, while the log reduction on steel, with 1.0% delmopinol) was approximately 3.8 log higher than with 0.01% NaOCl. The 0.5% delmopinol solution produced log reductions of 4.82 and 3.87 on HDPE plastic and steel. Even though this concentration was less effective in reduction compared to 1.0%

delmopinol, it was still significant. The log reduction on the chicken breast surface when 0.5% delmopinol was used was 2-3 logs less than the log reduction observed on HDPE plastic and steel. Also, the log reduction for 1.0% delmopinol on chicken breast surface was also significantly lower ($p<0.05$) than the measured reduction on the other two surfaces using the same delmopinol concentration). This may be due to the composition of animal tissue being extremely different in comparison to industrial surfaces, for it is much more porous. The bacteria may have been absorbed deep within the animal tissue, so more cells attached to the surface which resulted in lower log reductions.

Application of bacteria after the test chemicals

When *Campylobacter jejuni* was inoculated after the test chemical sprays were applied, the 0.5% and 1.0% delmopinol solutions produced significantly ($p<0.05$) greater log reductions than 0.01% NaOCl or distilled water among all surfaces tested (Table 2). For each material, these reductions were at least 3 log higher for the delmopinol solutions as compared to 0.01% NaOCl or distilled water. The highest log reductions of *C. jejuni* (5.86 and 5.39 log CFU/mL) occurred on steel in the presence of 0.5 or 1.0% delmopinol, respectively. Also, the log reductions on steel (in the presence of delmopinol) were significantly higher than the log reductions on chicken and HDPE.

The log reductions for 1.0% delmopinol on chicken breast surface and HDPE plastic (3.63 and 4.28 log CFU/mL, respectively) were significantly lower than the log reduction measured from steel at the same concentration (3.63 log CFU/mL). Similarly, the 0.5% delmopinol solution application resulted in *C. jejuni* log reductions on chicken

breast and HDPE that were significantly lower than the log reduction observed on stainless steel.

Chemical contact time effect

For each sprayed solution and material surface, inoculated *C. jejuni* remained in contact with a spray solution for 1, 10 or 20 min. No significant difference ($p>0.05$) in *C. jejuni* log reductions were observed between the three contact times when the bacteria was inoculated either before or after the chemical sprays (Table 3). Furthermore, the mean reduction of *C. jejuni* varied by less than 0.3 log between the 1, 10 and 20 min contact times for all spray applications on chicken meat and steel and for 1.0% delmopinol on HDPE. Test results for the three contact times were averaged for the data presented in tables 1 and 2. Even with these two treatment methods being averaged, there was no more than a 0.50 log reduction difference in the contact times (1 minute, 10 minutes, 20 minutes) for a particular chemical/material combination observed. When looking at contact time, recovery was slightly lower for 10 or 20 minutes vs 1 minute. This was expected, but it is to note that the delmopinol solutions still produced significant log reductions at the 1 minute contact time.

DISCUSSION

In this experiment, the first phase involved the inoculation of *Campylobacter jejuni* on the chicken skin or industrial surfaces before the chemical treatment is applied by a spray bottle. The second phase was the inoculation of the bacteria after the chemical is applied. Prior research studies show that each method produces different results for each chemical treatment used. In other words, the initial application of a chemical to a surface may provide a greater opportunity for the chemical to prevent the attachment of bacteria. Alternatively, when an antimicrobial or surfactant chemical is applied to a surface after bacteria are inoculated, then the ability of a chemical to remove loosely attached or firmly attached cells can vary.

The concentration and applied quantity of these solutions also plays a vital role in the effectiveness of the treatment. For instance, in one research study, where the inoculation of *C. jejuni* on chicken skin occurred before a 0.5% cetylpyridinium chloride (CPC) treatment was applied, a $\log_{10}$ reduction of 2.89 cfu/skin was achieved (Arritt et al., 2002), while a lower concentration of CPC (0.1%) resulted in a log reduction of 1.42 cfu/skin (Arritt et al., 2002). In Arritt's study, the chemical effectiveness was defined as the degree of bacterial population reduction (inactivation or preventing attachment to skin) caused by the chemical. When the study evaluated bacterial inocula application after a chemical treatment was applied, the results were a little different. The 0.5% CPC treatment ($\log_{10}$ reduction of 4.67cfu/skin) was again considerably more effective than the 0.1% CPC where a mean $\log_{10}$ reduction of 0.77 cfu/skin was observed (Arritt et al., 2002). The main difference occurred when the overall results were compared to the first set of results. While the application of 0.5% CPC to skins after inoculation with

bacteria increased inhibitory effect (4.67 vs 2.89 mean log reduction), the application of 10% trisodium phosphate, 0.1 % acidified sodium chlorite, and 0.1% CPC were more effective if applied before bacterial contamination (Arritt et al., 2002).

Similar comparisons were made concerning these two methods in this delmopinol study. The two concentrations of 0.5% and 1.0% delmopinol had significant log reductions in comparison with 0.01% NaOCl and deionized water. This can be said for both treatment methods of the inoculation of *Campylobacter* "before" and "after" test chemicals were applied onto testing surfaces. For example, Table 1 showing the average mean log reduction of *C. jejuni* when applied BEFORE test chemicals displays 1% delmopinol having a >1.0 log reduction than deionized water (the control) on chicken and a >3.5 log reduction greater than deionized water on HDPE plastic and steel. Also, table 2 which reports the average mean log reduction of *C. jejuni* when applied AFTER test chemicals demonstrates the effectiveness of delmopinol as well. On average 0.5% and 1.0% delmopinol had a 2.5+log reduction greater than both 0.01 NaOCl and deionized water on chicken, a 2.0+log reduction greater than both 0.01 NaOCl and deionized water on HDPE plastic, and a 4.0+log reduction greater than both 0.01 NaOCl and deionized water on steel. These results imply that delmopinol has the potential to reduce the level of an important foodborne pathogen on chicken meat and two common food contact surfaces. The pH may also play a role in its effectiveness, for the pH range of delmopinol is 4.0-5.0. The pH values for 0.5% and 1.0% concentrations were measured as 4.32 and 4.20, respectively. The acidity of these solutions can help break down bacteria and organic compounds to some degree.

The methods of chemical application have importance here as well. When the inoculation of *C. jejuni* on testing surfaces was performed after the test chemicals were applied, higher log reductions were achieved for the two concentrations of delmopinol for chicken and steel (0.5% delmopinol/chicken = 3.73, 1.0% delmopinol/chicken = 3.63, 0.5% delmopinol/steel = 5.86, and 1.0% delmopinol/steel = 5.39). In comparison, the inoculation of *C. jejuni* on testing surface that was performed before test chemicals were applied exhibited lower log reductions for the same delmopinol/material combination (~0.10 to 2.0 logs in difference). The higher the log reduction means the lower the recovery of loosely attached cells on the tested surface. Because the "AFTER" method produced such high log reductions when using the delmopinol solutions on chicken and steel, it is possible to conclude that the delmopinol is acting as a surfactant against the bacteria. In general, the surfactants in cleansers and disinfectants solubilize hydrophobic materials into the aqueous phase and enable their subsequent removal from a particular surface. The amphiphilic structure of surfactants, consisting of both a hydrophilic polar head group and a nonpolar lipophilic tail, drives surfactants to oil/water interfaces to facilitate cleansing (Walters et al., 2012). Also, recent studies reported in the dental literature on delmopinol support this hypothesis as well. For example, Bruhn (2011) observed that delmopinol's mechanism of action breaks down bacterial plaque and makes it less adhesive – forming a barrier that prevents plaque biofilm from sticking to teeth and gingiva. Delmopinol inhibits plaque by interfering with the enzymes responsible for biofilm formation.

This could very well be the case for this treatment method, for it is acting in the manner of a surfactant. Nevertheless, in this study, the log reduction of the delmopinol/

HDPE plastic combinations did not exhibit the same “surfactant” trend as the other two surfaces tested with delmopinol. Log reduction values for 0.5% and 1% delmopinol were actually higher on HDPE plastic when *C. jejuni* was applied BEFORE the chemicals compared to when *C. jejuni* was applied AFTER the chemicals (0.5% = 4.82, 1.0% = 5.60 vs 0.5% = 3.42, 1.0% = 4.28). A possible reason that this could have occurred is because the selected HDPE plastic is porous and the trapped bacteria had antimicrobial interaction with the delmopinol solutions. Even with a density of 0.941-0.965 g/cm^3, the plastic could have been susceptible to trapping the bacteria within itself (U.S. Plastic Corp., 2013). This could have led to less loosely attached cells that were recovered, which resulted in higher log reductions in the “BEFORE” method.

The contact time of antimicrobial solutions plays an important part in the effectiveness of reducing bacterial populations on a surface or in solution. One would hypothesize that with a longer contact time of a specific antimicrobial compound, a greater amount of removal would occur with the bacteria. Some antimicrobial chemicals, including chlorine solutions, are more effective if they are allowed to have a contact time of at least 30 seconds, and for not more than 10 minutes. This is mainly due to the fact that the antimicrobial needs to have a longer time to penetrate, react with or bind with the bacteria. In the study by Arritt et al. (2002) mentioned before, when *C. jejuni* was applied prior to chemical application, the effect of contact time (0.5, 3, or 10 min) was not significant. However, when the bacteria were applied after chemical sprays were used, the main effects of contact time were statistically significant. For example, the mean log reduction after 10 min contact time (1.42 cfu/skin) was significantly different than the log reduction achieved after 0.5 min contact time (0.70

cfu/skin) for all treatments combined (Arritt et al., 2002). In another study, a significant difference due to contact time was also obtained with respect to the inhibition of plaque growth on teeth using delmopinol. The mean areas of the teeth covered with plaque after the test periods (0 (placebo), 15, 30 and 60 seconds) were 41%, 29%, 23%, and 18%, respectively. Statistical analysis showed that rinsing with delmopinol for 30 or 60 seconds differed significantly ($P < 0.05$) from the placebo, and there was also a significant difference between rinsing for 15 and 60 seconds (Sjödin, 2011).

This was observed in this delmopinol study as well. Three different contact times were tested for each chemical/surface combination for the two types of treatment methods. No statistical significance or difference was found in any of the contact times for each chemical/material combination. One possible reason that the mode of action for delmopinol takes longer to complete. GUM PerioShield (a commercially available oral rinse solution that contains delmopinol) states that for the best results, use twice a day and avoid eating or drinking for about 30 minutes (Sunstar Americas, Inc., 2013). It may take 30 minutes or more for the solution to completely interact with gingivitis, enzymes, and bacteria. If this is the case, then higher log reductions could occur at contact times longer than 20 minutes (the longest contact time used in this research). Even though this study did not allow time for a sufficient biofilm to form, chemical contact time is still an important factor to study.

CONCLUSIONS

In conclusion, this study showed that delmopinol was significantly effective in preventing the attachment of *Campylobacter jejuni* on various surfaces under certain conditions. Two types of treatment methods were tested and when *C. jejuni* was inoculated before spray treatments, the 1% delmopinol application led to mean log reductions of 1.26, 3.70, and 3.72 log CFU/mL, greater than distilled water, for chicken, steel and HDPE respectively. When *C. jejuni* was inoculated after spray treatments, 1% delmopinol reduced *C. jejuni* by 2.72, 3.20, and 3.99 mean log CFU/mL more than distilled water for chicken, steel and HDPE respectively. It was demonstrated that this new alternative may have a place among the more common antimicrobials, due to its positive results in testing. This study can provide new and valuable information on how to combat the threat of *Campylobacter* in poultry processing and preparation. Additional research is needed that considers the effect of other chemical concentration, contact time, chemical application volume and method of application in commercial food processing to ascertain the effectiveness of delmopinol solutions against *Campylobacter* spp. and other foodborne pathogens.

Table 1. Mean Log reduction of *C. jejuni** when applied before test chemicals

Solution Type	**Surface Type**		
	Chicken	*HDPE Plastic*	*Steel*
0.5% delmopinol	1.87 ±0.40^{Bbc}	4.82 ±1.16^{Aab}	3.87 ±0.70^{Ab}
1.0% delmopinol	3.53 ±0.60^{Ca}	5.60 ±0.46^{Aa}	4.71 ±0.62^{Ba}
0.01% NaOCl	1.58 ±1.09^{Bc}	3.70 ±0.47^{Abc}	0.87 ±0.15^{Bc}
distilled water	2.27 ±1.26^{Ab}	1.90 ±1.25^{Ac}	0.99 ±0.42^{Bc}

n = 36 for total surface samples

* *C. jejuni* inoculated 10 min before chemical application

* Reductions given as $\log_{10}$ CFU/ml

Significant differences ($p<0.05$) in Tukey HSD statistic between rows are designated with a lower case superscript letter.

Significant differences ($p<0.05$) in Tukey HSD statistic between columns are designated with an upper case superscript letter.

Table 2. Mean Log reduction of *C. jejuni** when applied after test chemicals

Solution Type	**Surface Type**		
	Chicken	*HDPE Plastic*	*Steel*
0.5% delmopinol	3.73 ±0.35^{Ba}	3.42 ±0.33^{Ba}	5.86 ±0.27^{Aa}
1.0% delmopinol	3.63 ±0.15^{Ba}	4.28 ±0.85^{Ba}	5.39 ±0.44^{Aa}
0.01% NaOCl	0.96 ±0.03^{Ab}	1.29 ±0.62^{Ab}	1.28 ±0.36^{Ab}
distilled water	0.91 ±0.08^{Bb}	1.08 ±044^{Bb}	1.40 ±0.26^{Ab}

n = 36 for total surface samples

* Chemicals applied 10 min before *C. jejuni* inoculation

* Reductions given as log10 CFU/ml

Significant differences ($p<0.05$) in Tukey HSD statistic between rows are designated with a lower case superscript letter.

Significant differences ($p<0.05$) in Tukey HSD statistic between columns are designated with an upper case superscript letter.

Table 3. Mean Log reduction of *C. jejuni* by chemical solution contact time

Solution and Contact Time	**Surface Type**		
	Chicken	HDPE	Steel
0.5% delmopinol			
1min	2.80 ±0.47	4.64 ±0.07	4.89 ±0.40
10min	2.88 ±0.36	4.16 ±0.57	4.81 ±0.64
20min	2.76 ±0.24	3.57 ±0.49	4.90 ±0.66
1.0% delmopinol			
1min	3.42 ±0.15	4.91 ±0.54	5.04 ±0.76
10min	3.63 ±0.34	4.98 ±0.75	5.19 ±0.62
20min	3.70 ±0.30	4.94 ±0.80	4.91 ±0.27
0.01% NaOCl			
1min	1.34 ±0.63	2.00 ±0.77	1.17 ±0.26
10min	1.32 ±0.67	2.57 ±0.33	1.08 ±0.31
20min	1.14 ±0.83	2.91 ±0.22	0.97 ±0.11
distilled water			
1min	1.61 ±0.93	1.18 ±1.15	1.32 ±0.37
10min	1.52 ±0.84	1.54 ±0.69	1.06 ±0.19
20min	1.63 ±0.79	1.74 ±0.99	1.21 ±0.41

n = 72 for total surface samples

Figures presented are averages of before and after treatment log reduction values for each chemical/material combination

* Reductions given as log10 CFU/ml

No statistical significance between contact times for any combination of chemical and material

REFERENCES

Abu-Ruwaida, A.S., W.N. Sawaya, B.H. Dashti, M. Murad, H.A. Al-Othman. 1994. Microbiological quality of broilers during processing in a modern commercial slaughterhouse in Kuwait. Journal of Food Protection. 57(10):887-892.

Altekruse, S.F., N.J. Stern, P.I. Fields, D.L. Swerdlow. 1999. *Campylobacter jejuni* – An emerging foodborne pathogen. Emerging Infectious Diseases. 5: 28-35.

Arnold, J.W., G.W. Bailey. 2000. Surface finishes on stainless steel reduce bacterial attachment and early biofilm formation: Scanning electron and atomic force microscopy study. Poultry Science. 79:1839-1845.

Arritt, F. M., J.D. Eifert, M.D. Pierson, S.S. Sumner. 2002. Efficacy of antimicrobials against *Campylobacter jejuni* on chicken breast skin. Journal of Applied Poultry Research. 11: 358-366.

Bashor, M.P., P.A. Curtis, K.M. Keener, B.W. Sheldon, S. Kathariou, J.A. Osborne. 2004. Effects of carcass washers on *Campylobacter* contamination in large broiler processing plants. Poultry Science 83(7):1232-1239.

Bender, F. G., E. Brotsky. 1992. Process for treating poultry carcasses to control Salmonellae growth. U.S. Patent 5,143,739.

Bin Jasass, F.M. 2008. Effectiveness of trisodium phosphate, lactic acid, and acetic acid in reduction of *E. coli* and microbial load on chicken surfaces. African Journal of Microbiology Research. 2(3):050-055.

Blaser M.J., P.F. Smith, W.L. Wang, J.C. Hoff. 1986. Inactivation of *Campylobacter jejuni* by chlorine and monochloramine. Applied and Environmental Microbiology 51(2):307-11.

Bolder, N.M. 1998. The microbiology of the slaughter and processing of poultry. Chapter 5 In: A. Davies and R. Board (eds.), The Microbiology of Meat and Poultry. Blackie Academic & Professional, London.

British Stainless Steel Association (BSSA). 2013. Selection of stainless steels for the food processing industries. Available at: http://www.bssa.org.uk/topics.php?article=45. Accessed 10 December 2012.

Bruhn, A.M. 2011. Biofilm barrier: a new ingredient available in a therapeutic mouthrinse can help patients improve their plaque control regimen. Sunstar Spotlight. September 2011:1-5.

Canadian Food Inspection Agency. 2012. Microbial control agents reviewed by Health Canada (HC) and listed for use on raw poultry carcasses or parts. Available at:

http://www.inspection.gc.ca/english/ anima/meavia/mmopmmhv/chap4/4.5e.shtml#4.5. Accessed December 2012.

Collaert, B., R. Attstrom, H. De Bruyn, R. Movert. 1992. The effect of delmopinol rinsing on dental plaque formation and gingivitis healing. Journal of Clinical Periodontology. 34:58-65.

Cox, J.M. and A. Pavic. 2009. Advances in enteropathogen control in poultry production. Journal of Applied Microbiology 108(3):745-755.

Edstrom Industries, Inc. 2013. Biofilm: The key to understanding and controlling bacterial growth in automated animal drinking water systems. Available at: http://www.edstrom.com/file.aspx?DocumentId=21. Accessed 10 December 2012.

EFSA. 2005. Opinion of the Scientific Panel on food additives, flavorings, processing aids and materials in contact with food (AFC) on a request from the Commission related to Treatment of poultry carcasses with chlorine dioxide, acidified sodium chlorite, trisodium phosphate and perooxyacids. The EFSA Journal. 297:1-27.

Eifert, J.D., and Sanglay, G.C. 2002. Chemistry of chlorine sanitizers in food processing. Dairy, Food and Environmental Sanitation. 22(7): 534-538.

Food and Drug Administration (FDA). 1998. In: Subcommittee report on cetylpyridinium chloride. Available at: http://www.fda.gov/. Accessed May 2013.

FSIS. 2010. Compliance Guideline for Controlling *Salmonella* and *Campylobacter* in Poultry, 3rd edition. Available at: http://www.fsis.usda.gov/PDF/Compliance_Guide_Controling_Salmonella_Campylobacter_Poultry_0510.pdf. Accessed April 2013.

Gunsolley, J.C. 2010. Clinical efficacy of antimicrobial mouthrinses. Journal of Dentistry. 38:S6-S10.

Giese, J. 1992. Experimental process reduces *Salmonella* on poultry. Food Technology. 46:112.

Haps, S., D.E. Slot, C.E. Berchier, G.A. Van der Weijden. 2008. The effect of cetylpyridinium chloride- containing mouth rinses as adjuncts to tooth brushing on plaque and parameters of gingival inflammation: a systematic review. International Journal of Dental Hygiene. 6(4):290-303.

Hernestam, S., B. Thelin, E. Seifert, A. Nilsson. 1993. Process for preparing delmopinol via the intermediates isoxazolidines and isoxazolines. United States Patent # 5212305. Available at: http://patft.uspto.gov/netahtml/PTO/index.html. Accessed 01 April 2013.

Jacobs-Reitsma W.F., U. Lyhs, J. Wagenaar. 2008. *Campylobacter* in the Food Supply, p. 627-644. In I. Nachamkin, C. M. Szymanski, and M. J. Blaser (ed.), Campylobacter, 3rd ed. American Society for Microbiology, Washington, DC.

Kim, J.W., M.F. Slavik. 1994. Trisodium phosphate (TSP) treatment of beef surfaces to reduce *Escherichia coli* O157:H7 and *Salmonella* Typhimurium. Journal of Food Science. 59:20-22.

Kim, J.W., M.F. Slavik. 1996. Cetylpyridinium chloride (CPC) treatment on poultry skin to reduce attached *Salmonella*. Journal of Food Protection. 59(3):322-6.

Lang, N.P., J.C. Hase, M. Grassi, C.H. Hämmerle, C. Weigel, E. Kelty, F. Frutig. 1998. Plaque formation and gingivitis after supervised mouthrinsing with 0.2% delmopinol hydrochloride, 0.2% chlorhexidine diguluconate and placebo for 6 months. Oral Disease. 4:105-113.

Lee, R. M., P. A. Hartman, D. G. Olsen, F. D. Williams. 1994. Metal ions reverse the inhibitory effects of selected food-grade phosphates in *Staphylococcus aureus*. Journal of Food Protection. 57:284-288.

Levin, Robert E. 2007. *Campylobacter jejuni*: A review of its characteristics, pathogenicity, ecology, distribution, subspecies, characterization and molecular methods of detection. Food Biotechnology. 21:271-347.

Luber, P., S. Brynestad, D. Topsch, K. Scherer, E. Bartelt. 2006. Quantification of *Campylobacter* species cross-contamination during handling of contaminated fresh chicken parts in kitchens. Applied and Environmental Microbiology. 72(1):66-70.

Marsh, P.D. 2010. Controlling the oral biofilm with antimicrobials. Journal of Dentistry. 38:S11-S15.

Mayette, D.C. 1992. The existence and significance of biofilms in water. Water Review, pp. 1-3, Water Quality Research Council, Lisle, IL.

Mendonca, A. F., T. L. Amoroso, S. J. Knabel. 1994. Destruction of gram-negative food-borne pathogens by high pH involves disruption of the cytoplasmic membrane. Applied and Environmental Microbiology. 60:4009-4014.

Nagelberg, R.H. 2013. Supplemental use of therapeutic mouthrinses. Available at: http://www.cdeworld.com/courses/4609. Accessed April 2013.

Naiara, M.Z., G.F. Elena, H. Barbara, I. Carolina. 2010. Efficacy of acidified sodium chlorite against *Campylobacter jejuni* attached to poultry skin during refrigerated

storage. Abstract from presentation at the XIIIth European Poultry Conference, Tours, France.

Netdoctor. 2013. Merocaine Lozenges. Available at: http://www.netdoctor.co.uk/aches-and-pains/medicines/merocaine-lozenges.html. Accessed 16 April 2013.

ROSS. 2013. Understanding surfactant and new methods of dispersing. Available at: http://www.mixers.com/whitepapers/dispersing%20surfactants.pdf. Accessed January 2013.

Russell, S.M. 2009. Chlorine: Still the most popular sanitizer in the poultry industry. University of Georgia: Poultry Tips. Available at: http://www.poultry.uga.edu/extension/tips/tipsbysubject.htm.

Salager, J. 2002. Surfactants: Types and Uses. Available at: http://www.nanoparticles.org/pdf/Salager-E300A.pdf. Accessed January 2013.

Silvia, J., D. Leite, M. Fernandes, C. Mena, P.A. Gibbs, and P. Teixeira. 2011. *Campylobacter* spp. as a foodborne pathogen: A review. Frontiers in Microbiology. 2:1-12.

Sjodin, T., J. Hakansson, B. Sparre, I. Ekman, M. Astrom. 2011. Pharmacokinetics and clinical efficacy of delmopinol in an open rinse time study in healthy volunteers. American Journal of Denistry. 24(6):383-8.

Simonsson, T., E.B. Hvid, J. Rundegren, S. Edwardsson. 1991. Effect of delmopinol on in vitro dental plaque formation, bacterial acid production and the number of microorganism in human saliva. Oral Microbiology and Immunology. 6: 305-9.

Sinclair Pharmaceuticals. 2008. Decapinol. Available at: http://www.decapinol.com/. Accessed April 2013.

Sunstar Americas Inc . 2013. GUM® PerioShield™ Oral Health Rinse. Available at: http://www.gumbrand.com/departments/accessories/gum-perioshield-oral-health-rinse/products/gum-perioshield-oral-health-rinse/. Accessed April 2013.

Svensson, O., T. Halthur, T. Sjodin, T. Arnebrant. 2010. Adsorption of delmopinol at the solid/liquid interface – the role of the acid-base equilibrium. Journal of Colloid and Interface Science. 350: 275-281.

U.S. Department of Agriculture (USDA). 1996. Use of trisodium phosphate on raw, chilled poultry carcasses, Final Rule. Federal Register. 61(146): 39273-39278.

U.S. Patent and Trademark Office (USPTO). 2012. Available at: http://patft.uspto.gov/netacgi/nph-Parser?d=PALL&p=1&u=%2Fnetahtml%2FPTO%2Fsrchnum.htm&r=1&f=G&l=50&s1=8143463.PN.&OS=PN/8143463&RS=PN/8143463. Accessed 22 April 2013.

U.S. Plastic Corp. 2008. What are the differences between HDPE, LDPE, XLPE, LLDPE, and UHMWPE? Available at: http://www.usplastic.com/knowledgebase/article.aspx?contentkey=508. Accessed 16 April 2013.

Vassilakos, N., T. Arnebrant, J. Rundegren. 1993. In vitro interaction of delmopinol hydrochloride with salivary films adsorbed at solid/liquid interfaces. Caries Research. 27:176-182.

Walters, R.M., G. Mao, E.T. Gunn, S. Hornby. 2012. Cleansing formulations that respect skin barrier integrity. Dermatology Research and Practice. 2012:1-9.

Wieczorek, K., R. Szewczyk, J. Osek. 2012. Prevalence, antimicrobial resistance, and molecular characterization of *Campylobacter jejuni* and *C. coli* isolated from retail raw meat in Poland. Veterinarni Medicina. 57:293-299.

Williams, A., O.A. Oyarzabal. 2012. Prevalence of *Campylobacter* spp. in skinless, boneless retail broiler meat from 2005 through 2011 in Alabama, USA. BMC Microbiology. 12:1-7.

Zhao, S., S.R. Young, E. Tong, J.W. Abbott, N. Womack, S.L. Freidman, P.F. McDermott. 2010. Antimicrobial resistance of *Campylobacter* isolates from retail meat in the United States between 2002 and 2007. Applied and Environmental Microbiology. 76:7949-56.

APPENDIX A

Experimental Design Grouping –

(Main groups include chicken, stainless steel, and HDPE. Two concentration replications were performed for each contact time, which helped determined the log reduction values.)