DISSERTATION

SURVIVAL AND INACTIVATION OF *LISTERIA MONOCYTOGENES*, SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*, AND MULTIDRUG-RESISTANT AND SUSCEPTIBLE *SALMONELLA* SEROVARS EXPOSED TO HEAT AND ANTIMICROBIALS ON FOOD CONTACT AND FOOD SURFACES

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ABSTRACT

SURVIVAL AND INACTIVATION OF *LISTERIA MONOCYTOGENES*, SHIGA TOXIN-PRODUCING *ESCHERICHIA COLI*, AND MULTIDRUG-RESISTANT AND SUSCEPTIBLE *SALMONELLA* SEROVARS EXPOSED TO HEAT AND ANTIMICROBIALS ON FOOD CONTACT AND FOOD SURFACES CONDITIONS

Compared to planktonic cells, bacterial biofilms are more resistant to sanitizing agents, causing crucial challenges for their inactivation in various food environments. The first study of this dissertation investigated biofilm formation of seven pathogenic *Escherichia coli* serogroups (i.e., O157, O26, O45, O103, O111, O121, O145) and two or three phenotypes of *Salmonella* Newport and *S.* Typhimurium (i.e., susceptible, multidrug-resistant [MDR], and/or multidrug-resistant with acquired *ampC* gene [MDR-AmpC]). One-week mature biofilms were also exposed to water and to two commercially available quaternary ammonium compound-based (QAC) and acid-based (AB) sanitizers. Specifically, seven strain mixtures of the above-mentioned pathogen groups were separately spot-inoculated onto the surface of stainless steel coupons for target inoculation of 2 log CFU/cm^2. Coupons were then stored statically and partially submerged in non-sterilized meat (10% w/v) homogenate at 4, 15, and 25°C. Microbial counts on days 0, 1, 4, and 7 and survivors after exposure (submersion in 30 ml for 1 min) to water, QAC and AB of one-week mature biofilms were enumerated using selective and non-selective media. At 4°C, pathogen counts on inoculation day ranged from 1.6±0.4 log CFU/cm^2 to 2.4±0.6 and changed to 1.2±0.8 to 1.9±0.8 on day-7 with no appreciable differences among the pathogen groups. After treatment with QAC and AB on day-7, counts were reduced (P<0.05) to less than 0.7±0.6 and 1.2±0.5, respectively, with similar trends among the inoculated pathogen groups. Biofilm formation at higher temperatures was more enhanced. *E. coli* O157:H7, as an

example, increased (P<0.05) from 1.4±0.6 and 2.0±0.3 on day-0 to 4.8±0.6 and 6.5±0.2 on day-7, at 15 and 25°C, respectively. As compared to 4°C, after sanitation, more survivors were observed for 15 and 25°C treatments with no appreciable differences among the seven pathogen groups. Overall, similar patterns of growth and susceptibility to QAC and AB sanitizers were observed for the seven pathogen groups.

The second study of this dissertation compared the lactic acid resistance of individual strains of six serogroups of wild-type and rifampicin resistant non-O157 shiga toxin-producing *Escherichia coli* and susceptible, multidrug-resistant (MDR), and/or MDR with acquired *ampC* gene (MDR-AmpC) *Salmonella* Newport and Typhimurium against *E. coli* O157:H7 (5-strain mixture). After inoculation (6 log CFU/ml) of a sterile 10% (w/w) beef homogenate (28 ml homogenate, 0.3 ml inoculum), lactic acid (1.7 ml, 88%) was added for a target concentration of 5%. Before acid addition (control), a 2 seconds after acid addition (time-0), and 2, 4, 6 and 8 min after addition of acid, aliquots were analyzed (two repetitions, three acid challenges per strain/mixture) for survivors. Inoculated counts (6.1±0.1 log CFU/ml) of wild-type *E. coli* O157:H7 mixture were reduced (P<0.05) to 1.1±0.2 by the end of the 8-min acid challenge, while the rifampicin-resistant *E. coli* O157:H7 mixture, inoculated at 5.9±0.2, reached the detection limit (<1.0 log CFU/ml) after 6 min of exposure. Of the wild-type non-O157 and of the rifampicin-resistant variant strains, irrespective of serogroup, 85.7% (30 out of 35 strains) and 82.9% (29 out of 35) reached the detection limit within 0 to 6 min of exposure, respectively. Of the *Salmonella* strains, 87.9% (29 out of 33 isolates) reached the detection limit within 0 to 4 min of exposure, irrespective of serovar or antibiotic resistance phenotype. Results of non-log-linear microbial survivor curve analyses indicated that the non-O157 *E. coli* serogroups and multidrug-resistant and susceptible *Salmonella* strains required less time for a 4D reduction compared to *E.*

coli O157:H7. Overall, the results of this acid challenge showed, for nearly all strains and time intervals, that individual strains of wild-type and rifampicin-resistant non-O157 *E. coli* and of *S.* Newport and Typhimurium were less ($P<0.05$) acid tolerant than the *E. coli* O157:H7. The vast majority of non-O157 *E. coli* strains showed similar ($P\geq0.05$) acid tolerance and also the majority of drug resistant and susceptible *Salmonella* strains were similarly ($P\geq0.05$) acid tolerant.

The third study of this dissertation investigated decontamination of beef trimming inoculated with shiga toxin-producing *Escherichia coli* and *Salmonella* using lactic acid (LA). The efficacy of LA was compared against (i) six non-O157 Shiga toxin-producing *E. coli* (nSTEC) serogroups and (ii) antibiotic susceptible and multidrug resistant *S.* Newport and *S.* Typhimurium serovars. The antimicrobial effects against these pathogens were compared to those obtained against *E. coli* O157:H7. Four-strain mixture inocula of rifampicin-resistant *E. coli* O157:H7, O26, O45, O103, O111, O121 and O145, and antibiotic susceptible and multidrug resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium were evaluated on beef trimmings (100-g pieces). The inoculated (3 log CFU/cm^2) trimmings were immersed (30 s) in solutions of LA (5%, 25 or 55°C). Pathogen populations on untreated and treated samples were enumerated (two or three repetitions, three samples each), and data were analyzed as a complete randomized block design. Initial levels (3.1-3.3 log CFU/cm^2) of *E. coli* O157:H7 and nSTEC serogroups were reduced ($P<0.05$) by 0.7 (*E. coli* O157:H7) and 0.4-0.9 (nSTEC) log CFU/cm^2 in 25°C LA-treated samples, and 1.4 (*E. coli* O157:H7) and 1.0-1.3 (nSTEC) log CFU/cm^2 in 55°C LA-treated samples. No differences ($P\geq0.05$) were obtained between surviving counts of the six nSTEC serogroups and those of *E. coli* O157:H7. LA at 25°C and 55°C reduced ($P<0.05$) *Salmonella* counts (3.0-3.3 log CFU/ cm^2) by 1.2-1.5 and 1.5-1.9 log CFU/cm^2, respectively, while

corresponding *E. coli* O157:H7 reductions were 0.5 and 1.2 log CFU/cm^2, respectively. Reductions of *Salmonella* counts were not influenced by serovar or antibiotic resistance phenotype, and were similar ($P \geq 0.05$) or higher ($P < 0.05$) than reductions of *E. coli* O157:H7. The results indicated that LA decontamination of beef trimmings can be as effective against the six nSTEC serogroups and antibiotic susceptible and multidrug resistant *S.* Newport and *S.* Typhimurium as it is against *E. coli* O157:H7.

The fourth study of this dissertation was conducted with the objective of evaluating survival and multiplication of *L. monocytogenes* inoculated on cooked chicken breasts which were stored aerobically at 7°C for 7 days. Reduction of pathogen cells by microwave, domestic oven, and stove top reheating was also evaluated. *L. monocytogenes* populations increased from 3.7±0.1 to 7.8±0.2 log CFU/g after 7 days. Microwave oven reheating for 90 s, and stove-top and oven-reheating to 70°C internal temperature reduced pathogen populations to <0.4-2.6, <0.4-4.8, and 1.4-5.9 log CFU/g, respectively; numbers of survivors after reheating were higher ($P < 0.05$) in products stored for increasing length of time up to 7 days. At shorter microwaving times and lower product internal temperatures (stove-top and oven-reheating), similar reduction trends were observed but with higher levels of survivors after treatment. Although reheating methods in this study reduced *L. monocytogenes* contamination by 2-5 log CFU/g, growth of the pathogen during previous storage allowed high numbers of survivors after reheating, especially after 2 days of storage. This indicates that storage period, and type and intensity of reheating need to be considered for safe consumption of leftovers.

The last study of this dissertation was designed to evaluate growth of *L. monocytogenes* inoculated on cooked chicken meat with different marinades and survival of the pathogen as affected by microwave oven reheating during aerobic storage at 7°C. Raw chicken breast meat

samples were treated with three commercially-formulated and three domestically-available marinades, and then cooked (74.4°C internal temperature), cooled to 4°C, and surface-inoculated with *L. monocytogenes.* During storage at 7°C, on days 0, 1, 2, 4, and 7, samples were reheated by microwave oven (1100 W) for 45 or 90 s and analyzed microbiologically. *L. monocytogenes* counts on non-marinated control samples increased ($P<0.05$) from 2.7±0.1 to 6.9±0.1 log CFU/g during storage. At day-7 of storage, pathogen levels on samples marinated with tomato juice were not different ($P\geq0.05$; 6.9±0.1 log CFU/g) from those of the control, whereas for samples treated with the remaining marinades, pathogen counts were 0.7 to 2.0 log CFU/g lower ($P<0.05$) than those of control samples. Microwave reheating reduced *L. monocytogenes* by 1.9 to 4.1 (at 45 s) and 2.1 to 5.0 (at 90 s) log CFU/g. With similar trends across different marinates, the high levels of *L. monocytogenes* survivors found after microwave reheating, especially after two days of storage, indicate that length of storage and reheating time need to be considered for safe consumption of leftover cooked chicken.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

With advancements of molecular subtyping methods and available databases in North America, such as *FoodNet* and *PulseNet,* few cases of foodborne illnesses, hundreds of miles apart, can be associated with each other and the food source within days, preventing additional spread of illness. From a practical standpoint, contamination of food during commercial production, transportation, and processing is unavoidable; however, advancements in food microbiology in the last century have resulted in major public health improvements and reduction in economical losses due to spoilage microorganisms and foodborne illness episodes by pathogenic microorganisms (*Davidson et al., 2005*).

Among more than 200 known diseases that could potentially be transmitted through food, few pathogens are of major concern in commercial food processing. Earlier estimates considered *Campylobacter*, *Salmonella* serovars, *Escherichia coli* O157:H7, *Listeria monocytogenes* as major agents responsible for foodborne illnesses with over 75 million cases and 300,000 hospitalizations, and more than 5,000 deaths per year in the United States due to known (75%) and unknown (25%) pathogens (*Mead et al., 1999*). More recent epidemiological investigations, in 2011, estimated foodborne agents to cause 9.4 million illness episodes, about 56,000 hospitalizations, and 1,351 deaths per year with Norovirus, *Salmonella* serovars, *Clostridium perfringens* and *Campylobacter* as leading causes of foodborne illness, *Salmonella* serovars, Norovirus, *Campylobacter* spp. and *Toxoplasma gondii* as leading causes of hospitalizations and *Salmonella* serovars, *T. gondii*, *L. monocytogenes* and Norovirus as leading causes of foodborne deaths in the U.S. (*Scallan et al., 2011a; 2011b; Morris, 2011*).

Among the many pathogens that can survive, multiply, and proliferate on meat products, members of *Enterobacteriaceae* are of major concern due to their ability to survive in a wide array of environmental conditions and their potential pathogenicity to humans (*Jay, 2005*). Among members of this family, *E. coli* O157:H7, other shiga toxin-producing *E. coli*, and *Salmonella* serovars are major concerns in primary processing of meat products. Due to the ubiquitous and halophilic nature as well as its ability to proliferate at refrigerated temperatures, *L. monocytogenes* is considered as a major concern for further processed, value-added, and ready-to-eat meat products (*Juneja and Sofos, 2010*).

As one of the most researched pathogens, inactivation of *Salmonella* by various interventions has been the subject of research for more than 100 years (*FDA 2009, 2010*). More than 30 salmonellosis outbreaks in the United States and around the world have been associated with fresh meat as well as processed low-moisture food products in the last decade (*FDA, 2009*). In addition to *Salmonella* serovars, emergence of multiple drug resistant *Salmonella* strains in the food chain has been of a great concern and subject of recent investigations (*Bosilevac et al., 2009; Arthur et al., 2008*).

L. monocytogenes is widely distributed in the environment. Although rare in occurrence, as compared to other major foodborne diseases, listeriosis is a severe illness, being responsible for 3.8 % of foodborne hospitalizations and 27.4% of foodborne disease deaths in the United States. The very young, elderly, pregnant women, and the immunocompromized are among the most susceptible groups. Due to the presence of *L. monocytogenes* in a wide array of environments, its halophilic nature, its potential to form biofilms, and its ability to survive and multiply at refrigeration temperatures, it has been of special interest in academic and industrial research in ready-to-eat food products (*Sofos and Geornaras, 2010*).

Most *Escherichia coli* strains are not capable of causing foodborne illness in humans. However, Enterohemorrhagic *E. coli* has the potential of secreting shiga toxins, and cause Hemolytic Uremic Syndrome (HUS) in infected human host (*Wells et al., 2009; Elder et al., 2000*). The O157:H7 serotype as well as six non-O157 Shiga toxin-producing *E. coli* (O26, O45, O103, O111, O121, and O145 serotypes) are responsible for the majority of *E. coli* related foodborne illnesses in the United States (*Juneja and Sofos, 2010*).

This dissertation examined control of significant foodborne pathogens in food processing. The first study of this dissertation investigated biofilm formation and inactivation by sanitizers of foodborne pathogenic *E. coli* and *Salmonella* serovars. The second and third studies were designed to investigate the lactic acid resistance of individual strains as well as mixtures of non-O157 STEC strains and serogroups, and resistant and susceptible *Salmonella* serovars as compared to *E. coli* O157:H7 in a meat homogenate and beef trimmings. The objective of these investigations was to determine whether currently implemented lactic acid interventions in primary processing of red meat, known to be efficient against *E. coli* O157:H7, are also capable of reducing and/or eliminating non-O157 STEC, and antibiotic resistant and susceptible *Salmonella* serovars. The last two studies of this dissertation investigated reheating as an intervention and the antimicrobial properties of marinating ingredients against post-cooking inoculated *L. monocytogenes* to assure the safety of leftover cooked chicken meat during refrigerated storage.

CHAPTER II

REVIEW OF LITERATURE

Shiga toxin-producing *Escherichia coli,* and antibiotic resistant and susceptible *Salmonella* Typhimurium and Newport

Among the pathogens that can survive, multiply, and proliferate on the surface of meat products, some members of *Enterobacteriaceae* are of major concern due to potential pathogenicity to humans (Jay, 2005). *Escherichia coli* O157:H7, other shiga toxin-producing *E. coli*, and *Salmonella* serovars are major concerns in primary processing of meat products (Wells *et al.,* 2009).

Most *E. coli* serotypes are not capable of causing severe foodborne illness in humans. However, enterohemorrhagic *E. coli* have the potential of secreting shiga toxins, and cause Hemolytic Uremic Syndrome (HUS) in infected human hosts (Wells *et al.,* 2009; Elder *et al.,* 2000). After the multi-State Pacific Northwest outbreak of *E. coli* O157:H7 (Bell *et al.,* 1994) this serotype became the first microbial agent to be declared as an adulterant in food products by the U.S. Food Safety Inspection Service (FSIS, 1999). Along with serotype O157:H7, six non-O157 Shiga toxin producing *E. coli* (O26, O45, O103, O111, O121, and O145) serotypes are responsible for the majority of *E. coli* related foodborne illnesses in the United States (Johannes and Romer, 2010).

E. coli naturally reside in the lower intestine of many warm-blooded animal species and can benefit the host through protecting the gastrointestinal area against pathogenic organisms as well as producing absorbable vitamin K (Jay, 2005). Most *E. coli* are not capable of causing health

complications in human through oral ingestion and are not involved in foodborne illness episodes, however some subgroups of this organism are capable of causing health complications in humans. Enterohemorrhagic *E. coli* (EHEC) strains, are capable of producing shiga toxins that are responsible for causing Hemolytic Uremic Syndrome (HUS). HUS is characterized by hemolytic anemia and acute renal failure and is primarily a concern in children, the elderly, and individuals with suppressed immune system (Ahn *et al.,* 2009; Jahanner and Romer, 2010).

After outbreaks of gastrointestinal illnesses in 1983 (Bell *et al.*, 1994), *E. coli* O157:H7 was first recognized as a pathogenic agent capable of causing HUS. After major outbreaks, including a multistate Pacific Northwest outbreak of *E. coli* O157:H7 in 1992-1993 (Bell *et al.*, 1994), and an outbreak of ground beef and beef trimming contaminated with *E. coli* O157:H7 in August 1997 and July 2002, this pathogen became the first microbial agent to be declared as an adulterant in ground beef by the U.S. Food Safety Inspection Service (FSIS, 1999). Emergence of this pathogen has led to much advancement in food regulation and production systems including mandatory Hazards Analysis Critical Control Point (HACCP) based food safety management systems for meat processors (Sofos, 2009; Oliver *et al*., 2009). Most recent epidemiological investigations estimate every year in the United States *E. coli* O157:H7 is responsible for 3,268 illness episodes with 46.2% and 0.5% hospitalization and death rates, respectively (Scallan, *et al*., 2011).

Along with *E. coli* O157:H7, in recent years other serotypes of EHEC have been involved in foodborne episodes. A recall of ground beef associated with *E. coli* O26 by a Pennsylvania company (USDA-FSIS, 2011), a recall of ground beef contaminated with *E. coli* O111 in Japan (USDA-FSIS, 2011), and an outbreak of beef sausage contaminated with *E. coli* O26 in Denmark (Ethelberg *et al.*, 2009) are some of the most recent examples. *E. coli* O26, O45, O103,

O111, O121, and O145 serotypes are responsible for the majority of episodes of foodborne illness and HUS, associated with non-O157 shiga toxin-producing *E. coli* in the United States (Bethelheim, 2007; Mathusa *et al.*,2010; Grant *et al.*, 2011). It is estimated that 1,579 illness episodes, with a 12.8% hospitalization rate and a 0.3% death rate are caused by non-O157 STEC every year (Scallan *et al*., 2011).

As one of the most researched pathogens, inactivation of *Salmonella* by various interventions has been the subject of research for more than 100 years (FDA 2009, 2010). More than 30 salmonellosis outbreaks in the United States and around the world have been associated with fresh meat as well as processed low-moisture food products in the last decade (FDA, 2009). In addition to *Salmonella* serovars, emergence of multiple drug resistant *Salmonella* in the food chain has been of a great concern and of recent investigations (Bosilevac *et al.*, 2009; Arthur *et al.*, 2008).

Due to prevalence of shiga toxin-producing *E. coli* and antibiotic susceptible and resistant *Salmonella* serovars (Barkocy-Gallagher *et al*., 2003; Bosilevac *et al.*, 2009), these pathogens have been the subject of recent investigations (Sofos, 2009; Oliver *et al*., 2009). Recent studies indicate that MDR *Salmonella* is reduced at least as effectively as *E. coli* O157:H7 and susceptible *Salmonella* when treated with antimicrobial interventions currently in use in U.S. beef processing plants (Arthur *et al*., 2008).

Foodborne episodes with STEC in meat

Consumption of undercooked ground beef, roast beef, smoked meat products, sausages and nonintact steaks contaminated with O157 and non-O157 serotypes of *E. coli* has caused a large number of reported outbreaks and sporadic cases of human illness (Hussein, 2007). *E. coli*

related foodborne illness includes a range from mild diarrhea, abdominal pain, vomiting, and bloody diarrhea to hemorrhagic colitis (HC) and HUS (Nataro and Kaper, 1998). Although rare in occurrence, HUS can affect infected host, such as infants, children and the elderly, and is characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure (Hussein, 2007). From 1984 to 2007, 146 STEC outbreaks and sporadic cases of human illness have been traced to consumption of beef contaminated with O157 and non-O157 serotypes of *E. coli*, with 88% of the cases traced back to ground and nonintact beef (Hussein and Bollinger, 2005a).

Cattle as reservoir of STEC

Although STEC serotypes have been isolated from a variety of animals (Beutin *et al*., 1993; 1995), they have been associated primarily with ruminants (Hussein, 2007). *E. coli*-related human illnesses have been traced primarily to beef or other edible parts of cattle and food products contaminated with cattle manure (Wilson *et al*., 1992; Bielaszewska *et al*., 2000). STEC serotypes have been isolated from bulls, cows, heifers, steers, and calves (Cizek *et al*., 1999; Hussein *et al*., 2003; Ezawa *et al*., 2004) as well as feedlot cattle and cattle in grazing conditions (Padola *et al*., 2004).

Prevalence of STEC in cattle

Prevalence of STEC serotypes in feed yards and grazing conditions, and human related foodborne illness cases, have been shown to be higher in warmer months (Hussein, 2007). In general, it is estimated that 0.3 to 19.7% of feedlot cattle, 0.7 to 27.3% in irrigated pasture, and 0.9 to 6.9% of cattle in rangeland forages carry STEC serotypes in their gastrointestinal area. At slaughtering facilities, prevalence of O157 STEC serotypes ranges from 0.2 to 27.8% (Hussein

and Bollinger, 2005a). In an one year study in three beef processing plants (Barkocy-Gallagher *et al*., 2003) it was shown that 60.6% and 56.6% of cattle hides, 5.9% and 19.4% of cattle manure samples, and 26.7% and 58.0% of carcasses were contaminated with O157 and non-O157 STECS, respectively.

Prevalence of STEC in beef

It is estimated that 0.1 to 43.0% of carcasses in packing plants, 0.01 to 43.3% of sub-primal cuts in packing plants, 0.1 to 4.4% of meat cuts in supermarkets, an average of 2.4% meat products used in fast food chains, 0.1 to 54.2% of ground beef, and 0.1 to 4.4% of sausage samples in supermarkets can be contaminated with O157 STEC (Hussein and Bollinger, 2005b). Similarly, 1.7 to 58.0% of sub-primal cuts in packing plants, 3.0 to 62.5% in supermarkets, an average of 3.0% of meat products used in fast food chains, 2.4 to 30.0% of ground beef, 17.0 to 49.2% of sausage samples, and 1.7 to 58.0% of carcasses in packing plants were contaminated with non-O157 STEC (Hussein and Bollinger, 2005b).

Control of STEC in beef

It is estimated that non-O157 STEC are more common in beef products than O157 STEC serotypes (Grant *et al*., 2011). Although some studies have indicated that non-O157 STEC are no more resistant than O157 STEC serotypes in response to common commercial processing procedures in beef processing, it appears that more investigation is needed for control of non-O157 STEC serotypes on carcasses, meat cuts, and meat products (Johnson *et al*., 2006; Bettelheim, 2007; Gyles, 2007).

Biofilm decontamination

In addition to planktonic cells, biofilm communities of pathogens are of significant importance to the food industry. Bacterial biofilms were first described in 1943 (Zobell, 1943) and currently are considered as a major challenge in sanitation and hygiene at food processing (Simoes *et al.*, 2010; Shi and Zhu, 2009; Chmielewski and Frank, 2003). Biofilms are a community of viable and noviable cells of a single or a collection of species anchored to a surface and covered with a layer of extracellular polymeric substances that makes biofilms potentially resistant to cleaning and sanitation practices in food processing areas (Shi and Zhu, 2009; Chmielewski and Frank, 2003). Improper cleaning and sanitation of food contact surfaces has been a contributing factor to many foodborne illness episodes (Shi and Zhu, 2009; Chmielewski and Frank, 2003).

***Listeria monocytogenes* and its inactivation by reheating interventions and marination**

Among more than 200 known diseases that could potentially be transmitted through food routes, few pathogens are of major concern in food processing. Previous estimates were considering *Campylobacter*, *Salmonella* serovars, *E. coli* O157:H7, *L. monocytogenes* as major agents responsible for foodborne illnesses with over 75 million illness and 300,000 hospitalizations, and more than 5,000 deaths per year in the United States due to known (75%) and unknown (25%) pathogens (Mead *et al*., 1999). More recent epidemiological investigations in 2011 revealed similar trends for foodborne illness with 9.4 million episodes of food safety illness, about 56,000 hospitalizations, and 1,351 deaths per year, with Norovirus, *Salmonella* serovars, *Clostridium perfringens* and *Campylobacter* as leading causes of foodborne illness, *Salmonella* serovars, Norovirus, *Campylobacter* and *Toxoplasma gondii* are leading causes of hospitalizations, and *Salmonella* serovars, *T. gondii*, *L. monocytogenes* and Norovirus leading

causes of foodborne deaths in the U.S. (Scallan *et al*., 2011a; 2011b; Morris, 2011). Due to its ubiquitous and halophilic nature and its ability to proliferate at refrigerated temperatures, *L. monocytogenes* is considered as a major concern for further processed, value-added, and ready-to-eat meat products (Juneja and Sofos, 2010).

Similar to ready-to-eat products, cooked leftover food, both in the domestic environment and at commercial establishments, has reduced levels of background microflora and in case of cross-contamination during refrigerated storage may harbor and support growth of foodborne pathogens (*Murphy and Berrang, 2002; Murphy et al., 2001; 2003*), especially psychotropic pathogens such as *L. monocytogenes* and *Yersinia enterocolitica* (*Jay, 2005*).

Although generally foodborne diseases are linked to food consumed outside the home (*Kennedy et al., 2005*), epidemiological investigations indicate that poor hygienic practices in the domestic environment may also be responsible for foodborne disease episodes (*Redmond et al., 2003*). Infrequent hand washing, poor hand-washing technique, lack of hand-washing prior to food preparation, inadequate cleaning of kitchen surfaces, involvement of pets in the kitchen, touching of face, mouth, nose and/or hair during preparation of food (*Jay et al., 1999*), as well as improper storage, and inadequate cooking and reheating (*James and Evans 1992; Griffith et al., 1994*) are some of the practices that could potentially result in introduction of pathogens of public health concern into food products, and allow their survival and multiplication to levels of concern. In recent years, various studies have detected foodborne pathogens, including *L. monocytogenes*, on kitchen surfaces, dish cloths, sinks, drains, and refrigerators (*Jackson et al., 2007; Azevedo et al., 2003; Kilonzo-Nthenge et al., 2008*). These scenarios may also happen in commercial catering establishments. It has been shown that the microbiological profile of cooked

chicken stored in refrigerators is very similar to microbial profiles of swabs taken from the kitchen environments (*Toule and Mutphy, 1978*).

L. monocytogenes is widely distributed in the environment including the domestic environment and commercial food preparation areas as well as different processed and raw food products (*Gandhi and Chikindas, 2007*). Although rare in occurrence, as compared to other major foodborne diseases, listeriosis caused by *L. monocytogenes* is an important severe illness, being responsible for 3.8 % of foodborne hospitalizations and 27.4% of foodborne disease deaths in the United States (*Ryser and Marth, 2007*). The very young, elderly, pregnant women and the immunocompromized are the most susceptible groups (*Ryser and Marth, 2007*). Due to the presence of *L. monocytogenes* in a wide array of environments, its halophilic nature, its potential to form biofilms, and its ability to survive and multiply at refrigeration temperatures it has been of special interest in academic and industrial research in ready-to-eat food products *(Sofos and Georgnaras, 2010; Sofos 2008; 2009*).

L. monocytogenes has been isolated both from domestic and industrial refrigerated areas as well foods stored under refrigeration conditions (*Sergelidis et al., 2007; Azevedo et al., 2003; Walker et al., 1990*). The multiplication ability of *L. monocytogenes* inoculated on cooked food has been explained by increased hydrolysis of macromolecules and bioavailability of nutrients on the surface of cooked chicken as a result of cooking (*Damodaran et al., 2008*).

The effectiveness of microwave treatments against surface inoculated *L. monocytogenes* has been investigated in some recent studies. Rodriguez-Marval *et al*. (*2009*) demonstrated that inoculated *L. monocytogenes* on surface of frankfurters could be reduced by 3.7 log CFU/g after

75 s of microwaving. Similarly, Shen *et al*. *(2009*) showed that 30 s of microwaving resulted in 0.8-1.3 log CFU/g reduction of inoculated *L. monocytogenes.*

For investigating the effects of the stove top reheating method, Adekunle *et al.* (*2009*) have shown that pan-frying methods during storage of pork scrapple resulted in more than 6 log CFU/g reduction of inoculated *L. monocytogenes.* Previous studies have also investigated effectiveness of different reheating instruments. Different pathogen reduction potential of various instruments have been explained by different heat transfer principles wherein oven treatment, convection and in stove top treatment conduction are the primary modes of heat transfer (*Adler et al., 2009*).

In addition to adding value and improving palatability, marinating ingredients may provide improvement in shelf life through their antimicrobial properties. Due to presence of *L. monocytogenes* in a wide array of environments, its halophilic nature, the potential to form biofilms, and its ability to survive and multiply at refrigeration temperature, this pathogen has been linked to various recent outbreaks and has been isolated from many food processing and domestic environments (*Jay, 2005*).

While some marinating formulations have been shown to have little to no effects on reducing microbial load of poultry products during storage (Perko-Makela *et al.*, 2000), some studies suggest marinating ingredients can reduce foodborne pathogens after application and during storage. Bjorkroth *et al.* (2000) showed tomato-based marination can inhibit the growth of lactic acid bacteria associated with gaseous spoilage of modified-atmosphere-packaged raw chicken meat. In two similar studies, Pathania *et al.* (2010a, 2010b) showed teriyaki and lemon

pepper marinates could both reduce the *Salmonella* load on chicken skin and red meat during aerobic storage.

Objectives of this dissertation are:

- Compare biofilm formation of *E. coli* O157:H7, a mixture of all six *E. coli* O26, O45, O103, O111, O121, and O145, and two or three phenotypes (drug susceptible, MDR, and MDR-AmpC) of *S.* Typhimurium and S. Newport at temperatures of 4, 15, and 25°C.
- Decontamination of one-week mature biofilms of the above-mentioned pathogens using water and quaternary ammonium compound-based and acid-based sanitizers.
- Compare the lactic acid resistance of individual strains of wild-type and spontaneous rifampicin-resistant variants of six STEC serogroups, O26, O45, O103, O111, O121 and O145, and antibiotic susceptible and multidrug resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium, to that of a wild-type and rifampicin-resistant variants of 5 strains of *E. coli* O157:H7 tested as a mixture.
- Determine whether lactic acid resistance of non-O157 STEC as well as multidrug resistant and susceptible *Salmonella* is different than *E. coli* O157:H7 inoculated on beef trimming.
- Investigate survival and multiplication of background microflora and inoculated *L. monocytogenes* on cooked boneless skinless chicken breasts stored aerobically at 7 °C and investigate the effects of three reheating methods, applied at 0, 1, 2, 4, and 7 days of storage, against the pathogen and background microflora of the inoculated chicken meat.
- Evaluate the effect of three commercially formulated (tomato-based, soy sauce-based, and lemon based), and three domestically available (tomato juice, soy sauce, and lemon

juice) marinades against post-cooking inoculated *L. monocytogenes* on chicken breasts, during a 7-day aerobic storage period at 7°C.

- Investigate pathogen survival after microwave oven reheating of cooked and stored marinated chicken.

CHAPTER III

Biofilm formation by O157 and non-O157 Shiga toxin-producing *Escherichia coli* and multidrug-resistant and susceptible *Salmonella* Typhimurium and Newport at 4, 15, and 25°C and their inactivation by acid- and quaternary ammonium compound-based sanitizers

SUMMARY

Compared to planktonic cells, bacterial biofilms are more resistant to sanitizing agents, causing crucial challenges for their inactivation on various food environments. This study compared biofilm formation by seven serogroups of *Escherichia coli* (i.e., O157, O26, O45, O103, O111, O121, O145) and two or three phenotypes of *Salmonella* Newport and *S.* Typhimurium (i.e., susceptible, multidrug-resistant [MDR], and/or multidrug-resistant with acquired *ampC* gene [MDR-AmpC]). One-week mature biofilms were also exposed to water and two commercially available quaternary ammonium compound-based (QAC) and acid-based (AB) sanitizers. Seven strain mixtures of the above-mentioned pathogen groups were separately spot-inoculated onto the surface of stainless steel coupons for target inoculation of 2 log CFU/cm^2. Coupons were then stored statically, partially submerged in 10% (w/v) non-sterilized meat homogenate at 4, 15, and 25°C. Microbial counts on days 0, 1, 4, and 7 and survivors after exposure (submersion in 30 ml for 1 min) to water, QAC and AB of one-week mature biofilms were enumerated using selective and non-selective media. At 4°C, pathogen counts on inoculation day ranged from 1.6±0.4 to 2.4±0.6 log CFU/cm^2 and changed to 1.2±0.8 to 1.9±0.8 on day-7 with no appreciable difference among the pathogen groups. After treatment with QAC

and AB on day-7, counts were reduced (P<0.05) to less than 0.7±0.6 and 1.2±0.5, respectively, with similar trends among the inoculated pathogen counts. Biofilm formation was more enhanced at higher temperatures. *E. coli* O157:H7, as an example, increased (P<0.05) from 1.4±0.6 and 2.0±0.3 on day-0 to 4.8±0.6 and 6.5±0.2 on day-7 at 15, and 25°C, respectively. As compared to 4°C, after sanitation, more survivors were observed for 15 and 25°C treatments with no appreciable differences among the seven pathogen groups. MDR-AmpC *S.* Newport, as an example, were reduced to <1.6±1.3 and 1.9±0.9 after exposure to QAC and AB at 15°C, while at 25°C they were reduced to 5.5±0.5 and 6.0±0.4, respectively. Overall it was observed that patterns of growth and susceptibility to QAC and AB sanitizers were similar among the seven tested pathogens, with enhanced biofilm formation capability and high numbers of survivors at higher temperatures.

INTRODUCTION

Foodborne pathogenic *E. coli* and *Salmonella* serovars are of major concern in primary and further processing of foods (Ahn *et al.,* 2009; Jahanner and Romer, 2010). Recent epidemiological investigations estimate that every year in the United States *E. coli* O157:H7 is responsible for 3,268 illness episodes with 46.2% and 0.5% hospitalization and death rates, respectively (Scallan *et al.,* 2011). Along with *E. coli* O157:H7, in recent years other serogroups of shiga toxin-producing *E. coli* (STEC) have been involved in foodborne episodes. They include *E. coli* O26, O45, O103, O111, O121, and O145 serogroups which are responsible for the majority of episodes of non-O157 STEC related foodborne illnesses in the United States (Bettelheim, 2007; Mathusa *et al.,* 2010; Grant *et al.,* 2011). It is estimated that 1,579 illness episodes, with a 12.8% hospitalization rate and a 0.3% death rate are caused by non-O157 STEC every year (Scallan *et al.,* 2011).

Pathogenic *Salmonella* (nontyphoidal) is the leading cause of foodborne deaths in the United States, being responsible for 1,229,007 illness episodes, with a 27.2% hospitalization rate and a 0.5% death rate (Scallan *et al.,* 2011). In addition, *Salmonella*, multidrug resistant (MDR) and MDR with acquired *ampC* gene (MDR-AmpC) *Salmonella* serovars in the food chain cause challenges in antibiotic treatment of salmonellosis in hospitals (Bosilevac *et al.*, 2009; Arthur, *et al.,* 2004; Boyle *et al.*, 2007; Anonymous, 2012). Recently it has been shown that as high as 0.6% of ground meat samples may harbor drug-resistant *Salmonella* (Bosilevac *et al.*, 2009) with approximately 7% of them displaying the MDR-AmpC phenotype (Zhao *et al.,* 2009).

Bacterial biofilms were first described in 1943 (Zobell, 1943) and currently are considered as a major challenge in sanitation and hygiene of food processing (Simoes *et al.,* 2010; Shi and Zhu, 2009; Chmielewski and Frank, 2003). Biofilms are a community of viable and noviable cells of a single or a collection of species anchored to a surface and covered with a layer of extracellular polymeric substances that makes biofilms potentially resistant to cleaning and sanitation practices in food processing areas (Shi and Zhu, 2009; Chmielewski and Frank, 2003). Improper cleaning and sanitation of food contact surfaces has been a contributing factor to many foodborne illness episodes (Shi and Zhu, 2009; Chmielewski and Frank, 2003).

Quaternary ammonium compound- and acid- based sanitizers are among the most common sanitizers used in food processing (Hegstad *et al.,* 2012; Farrell *et al.,* 1998) and have been extensively investigated against foodborne pathogens (Fratamico *et al.,* 1996; Farrell *et al.,* 1998; Sundheim *et al.,*1998; Joseph *et al.,*2001; Ellebracht *et al.,* 2005; Uhlich *et al.,* 2006).

Although some recent studies have targeted inactivation of *E. coli* O157:H7 and *Salmonella* biofilms on food processing surfaces (Hood and Zottola, 1997; Stopforth *et al.,* 2003; Silagyi *et*

al., 2009; Bodur and Cagri-Mehmetoglu, 2012; Beauchamp *et al.,* 2012; Wang *et al.,* 2012), only limited work is available comparing biofilm formation and inactivation of pathogenic *E. coli* and *Salmonella* at various temperatures. In addition, due to involvement of *E. coli* O157:H7 in numerous national and international foodborne episodes in the last few decades, this pathogen has been the primary target for control in food processing environments (Sofos, 2009; Oliver *et al*., 2009) and thus limited literature is available on biofilm formation and inactivation of emerging non-O157 serotypes of shiga toxin-producing *E. coli* and multidrug resistant *Salmonella* as compared to *E. coli* O157:H7.

This study was a comparative investigation of biofilm formation of *E. coli* O157:H7, a mixture of all six *E. coli* O26, O45, O103, O111, O121, and O145, and two or three phenotypes (drug susceptible, MDR, and MDR-AmpC) of *S.* Typhimurium and S. Newport. In addition to biofilm formation at temperatures of 4, 15, and 25°C, three decontamination interventions (water, quaternary ammonium compound-based and acid-based) were tested against one-week mature biofilms of the above-mentioned pathogen groups. It should be noted that chemical sanitizing agents are recommended to be used after thorough cleaning of equipment (Beauchamp *et al.*, 2012). In this study the target was to determine any differences in sensitivity to sanitizers among the pathogen groups when they might present in conditions that might not be easy to clean.

MATERIALS AND METHODS

Bacterial strains. To facilitate selective enumeration of *E. coli* strains in the presence of the natural meat homogenate microflora, rifampicin-resistant (100 μg/ml) variants of wild-type *E. coli* strains were selected by a method described by Kaspar and Tamplin (1993). A four stain mixture of rifampicin-resistant *E. coli* O157:H7 and a six strain mixture (one from each

serogroup) of rifampicin-resistant *E. coli* O26, O45, O103, O111, O121, and O145 were used in this study. *Salmonella* strains represented two (susceptible, and MDR-AmpC for *S.* Newport) or three (susceptible, MDR, and MDR-AmpC for *S.* Typhimurium) antibiotic resistant phenotypes. Prior to this experiment, antibiotic resistance profiles of the *Salmonella* isolates was confirmed using the Sensititre® antimicrobial susceptibility system (Trek Diagnostic Systems, Cleveland, OH) as described by Geornaras *et al.* (2011). Four-strain mixtures of each of the antibiotic resistant phenotypes of *S.* Newport and Typhimurium were used in this study.

Both wild-type and rifampicin-resistant variants of *E. coli* O157:H7 strains needed for this study were available at the Pathogen Reduction Laboratory of the Center for Meat Safety and Quality of Colorado State University (CSU), while remaining wild-type *E. coli* and *Salmonella* strains used in this study were kindly provided by Dr. Chitrita DebRoy (*E. coli* Reference Center, The Pennsylvania State University, University Park, PA), Dr. Pina Fratamico (Eastern Regional Research Center, USDA-ARS-NAA, Wyndmoor, PA), Dr. Tommy Wheeler (U.S. Meat Animal Research Center, USDA-ARS-NPA, Clay Center, NE), Dr. Martin Wiedmann (Department of Food Science, Cornell University, Ithaca, NY) and Dr. Shaohua Zhao (Center for Veterinary Medicine, U.S. FDA, Laurel, MD). A list of these strains, their identification codes, and isolation sources are reported in a recent publication (Geornaras *et al.*, 2011).

Inocula preparation. Each isolate was individually prepared by transferring a loop-full from a stock kept frozen into 10 ml of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) (for *Salmonella* strains) or TSB supplemented with rifampicin (100 µg/ml, Sigma-Aldrich, St. Louis, MO; TSB+rif) (for *E. coli* strains). The inoculated tubes were stored at 35°C and subcultured after 20-24 h, by transferring 0.1 ml of bacterial suspension into 10 ml sterile TSB (for *Salmonella* strains) or TSB+rif (for *E. coli* strains). The subculture tubes were incubated at

35°C for 20-24 h and were then streak-plated onto xylose lysine deoxycholate agar (XLD; Acumedia, Lansing, MI) (for *Salmonella* strains) or tryptic soy agar (TSA; Acumedia, Lansing, MI) supplemented with 100 μg/ml rifampicin (TSA+rif) (for *E. coli* strains), and were incubated at 35°C for 24 h. These plates with purified cultures were stored at 4ºC and were used for preparation of the strain mixtures used in the study.

Prior to the experiment, for each strain individually, single colonies from the above-mentioned purified plates were activated by suspending each colony into 10 ml of TSB (for *Salmonella* strains) or TSB+rif (for *E. coli* strains), incubating at 35°C for 20-24 h, followed by subculturing the strains by transferring 0.1 ml of bacterial suspension into 10 ml sterile TSB (for *Salmonella* strains) or TSB+rif (for *E. coli* strains) and incubation at 35°C for 20-24 h. Individually sub-cultured strains were then combined to obtain seven inocula of (i) a four-strain mixture of rifampicin-resistant *E. coli* O157:H7, (ii) a six strain mixture of rifampicin-resistant *E. coli*, including single strains of *E. coli* O26, O45, O103, O111, O121, and O145 (non-O157 *E. coli*), (iii) a four-strain mixture of susceptible *S.* Typhimurium, (iv) a four-strain mixture of MDR *S.* Typhimurium, (v) a four-strain mixture of MDR-AmpC *S.* Typhimurium, (vi) a four-strain mixture of susceptible *S.* Newport, and (vii) a four-strain mixture of MDR-AmpC *S.* Newport. Each strain mixture was then centrifuged at 4,629 *g* for 15 min, washed with 10 ml phosphate-buffered saline (PBS, pH 7.4; 0.2 g/liter KH_2PO_4, 1.5 g/liter $Na_2HPO_4 \cdot 7H2O$, 8.0 g/liter NaCl, and 0.2 g/liter KCl), centrifuged (4,629 *g* at 4°C for 15 min) again, and resuspended in 10 ml PBS.

Preparation of homogenate and inoculation of stainless steel coupons. Previously used stainless steel coupons (type 304, #2b finish, [2 x 5 x 0.8 cm]) were washed and sterilized as described by Beauchamp *et al.* (2012). Specifically, coupons were washed and rinsed with soap,

water, 99% acetone (Fisher Scientific, Fair Lawn, NJ), and 70% ethyl alcohol (Fisher Scientific, Fair Lawn, NJ), to remove food residues, and then autoclaved for sterility. Dried and sterilized coupons were then spot-inoculated with 0.1 ml of 1:1000 diluted bacterial suspension of each of the above-mentioned strain mixtures and maintained under a biosafety cabinet for 60 min for attachment of the cells onto the surface of coupons. Inoculated coupons were then aseptically transferred into 50 ml polypropylene sterile tubes (Fisher Scientific, Fair Lawn, NJ) containing a 10% non-sterile meat homogenate. The homogenate was prepared by 2 min homogenization (Masticator, IUL Instruments, Barcelona, Spain) of lean fresh beef (5% fat content) with distilled water to yield a 10% (w/v) homogenate. Coupons remained half-submerged in the homogenate in an upright tilted position and were incubated statically under aerobic conditions at 4, 15, and 25ºC for seven days. Attachment time and conditions of this experiment were chosen based on preliminary experiments to optimize formation and attachment of biofilm mass on stainless steel coupons (date not shown).

Formation and inactivation of biofilms. For investigating formation of biofilms at 4, 15, and 25ºC, two hours after inoculation (day-0) and on days 1, 4, and 7, inoculated coupons were analyzed for microbial populations. Decontamination of the coupons was investigated on day-7 of the experiment, by submerging coupons in 30 ml of treatment solution for 60 seconds. These solutions were (i) sterilized water, (ii) Oasis-146®, Multi-Quat Sanitizer (Ecolab, Inc., St. Paul, MN), and (iii) Vortexx®-Acid Liquid Sanitizer (Ecolab, Inc., St. Paul, MN). These sanitizers were chosen due to wide-spread use of such compounds in food processing (Hegstad *et al.*, 2012; Farrell *et al.*, 1998). Manufacturer's of these two selected sanitizer products have multiple concentration recommendations, maximum general disinfection concentration for Oasis-146®, Multi-Quat Sanitizer (referred as QAC in the rest of this publication), and maximum

concentration for food contact surfaces for Vortexx®-Acid Liquid Sanitizer (referred as AB in the rest of this publication), were selected in this study. The QAC (Alkyl [C4, 50%; C12, 40%; C16, 10%]) had ingredients of dimethyl benzyl ammonium chloride (3.00%), octyl decyl dimethyl ammonium chloride (2.25%), octyl dimethly ammonium chloride (1.35%), dioctyl dimethyl ammonium chloride (0.90%), other ingredients (92.50%) and manufacturer's recommendation for general disinfection was 1 fl. Oz per gallon of water or (8 ml/liter). The AB had ingredients of hydrogen peroxide (6.9%), peroxy acetic acid (4.4%), octanoic acid (3.3%), inert ingredients (85.4%) and maximum manufacturer's recommendation for food contact surfaces are 2 fl. Oz per 6 gallon of water (2.6 ml/liter).

Microbiological and pH analyses. Prior to microbiological analysis, each side of each coupon was rinsed with 10 ml sterile water to remove loosely attached cells. For studying the formation of biofilms on days 0, 1, 4, and 7 and for studying the decontamination effects of the sanitizers on day 7, rinsed and/or treated coupons were placed inside a sterile test tube with 40 ml D/E neutralizing broth (Difco, Becton Dickinson, Sparks, MD) and with 10 sterile glass beads (4mm diameter). The tube was then vortexed for two min (3200 rpm) to remove attached biofilm cells (Stopforth *et al.*, 2002, Giaouris *et al.*, 2005). Immediately after vortexing, sample aliquots (1 ml) were 10-fold serially diluted in PBS and appropriate dilutions were spread-plated onto TSA (for enumeration of background microflora), TSA supplemented with 100 μg/ml rifampicin (TSA+rif) (for *E. coli* strain-mixtures), or Xylose-Lysine-Tergitol 4 (XLT-4) agar (Acumedia) (for *Salmonella* strain-mixtures). Colonies were counted after incubation of plates at 35°C for 24 h. The detection limit of the analysis was 0.3 log CFU/cm^2. Selective medium for enumeration of *Salmonella* strains were chosen after a preliminary experiment using different selective media (Comparing XLD and XLT-4 media) to assure selection and differentiation of *Salmonella*

colonies from background microflora (date not shown). On days 0, 1, 4, and 7, pH (Denver Instrument, Arvada, CO) of the homogenate was measured using standard laboratory procedures as described by Byelashov *et al.* (2010).

Design and statistical analyses. This study was conducted in two biologically independent repetitions each having three replicates per analysis and thus each reported value is a mean of six observations. Microbiological counts were converted to log CFU/cm^2 and the study was considered as a randomized complete block design with each biologically independent repetition as a blocking factor. The log-transformed bacterial counts were analyzed using analysis of variance (ANOVA) procedure followed by Tukey-adjusted mean separation to compare microbial counts of the seven pathogen groups on each storage day of analysis and after each treatment on day-7. A Tukey-adjusted ANOVA were also used to compare microbial counts of each of the seven inoculated pathogens on each day of storage (comparing counts of days 0, 1, 4, and 7 for each pathogen) or after each decontamination intervention (comparing counts of non-treated, water-washed, QAC-, and AB-treated samples for each pathogen). Data of the three storage temperatures (4, 15, and 25ºC) were obtained in three separated experiments thus dataset of each temperature was analyzed independently. All statistical analyses were performed using general linear model and mixed procedures of $SAS_{9.2}$ software (SAS Inst., Cary, NC). The analysis was conducted at type one error level of 5% (α= 0.05).

RESULTS AND DISCUSSION

Bacterial counts of homogenate and pH values. At 4°C, pH values of homogenates on days 0, 1, 4, and 7 were 5.47±0.11, 5.41±0.12, 4.04±0.86, and 4.78±0.16, respectively. Corresponding values were 5.17±0.52, 4.84±0.21, 6.00±0.34, and 7.14±0.53 for samples of 15°C

and 5.05±0.44, 4.08±0.48, 6.29±0.41, and 7.32±0.52 for samples of 25°C. Values of pH of non-inoculated homogenate, water, QAC and AB sanitizers were 5.29±0.36, 6.78±0.63, 5.50±0.81, and 3.17±0.47, respectively. Microbial counts of the meat used for preparation of homogenate were 5.6±0.5 CFU/g and counts of bacterial suspension before inoculation of coupons were 5.7±0.2, 6.0±0.1, 5.8±0.4, 5.9±0.4, 5.6±0.3, 5.9±0.4, and 5.8±0.5 log CFU/ml for *E. coli* O157:H7, Non-O157 *E. coli* serovars, Susceptible *S.* Typhimurium, MDR *S.* Typhimurium, MDR-AmpC *S.* Typhimurium, Susceptible *S.* Newport, and MDR-AmpC *S.* Newport, respectively.

Biofilm formation and inactivation at 4°C. Section A of Figure 3.1 (Appendix Tables A1 and A2) shows biofilm formation and inactivation of the pathogen groups at 4°C (counts of selective media). Pathogen counts on day-0 ranged from 1.6±0.4 log CFU/cm^2 (for *E. coli* O157:H7) to 2.4±0.6 (for MDR-AmpC *S.* Newport) with no differences ($P \geq 0.05$) among the seven pathogen groups. After seven days of storage these values ranged from 1.2±0.8 (for *E. coli* O157:H7) to 1.9±0.8 (for MDR-AmpC *S.* Newport). The differences in biofilm formation among the seven pathogen groups on day-7 were not statistically significant ($P \geq 0.05$). For all seven pathogen groups, there was no increase ($P \geq 0.05$) of biofilm mass from day-0 to day-7 at 4°C. At this temperature (4°C), except for susceptible *S.* Typhimurium which had lower ($P < 0.05$) biofilm counts after treatment with water compared to other pathogen groups, the remaining six pathogen groups were affected similarly ($P \geq 0.05$) by water washing. Treatment with QAC reduced ($P < 0.05$) all five *Salmonella* inocula to less than the detection limit and reduced O157:H7 and non-O157 *E. coli* to <0.7±0.6 and <0.4±0.1 log CFU/cm^2, respectively. For five out of seven pathogen groups (i.e., five *Salmonella* inocula), QAC was more effective than water for cell reduction ($P < 0.05$) at 4°C. At the chosen concentration, treatment with AB did not reduce

($P \geq 0.05$) any of the pathogens' biofilm counts compared to non-treated samples or samples treated with water (Table 3.1). It should be noted that the concentration chosen for QAC is the manufacturer's maximum recommendation for general disinfection and the concentration chosen for AB is the manufacturer's maximum recommended concentration for inactivation of food contact surfaces. It should be noted that chemical sanitizing agents are recommended to be used after thorough cleaning of equipment (Beauchamp *et al.*, 2012). In this study the target was to determine any differences in sensitivity to sanitizers among the pathogen groups when they might be present in conditions that might not be easy to clean.

In contrast to pathogen counts, those from non-selective media (i.e., TSA), representing both inoculated pathogen counts and background microflora of the meat homogenate, increased extensively during the seven day storage at 4°C. Section A of Figure 3.2 (Appendix Tables A3 and A4) shows the biofilm formation and inactivation of the pathogen groups and background microflora combined at 4°C (i.e., , counts of non-selective media). On day-0 and day-7, the non-selective biofilm counts were not statistically different ($P \geq 0.05$) among the seven pathogen groups. These counts ranged from 2.2±0.3 to 2.8±0.6 on day-0 and increased ($P < 0.05$) to 5.5±0.1 to 5.7±0.2 on day-7. Treatment with water did not reduce ($P \geq 0.05$) non-selective counts of any of the seven pathogen groups. Treatment with AB yielded results similar to water, except for one pathogen group (MDR-AmpC *S.* Newport); the remaining reductions of non-selective counts were not different ($P \geq 0.05$) than counts of coupons treated with water. Treatment with QAC reduced non-selective counts by 2.3-3.4 log CFU/cm^2 and had significantly ($P < 0.05$) lower counts than non-treated samples and samples treated with water and AB (Table 3.4).

Biofilm formation and inactivation at 15°C. Section B of Figure 3.1 (Appendix Tables A5 and A6) shows biofilm formation and inactivation of the pathogen groups at 15°C (counts of

selective media). On each of days 0, 1, 4, and 7, biofilm counts of the seven pathogen groups were not different ($P \geq 0.05$) indicating similar biofilm formation at this temperature. Counts on day-0 ranged from 1.3±0.8 log CFU/cm^2 (for susceptible *S.* Typhimurium) to 2.3±0.4 (for MDR *S.* Typhimurium). These counts increased ($P < 0.05$) on day-7 (for all seven pathogen groups) and ranged from 3.8±0.5 log CFU/cm^2 (for susceptible *S.* Typhimurium) to 4.9±0.6 (for non-O157 *E. coli*). Treatment with water on day-7 was not effective ($P \geq 0.05$) in reducing any of the seven pathogen groups. Treatment with AB was not also effective against the four pathogen groups (O157 and non-O157 *E. coli*, susceptible *S.* Typhimurium, and susceptible *S.* Newport), while it reduced ($P < 0.05$) the counts of MDR and MDR-AmpC *S.* Typhimurium and MDR-AmpC *S.* Newport by 1.0, 2.1, and 2.6 log CFU/cm^2, respectively. Treatment with QAC reduced all pathogen counts more effectively ($P < 0.05$) than non-treated and water-treated samples. The reductions caused by this treatment were 2.6, 3.0, 1.5, 2.2, 3.1, 2.9, and <2.9 log CFU/cm^2 for *E. coli* O157:H7, non-O157 *E. coli*, susceptible *S.* Typhimurium, MDR *S.* Typhimurium, MDR-AmpC *S.* Typhimurium, susceptible *S.* Newport, and MDR-AmpC *S.* Newport, respectively (Table 3.3).

Section B of Figure 3.2 (Appendix Tables A1 and A2) shows biofilm formation and inactivation of the pathogen groups and background microflora combined at 15°C (i.e., counts of non-selective media). Similar to 4°C, non-selective counts increased more extensively than pathogens during the seven-day storage. On day-0 these counts ranged from 2.2±0.2 (for susceptible *S.* Typhimurium) to 2.6±0.4 (for MDR *S.* Typhimurium) with no difference ($P \geq 0.05$) among the seven pathogen groups. These counts increased to 6.3±0.2 (for MDR-AmpC *S.* Newport) and 6.6±0.2 (for MDR-AmpC *S.* Typhimurium) with similar trends ($P \geq 0.05$) across the seven pathogen groups. Treatment with water on day-7 did not reduce ($P \geq 0.05$) counts. Similarly,

treatment with AB did not reduce ($P \geq 0.05$) non-selective counts of six out of seven pathogen groups. MDR-AmpC *S.* Typhimurium were reduced ($P < 0.05$) by this treatment showing reduction from ($P < 0.05$) 6.6±0.2 (non-treated counts) to 5.9±0.5 log CFU/cm^2 (AB-treated counts). QAC was effective ($P \geq 0.05$) against the seven pathogen groups and was more effective ($P < 0.05$) than non-treated samples and samples treated with water and AB, reducing the non-selective counts by 0.8 to 1.6 log CFU/cm^2 (Table 3.5).

Biofilm formation and inactivation at 25°C. Section C of Figure 3.1 (Appendix Tables A3 and A4) shows biofilm formation and inactivation of the pathogen groups at 25°C (counts of selective media). On day-0, these counts ranged from 1.5±0.8 log CFU/cm^2 (for susceptible *S.* Typhimurium) to 2.0±0.8 (for non-O157 *E. coli*), and were not different ($P \geq 0.05$) among the seven groups. By day-7 counts increased to 3.3±0.7, 2.9±0.5, 4.2±0.4, 4.3±0.4, 4.1±1.0, 4.3±0.4, and 4.3±0.5 log CFU/cm^2 for *E. coli* O157:H7, non-O157 *E. coli*, susceptible *S.* Typhimurium, MDR *S.* Typhimurium, MDR-AmpC *S.* Typhimurium, susceptible *S.* Newport, and MDR-AmpC *S.* Newport, respectively. Counts of all five *Salmonella* inocula were not statistically different ($P \geq 0.05$) from each other, but, they were greater ($P < 0.05$) than counts of *E. coli* O157:H7 and non-O157 *E. coli*. On day-7, treatment with water and AB resulted in no ($P \geq 0.05$) reduction compared to non-treated samples, while six out of seven (all except for non-O157 *E. coli*) were reduced ($P < 0.05$) due to treatment with QAC (Table 3.3).

Section C of Figure 3.2 (Appendix Tables A5 and A6) shows the biofilm formation and inactivation of the pathogen groups and background microflora combined at 25°C (i.e., counts of non-selective media). Non-selective counts of all seven pathogen groups were not different ($P \geq 0.05$) from each other on either days of 0, 1, 4, and 7, and they all increased ($P < 0.05$) during the seven day storage. These counts ranged from 2.0±0.5 log CFU/cm^2 (for susceptible *S.*

Typhimurium) to 2.3±0.4 (for *E. coli* O157:H7) on day-0 to 6.4±0.6 (for *E. coli* O157:H7) to 7.1±0.4 (for MDR *S.* Typhimurium) on day-7. Non-selective counts of any of the seven pathogen groups treated with water were not lower ($P \geq 0.05$) than non-treated counts. Similar to 4 and 15°C, non-selective counts of AB-treated samples of all seven pathogen groups were not different ($P \geq 0.05$) than counts of non-selective counts of water-treated samples. Except for non-O157 *E. coli,* all non-selective counts of all pathogen groups were reduced by ($P < 0.05$) 0.6 to 1.6 log CFU/cm^2 as a result of treatment with QAC (Table 3.6).

Overall comparison of pathogen types relative to biofilm formation and inactivation. Due to increased concerns about MDR and MDR-AmpC *salmonella* serovars presence in the food chain, control of these pathogens is among the most important concerns in microbiological safety of food (Bosilevac *et al.,* 2009; Arthur *et al.,* 2004). In our study, at vast majority of time intervals, temperatures and inactivation treatments, MDR and MDR-AmpC phenotypes of *Salmonella* were equally or less resistant to interventions than susceptible *Salmonella* serovars, indicating that validating a cleaning and sanitation procedure against susceptible *Salmonella* serovars will most probably be equally effective for the control of drug-resistant phenotypes as well. Low tolerance of drug resistant *Salmonella* relative to susceptible *Salmonella* serovars was discussed and investigated in study of Morosini *et al.* (2000). They showed that acquisition, maintenance, and expression of *amp*C and other drug resistant genes may cost *Salmonella* serovars reduction in lifestyle attributes making MDR and MDR-AmpC phenotypes of *Salmonella* potentially less resistant to antimicrobial intervention.

Control of foodborne pathogenic non-O157 serogroups of *E. coli* is also a concerntopics in microbiological food safety (Bettelheim, 2007; Mathusa *et al.,* 2010; Grant *et al.,* 2011). This

study indicated that control of these pathogen groups on stainless steel material can be achieved with similar interventions validated against *E. coli* O157:H7.

Overall temperature effect on biofilm formation and inactivation. Under the conditions of our study, reduced efficacy of the chemicals as a result of increased temperature of the environment during biofilm formation was observed. treatments with a quaternary ammonium compound-based sanitizer, were able to reduce biofilm mass of one-week mature biofilm to less than detection limited for most pathogen groups when tested at 4°C while same treatment at 25°C left behind over 100 pathogenic cell/cm^2 on the surface of stainless steel. A study by Ryu *et al*. (2004) showed similarly higher biofilm formation and lower susceptibility to sanitizers as temperature increases. This may indicate the need for cleaning protocols for different areas of processing, depending upon environment temperature.

Sanitizers tested in this study have been previously shown to be efficient in inactivation of planktonic cells. Farrell *et al.* (1998) and Sundheim *et al.* (1998) showed proper utilization of peroxyacetic acid-based and quaternary ammonium compounds-based sanitizers can reduce the counts of *E. coli* O157:H7 to below detection limit. Fratamico *et al.* (1996), Joseph *et al.* (2001), Uhlich *et al.* (2006), and Ellebracht *et al.* (2005) reached similar conclusions examining a selection of food processing sanitizers. In our study, we observed low efficacy of sanitizers against one-week mature biofilms at three tested temperatures. Nearly for all the seven tested pathogen groups grown at 4, 15, and 25°C, we observed low efficacy of the peroxyacetic-based sanitizer for inactivation of one-week mature biofilm, at the highest manufacturers recommended concentration for inactivation of food contact surfaces. The quaternary ammonium compound-based sanitizer tested in this study, especially at higher temperatures of biofilm formation, was not capable of complete sanitation of the surfaces as well when tested at the maximum

recommended concentration for general disinfection. This confirms and reinforces the need for incorporating proper cleaning including physical scrubbing before applying sanitizers for effective removal of biofilms from processing area. A recent study of biofilm formation and inactivation of *E. coli* O157:H7 also recommended use of mechanical forces and potentially higher concentration of sanitizers to achieve microbiological destruction during cleaning and sanitation in food processing areas (Beauchamp *et al.*, 2012).

Overall, for decontamination of stainless steel coupons from biofilms of foodborne pathogenic bacteria, low efficacy of quaternary ammonium compound-based and peroxyacetic-based chemical decontamination was observed. These sanitizers were previously validated to be effective in removal of planktonic cells. This decrease in efficacy was more notable as a result of increase in temperature of the biofilm environment. Seven tested pathogen groups in this study had similar biofilm formation and susceptibility to chemical decontamination indicating that a successful cleaning and sanitation program validated against *E. coli* O157:H7 will most probably be effective against the other tested non-O157 *E. coli* and *Salmonella* serovars as well.

Table 3.1. Biofilm decontamination at 4°C for the seven pathogen groups (selective media counts).

	Treatments on Day 7*			
	Control	Water	QUAT-146®	VORTEXX®
Escherichia coli O157:H7**	1.2±0.8 aA	1.2±0.5 abA	<0.7±0.6 aA	1.2±0.5 abA
Non-O157 *Escherichia coli* serovars	1.4±0.3 aA	2.0±0.5 aA	<0.4±0.1 aB	1.4±0.5 aA
Susceptible *Salmonella* Typhimurium	1.9±0.4 aA	0.6±0.0 bB	<0.3 aB	<0.5±0.2 bB
MDR *Salmonella* Typhimurium	1.7±1.0 aA	1.5±0.5 abA	<0.3 aB	<0.9±0.4 abAB
MDR-AmpC *Salmonella* Typhimurium	1.5±0.3 aA	1.5±0.8 abA	<0.3 aB	<0.9±0.6 abAB
Susceptible *Salmonella* Newport	1.4±0.6 aA	1.3±0.7 abAB	<0.3 aC	<0.6±0.3 bBC
MDR-AmpC *Salmonella* Newport	1.9±0.8 aA	1.2±0.7 abA	<0.3 aB	<1.0±0.4 abAB

* Control (no treatment) and complete immersion in 30 ml of treatment solution for 60 seconds of (1) sterilized water, (2) Oasis-146®, Multi-Quat Sanitizer. (Alkyl [C4, 50%; C12, 40%; C16, 10%]). Ingredients: Dimethyl benzyl ammonium chloride (3.00%), Octyl decyl dimethyl ammonium chloride (2.25%), Octyl dimethly ammonium chloride (1.35%), Dioctyl dimethyl ammonium chloride (0.90%), other ingredients (92.50%). Manufacturer's recommendation for General Disinfection: 1 fl. Oz per gallon of water or (8 ml/liter), and (3) Vortexx®. Ingredients: Hydrogen Peroxide (6.9%), Peroxy acetic acid (4.4%), Octanoic acid (3.3%), inert ingredients (85.4%). Maximum Manufacturer's recommendation for food contact surfaces: 2 fl. Oz per 6 gallon of water (2.6 ml/liter).

** Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table 3.2 . Biofilm decontamination at 15°C for the seven pathogen groups (selective media counts).

	Treatments on Day 7*			
	Control	Water	QUAT-146®	VORTEXX®
Escherichia coli O157:H7**	4.8±0.6 abA	4.0±1.1 aA	2.2±1.4 aB	3.5±0.8 aAB
Non-O157 *Escherichia coli* serovars	4.9±0.6 aA	4.3±0.9 aA	1.9±1.0 aB	3.3±1.1 abAB
Susceptible *Salmonella* Typhimurium	3.8±0.5 bA	3.9±0.8 aA	2.3±1.0 aB	3.0±0.4 abAB
MDR *Salmonella* Typhimurium	4.2±0.6 abA	4.1±0.5 aA	2.0±0.6 aC	3.2±0.3 abB
MDR-AmpC *Salmonella* Typhimurium	4.7±0.6 abA	4.3±0.6 aA	1.6±0.8 aB	2.6±1.1 abB
Susceptible *Salmonella* Newport	4.5±0.4 abA	4.1±0.3 aAB	2.7±1.1 aC	3.0±0.8 abBC
MDR-AmpC *Salmonella* Newport	4.5±0.8 abA	4.1±0.5 aA	<1.6±1.3 aB	1.9±0.9 bB

* Control (no treatment) and complete immersion in 30 ml of treatment solution for 60 seconds of (1) sterilized water, (2) Oasis-146®, Multi-Quat Sanitizer. (Alkyl [C4, 50%; C12, 40%; C16, 10%]). Ingredients: Dimethyl benzyl ammonium chloride (3.00%), Octyl decyl dimethyl ammonium chloride (2.25%), Octyl dimethly ammonium chloride (1.35%), Dioctyl dimethyl ammonium chloride (0.90%), other ingredients (92.50%). Manufacturer's recommendation for General Disinfection: 1 fl. Oz per gallon of water or (8 ml/liter), and (3) Vortexx®. Ingredients: Hydrogen Peroxide (6.9%), Peroxy acetic acid (4.4%), Octanoic acid (3.3%), inert ingredients (85.4%). Maximum Manufacturer's recommendation for food contact surfaces: 2 fl. Oz per 6 gallon of water (2.6 ml/liter).

** Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table 3.3 . Biofilm decontamination at 25℃ for the seven pathogen groups (selective media counts).

	Treatments on Day 7*			
	Control	Water	QUAT-146®	VORTEXX®
Escherichia coli O157:H7**	3.3±0.7 bcAB	2.6±0.7 bcAB	2.2±0.4 aB	3.5±0.9 aA
Non-O157 *Escherichia coli* serovars	2.9±0.5 cAB	2.3±0.6 cB	2.7±0.9 aAB	3.5±0.7 aA
Susceptible *Salmonella* Typhimurium	4.2±0.4 abA	4.0±0.6 aA	2.3±0.8 aB	4.0±0.8 aA
MDR *Salmonella* Typhimurium	4.3±0.4 aA	4.2±0.4 aAB	2.9±0.6 aB	4.4±0.7 aA
MDR-AmpC *Salmonella* Typhimurium	4.1±1.0 abA	3.7±0.5 abAB	3.0±0.4 aB	4.4±0.4 aA
Susceptible *Salmonella* Newport	4.3±0.4 abA	4.1±0.6 aA	3.0±0.6 aB	4.4±0.4 aA
MDR-AmpC *Salmonella* Newport	4.3±0.5 abA	3.9±0.8 aA	2.5±1.1 aB	4.3±0.6 aA

* Control (no treatment) and complete immersion in 30 ml of treatment solution for 60 seconds of (1) sterilized water, (2) Oasis-146®, Multi-Quat Sanitizer. (Alkyl [C4, 50%; C12, 40%; C16, 10%]). Ingredients: Dimethyl benzyl ammonium chloride (3.00%), Octyl decyl dimethyl ammonium chloride (2.25%), Octyl dimethly ammonium chloride (1.35%), Dioctyl dimethyl ammonium chloride (0.90%), other ingredients (92.50%). Manufacturer's recommendation for General Disinfection: 1 fl. Oz per gallon of water or (8 ml/liter), and (3) Vortexx®. Ingredients: Hydrogen Peroxide (6.9%), Peroxy acetic acid (4.4%), Octanoic acid (3.3%), inert ingredients (85.4%). Maximum Manufacturer's recommendation for food contact surfaces: 2 fl. Oz per 6 gallon of water (2.6 ml/liter).

** Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table 3.4. Biofilm decontamination at 4°C for the seven pathogen groups and background microflora (non-selective media counts).

	Treatments on Day 7*			
	Control	Water	QUAT-146®	VORTEXX®
Escherichia coli O157:H7**	5.5±0.2 aA	5.3±0.1 aA	3.2±0.6 aB	5.3±0.3 aA
Non-O157 *Escherichia coli* serovars	5.5±0.2 aA	5.3±0.1 aAB	2.7±0.4 aC	5.0±0.3 abB
Susceptible *Salmonella* Typhimurium	5.7±0.2 aA	5.3±0.2 aAB	2.8±0.5 aC	4.9±0.3 abB
MDR *Salmonella* Typhimurium	5.4±0.2 aA	5.3±0.2 aAB	2.2±0.6 aC	4.6±0.5 abB
MDR-AmpC *Salmonella* Typhimurium	5.7±0.4 aA	5.3±0.1 aA	2.8±0.4 aB	4.4±0.8 bC
Susceptible *Salmonella* Newport	5.5±0.1 aA	5.4±0.1 aA	2.9±0.5 aC	4.7±0.4 abB
MDR-AmpC *Salmonella* Newport	5.6±0.4 aA	5.1±0.4 aAB	2.2±0.6 aC	4.5±0.5 abB

* Control (no treatment) and complete immersion in 30 ml of treatment solution for 60 seconds of (1) sterilized water, (2) Oasis-146®, Multi-Quat Sanitizer. (Alkyl [C4, 50%; C12, 40%; C16, 10%]). Ingredients: Dimethyl benzyl ammonium chloride (3.00%), Octyl decyl dimethyl ammonium chloride (2.25%), Octyl dimethly ammonium chloride (1.35%), Dioctyl dimethyl ammonium chloride (0.90%), other ingredients (92.50%). Manufacturer's recommendation for General Disinfection: 1 fl. Oz per gallon of water or (8 ml/liter), and (3) Vortexx®. Ingredients: Hydrogen Peroxide (6.9%), Peroxy acetic acid (4.4%), Octanoic acid (3.3%), inert ingredients (85.4%). Maximum Manufacturer's recommendation for food contact surfaces: 2 fl. Oz per 6 gallon of water (2.6 ml/liter).

** Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table 3.5 . Biofilm decontamination at 15°C for the seven pathogen groups and background microflora (non-selective media counts).

	Treatments on Day 7*			
	Control	Water	QUAT-146®	VORTEXX®
Escherichia coli O157:H7**	6.5±0.2 aA	6.5±0.1 aA	5.2±0.7 aB	6.3±0.3 aA
Non-O157 *Escherichia coli* serovars	6.4±0.3 aA	6.5±0.1 aA	4.8±1.1 aB	6.4±0.3 aA
Susceptible *Salmonella* Typhimurium	6.4±0.3 aA	6.5±0.2 aA	5.3±0.6 aB	6.4±0.3 aA
MDR *Salmonella* Typhimurium	6.4±0.3 aA	6.6±0.2 aA	5.0±0.5 aB	6.3±0.2 aA
MDR-AmpC *Salmonella* Typhimurium	6.6±0.2 aA	6.6±0.3 aA	5.7±0.5 aB	5.9±0.5 aB
Susceptible *Salmonella* Newport	6.5±0.3 aA	6.7±0.3 aA	5.6±0.6 aB	6.4±0.3 aA
MDR-AmpC *Salmonella* Newport	6.3±0.2 aA	6.4±0.2 aA	5.5±0.5 aB	6.0±0.4 aAB

* Control (no treatment) and complete immersion in 30 ml of treatment solution for 60 seconds of (1) sterilized water, (2) Oasis-146®,, Multi-Quat Sanitizer. (Alkyl [C4, 50%; C12, 40%; C16, 10%]). Ingredients: Dimethyl benzyl ammonium chloride (3.00%), Octyl decyl dimethyl ammonium chloride (2.25%), Octyl dimethly ammonium chloride (1.35%), Dioctyl dimethyl ammonium chloride (0.90%), other ingredients (92.50%). Manufacturer's recommendation for General Disinfection: 1 fl. Oz per gallon of water or (8 ml/liter), and (3) Vortexx®. Ingredients: Hydrogen Peroxide (6.9%), Peroxy acetic acid (4.4%), Octanoic acid (3.3%), inert ingredients (85.4%). Maximum Manufacturer's recommendation for food contact surfaces: 2 fl. Oz per 6 gallon of water (2.6 ml/liter).

Table 3. 6. Biofilm decontamination at 25℃ for the seven pathogen groups and background microflora (non-selective media counts).

	Treatments on Day 7*			
	Control	Water	QUAT-146®	VORTEXX®
Escherichia coli O157:H7**	6.4±0.6 aA	6.9±0.7 aAB	5.7±0.9 aB	6.3±0.3 aAB
Non-O157 *Escherichia coli* serovars	6.5±0.6 aA	6.8±0.3 aA	5.9±0.8 aA	6.2±0.4 aA
Susceptible *Salmonella* Typhimurium	7.0±0.2 aA	7.1±0.8 aA	5.4±0.6 aB	6.4±0.4 aA
MDR *Salmonella* Typhimurium	7.1±0.4 aA	6.5±0.5 aAB	6.0±0.4 aB	6.7±0.5 aA
MDR-AmpC *Salmonella* Typhimurium	6.5±0.5 aA	6.7±0.5 aAB	5.9±0.5 aB	6.4±0.5 aAB
Susceptible *Salmonella* Newport	7.1±0.4 aA	7.1±0.3 aA	6.1±0.7 aB	6.5±0.7 aAB
MDR-AmpC *Salmonella* Newport	7.0±0.1 aA	6.4±0.3 aAB	6.0±0.5 aB	6.3±0.5 aB

* Control (no treatment) and complete immersion in 30 ml of treatment solution for 60 seconds of (1) sterilized water, (2) Oasis-146®, Multi-Quat Sanitizer. (Alkyl [C4, 50%; C12, 40%; C16, 10%]). Ingredients: Dimethyl benzyl ammonium chloride (3.00%), Octyl decyl dimethyl ammonium chloride (2.25%), Octyl dimethly ammonium chloride (1.35%), Dioctyl dimethyl ammonium chloride (0.90%), other ingredients (92.50%). Manufacturer's recommendation for General Disinfection: 1 fl. Oz per gallon of water or (8 ml/liter), and (3) Vortexx®. Ingredients: Hydrogen Peroxide (6.9%), Peroxy acetic acid (4.4%), Octanoic acid (3.3%), inert ingredients (85.4%). Maximum Manufacturer's recommendation for food contact surfaces: 2 fl. Oz per 6 gallon of water (2.6 ml/liter).

** Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

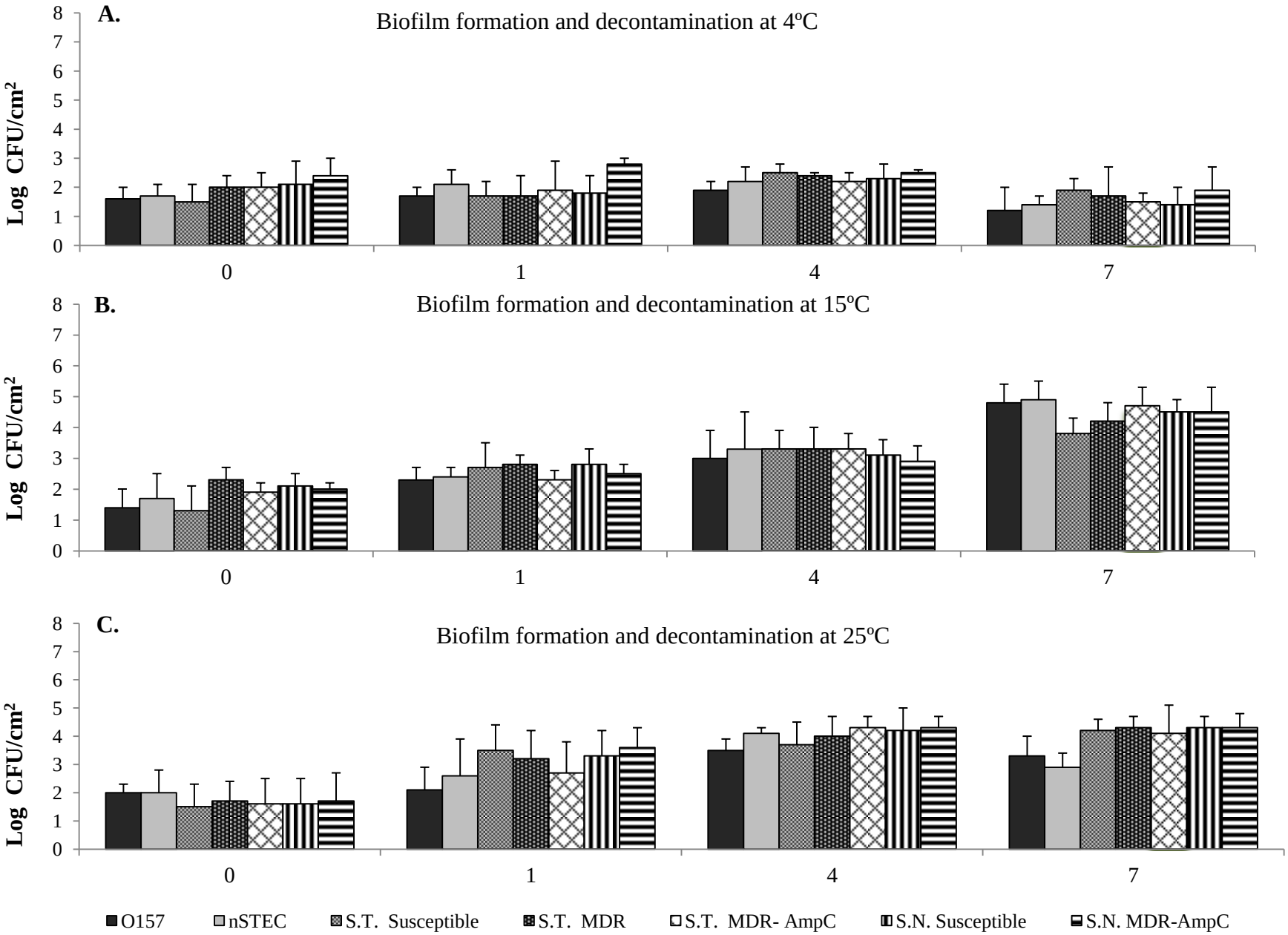
A.
Biofilm formation and decontamination at 4ºC
B.
Biofilm formation and decontamination at 15ºC
C.
Biofilm formation and decontamination at 25ºC
Log CFU/cm²
0
1
2
3
4
5
6
7
8
0
1
4
7
O157
nSTEC
S.T. Susceptible
S.T. MDR
S.T. MDR- AmpC
S.N. Susceptible
S.N. MDR-AmpC

Figure 3.1 (Data in Appendix Tables A1 to A3 Biofilm formation counts of selective media e.g. pathogen counts) of the selected foodborne pathogenic *E. coli* and *Salmonella* serovars at A. 4°C, B. 15°C, and C. 25 °C. Bars from left to right represent counts of *E. coli* O157:H7 (O157), six serogroups mixture of non-O157 shiga toxin-producing *E. coli* (nSTEC), drug susceptible *S.* Typhimurium (S.T. Susceptible), multidrug resistant S. Typhimurium (S.T. MDR), multidrug resistant with acquired *Amp*C gene *S.* Typhimurium (S.T. MDR-AmpC), drug susceptible *S.* Newport (S.N. Susceptible), and multidrug resistant with acquired *Amp*C gene *S.* Newport (S.T. MDR-AmpC).

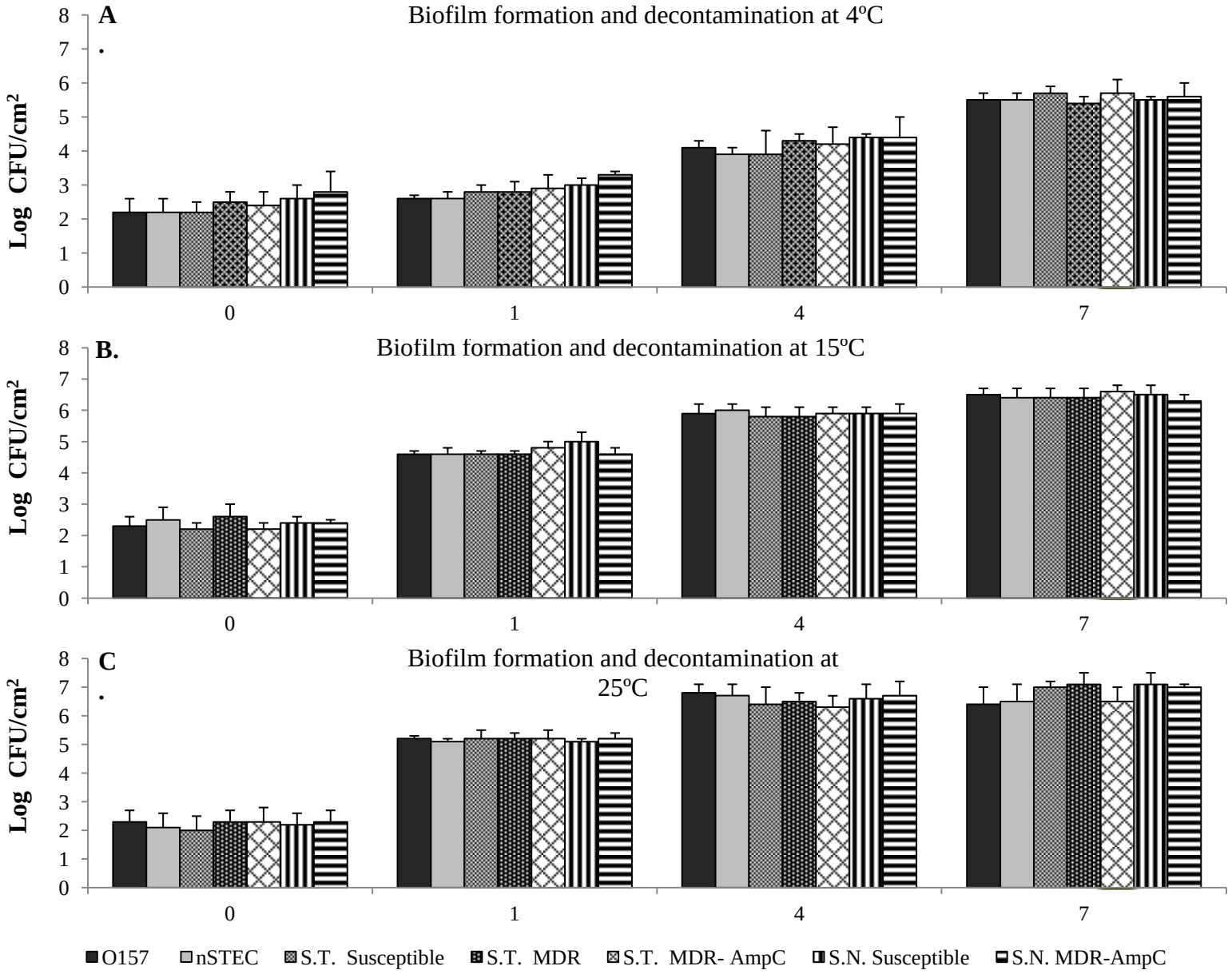
A.
Biofilm formation and decontamination at 4ºC
B.
Biofilm formation and decontamination at 15ºC
C.
Biofilm formation and decontamination at 25ºC
Log CFU/cm²
0
1
2
3
4
5
6
7
8
0
1
4
7
O157
nSTEC
S.T. Susceptible
S.T. MDR
S.T. MDR- AmpC
S.N. Susceptible
S.N. MDR-AmpC

Figure 3.2 (Data in Appendix Tables A4 to A6). Biofilm formation (counts of non-selective media e.g. background microflora and pathogen counts) at A. 4°C, B. 15°C, and C. 25°C. Bars from left to right represent counts of *E. coli* O157:H7 (O157), six serogroups mixture of non-O157 shiga toxin-producing *E. coli* (nSTEC), drug susceptible *S.* Typhimurium (S.T. Susceptible), multidrug resistant S.Typhimurium (S.T. MDR), multidrug resistant with acquired *Amp*C gene *S.* Typhimurium (S.T. MDR-AmpC), drug susceptible *S.* Newport (S.N. Susceptible), and multidrug resistant with acquired *Amp*C gene *S.* Newport (S.T. MDR-AmpC).

CHAPTER IV

Lactic acid resistance of Shiga toxin-producing *Escherichia coli* and multidrug-resistant and susceptible *Salmonella* Typhimurium and Newport in meat homogenate

SUMMARY

This study compared resistance to lactic acid of individual strains of six serogroups of wild-type and rifampicin resistant non-O157 shiga toxin-producing *Escherichia coli* and susceptible, multidrug-resistant (MDR), and/or MDR with acquired *ampC* gene (MDR-AmpC) *Salmonella* Newport and Typhimurium against *E. coli* O157:H7 (5-strain mixture). After inoculation (6 log CFU/ml) of a sterile 10% (w/w) beef homogenate (28 ml homogenate, 0.3 ml inoculum), lactic acid (1.7 ml, 88%) was added for a target concentration of 5%. Before acid addition (control), two seconds after acid addition (time-0), 2, 4, 6 and 8 min after addition of acid, aliquots were analyzed (two repetitions, three acid challenges per strain/mixture) for survivors. Inoculated counts (6.1±0.1 log CFU/ml) of wild-type *E. coli* O157:H7 mixture were reduced ($P<0.05$) to 1.1±0.2 by the end of the 8-min acid challenge, while the rifampicin-resistant *E. coli* O157:H7 mixture, inoculated at 5.9±0.2, reached the detection limit (<1.0 log CFU/ml) after 6 min of exposure. Of the wild-type non-O157 and of the rifampicin-resistant variants strains, irrespective of serogroup, 85.7% (30 out of 35 strains) and 82.9% (29 out of 35) reached the detection limit within 0 to 6 min of exposure, respectively. Of the *Salmonella* strains, 87.9% (29 out of 33 isolates) reached the detection limit within 0 to 4 min of exposure, irrespective of serovar or antibiotic resistance phenotype. Results of non-log-linear microbial survivor curve analyses

indicated that the non-O157 *E. coli* serogroups and multidrug-resistant and susceptible *Salmonella* strains required less time for a 4D reduction compare to *E. coli* O157:H7. Overall, the results of this acid challenge showed, for nearly all strains and time intervals, that individual strains of wild-type and rifampicin-resistant non-O157 *E. coli* and *S.* Newport and Typhimurium were less ($P<0.05$) acid tolerant than the *E. coli* O157:H7 5-strain mixture.

INTRODUCTION

E. coli O157:H7 and other shiga toxin-producing *E. coli* (STEC) serotypes are major concerns in primary and further processing of muscle foods as they have been involved in various foodborne episodes in recent years (Bosilevac *et al.,* 2009). Pathogenic *E. coli* serovars, and especially enterohemorrhagic *E. coli* (EHEC) strains, are capable of producing shiga toxins that may lead to Hemolytic Uremic Syndrome (HUS) a potentially life-threatening kidney complication in infected people. HUS is characterized by hemolytic anemia and acute renal failure and is primarily a concern for children, the elderly, and individuals with suppressed immune system (Ahn *et al.,* 2009; Jahanner and Romer, 2010).

After outbreaks of gastrointestinal illnesses in 1983, *E. coli* O157:H7 was first recognized as a pathogenic agent capable of causing HUS. After major outbreaks, including a multistate Pacific Northwest outbreak of *E. coli* O157:H7 in 1992-1993 (Bell *et al.,* 1994), and an outbreak of ground beef and beef trimming contaminated with *E. coli* O157:H7 in August 1997 and July 2002, this pathogen became the first microbial agent to be declared as an adulterant in food products by the U.S. Food Safety Inspection Service (FSIS, 1999). Emergence of this pathogen has led to much advancement in food regulation and production systems including mandatory Hazards Analysis Critical Control Point (HACCP) based food safety management systems for

meat producers (Sofos, 2009; Oliver *et al*., 2009). Most recent epidemiological investigations estimate that every year in the United States *E. coli* O157:H7 is responsible for 3,268 illness episodes with 46.2% and 0.5% hospitalization and death rates, respectively (Scallan *et al.*, 2011). Along with *E. coli* O157:H7 in recent years other serotypes of EHEC have been involved in foodborne episodes, including a recall of ground beef associated with *E. coli* O26 by a Pennsylvania company (USDA-FSIS, 2010), a recall of ground beef contaminated with *E. coli* O111 in Japan (USDA-FSIS, 2011), and an outbreak through consumption of beef sausage contaminated with *E. coli* O26 in Denmark (Ethelberg *et al.,* 2009). *E. coli* O26, O45, O103, O111, O121, and O145 serotypes are responsible for the majority of episodes of foodborne illness and HUS associated with non-O157 STEC in the United States (Bettelheim, 2007; Mathusa *et al.,* 2010; Grant *et al.,* 2011). It is estimated that 1,579 illness episodes, with a 12.8% hospitalization rate and a 0.3% death rate are related to non-O157 STEC every year (Scallan *et al.,* 2011).

Recent investigations show that 0.3 to 19.7% of feedlot cattle, 0.7 to 27.3% of cattle on irrigated pasture, and 0.9 to 6.9% of cattle in rangeland forage carry STEC serotypes in their gastrointestinal system, while at slaughtering facilities, prevalence of STEC serotypes ranges from 0.2 to 27.8% (Hussein and Bollinger, 2005a). In a one year study of beef processing plants (Barkocy-Gallagher *et al*., 2003) it was shown that 60.6% and 56.6% of cattle hides, 5.9% and 19.4% of cattle manures samples, and 26.7% and 58.0% of carcasses were contaminated with O157 and non-O157 STEC, respectively.

Pathogenic *Salmonella* (nontyphoidal) is the leading cause of foodborne deaths in the United States, being responsible for 1,229,007 illness episodes, with a 27.2% hospitalization rate and a 0.5% death rate (Scallan *et al.,* 2011). In addition, *Salmonella*, multidrug resistant (MDR) and

MDR with acquired *ampC* gene (MDR-AmpC) *Salmonella* serovars in the food chain cause challenges in antibiotic treatment of salmonellosis in hospitals (Bosilevac *et al.,* 2009; Arthur, *et al.,* 2004; Boyle *et al.,* 2007; Anonymous, 2012). Recently it has been shown that as high as 0.6% of ground meat samples may harbor drug-resistant *Salmonella* (Bosilevac *et al.,* 2009) with approximately 7% of them displaying a MDR-AmpC phenotype (Zhao *et al.,* 2009).

Due to involvement of *E. coli* O157:H7 in numerous national and international foodborne episodes in the last few decades, this pathogen has been the primary target for control in beef processing (Sofos, 2009; Oliver *et al*., 2009). Lactic acid is one of most common antimicrobial intervention in primary processing of fresh beef and its efficacy has been investigated in many recent studies (Cutter *et al*., 2000; Castillo *et al*., 2001; Harris *et al.,* 2006; Arthur *et al.,* 2008).

The purpose of this study was to compare the lactic acid resistance of individual strains of wild-type and spontaneous rifampicin-resistant variants of six STEC serogroups, O26, O45, O103, O111, O121 and O145, and antibiotic susceptible and multidrug resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium, to that of a wild-type and rifampicin-resistant variants of 5 strains of *E. coli* O157:H7 tested as a mixture.

MATERIALS AND METHODS

***E. coli* strains.** Four to seven individual strains from each of six non-O157 STEC serogroups (i.e., O26, O45, O103, O111, O121, and O145) were used in this study. They were kindly provided by Dr. Chitrita DebRoy (*E. coli* Reference Center, The Pennsylvania State University, University Park, PA), Dr. Pina Fratamico (Eastern Regional Research Center, USDA-ARS-NAA, Wyndmoor, PA), and Dr. Tommy Wheeler (U.S. Meat Animal Research Center, USDA-ARS-NPA, Clay Center, NE). A list of these strains, their identification codes, and isolation

sources are reported by Geornaras *et al.* (2011) and are presented in Table 4.1. In addition to these wild-type strains, to facilitate selective enumeration from the natural meat microflora, rifampicin-resistant (100 μg/ml) variants were selected by a method described by Kaspar and Tamplin (1993). Wild-type and rifampicin-resistant variants of *E. coli* O157:H7 strains used in this study were ATCC 43888, ATCC 43895, C1-057, C1-072, and C1-109 (Carlson *et al*., 2009) and were available in the Pathogen Reduction Laboratory of the Center for Meat Safety and Quality of Colorado State University. Lactic acid resistance was tested on both, wild-type (parental) and rifampicin-resistant strains, of non-O157 and O157 STEC as well as *Salmonella* strains.

***Salmonella* strains.** Individual strains of each of MDR and/or MDR-AmpC and susceptible *S.* Newport and *S.* Typhimurium were used in this study. They were kindly provided by Dr. Martin Wiedmann (Department of Food Science, Cornell University, Ithaca, NY) and Dr. Shaohua Zhao (Center for Veterinary Medicine, U.S. FDA, Laurel, MD). A list of these strains, their identification codes, and isolation sources are reported by Geornaras *et al.* (2011) and are presented in Tables 4.2 and 4.3. The antibiotic resistance profiles of the *Salmonella* isolates were confirmed using the Sensititre® antimicrobial susceptibility system (Trek Diagnostic Systems, Cleveland, OH), specifically panel CMV2AGNF which was designed for the National Antimicrobial Resistance Monitoring System (NARMS). With this panel, minimum inhibitory concentrations (MIC) were determined, in accordance with the manufacturer's instructions, for ampicillin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, kanamycin, nalidixic acid, streptomycin, sulfisoxazole, tetracycline, and trimethoprim/sulfamethoxazole. It should be noted that azithromycin was also included on the panel but no breakpoints were found for this antimicrobial (Geornaras *et al*., 2011).

Salmonella strains with a MDR phenotype were resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT), and strains with a MDR AmpC phenotype were resistant to at least ACSSuT, amoxicillin-clavulanic acid and ceftiofur, and had a decreased susceptibility to ceftriaxone (MIC ≥2 μg/ml) (CDC, 2009; Greene, 2008).

Preparation of strains and mixtures. Strains were individually prepared and subcultured at 35°C for 20-24 h in 10 ml tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) (for *Salmonella*, and wild-type STEC strains) or TSB supplemented with rifampicin (100 μg/ml, Sigma-Aldrich, St. Louis, MO; TSB+rif) (for rifampicin-resistant STEC strains). Broth cultures were then streak-plated onto tryptic soy agar (TSA; Acumedia, Lansing, MI) (for wild-type STEC strains), TSA supplemented with 100 μg/ml rifampicin (TSA+rif) (for rifampicin-resistant STEC strains), or xylose lysine deoxycholate (XLD) agar (Acumedia) (for *Salmonella* strains); plates were incubated at 35°C for 24 h. Inocula of each strain were prepared by suspending single colonies from the above-mentioned cultured plates into 5 ml phosphate-buffered saline (PBS, pH 7.4; 0.2 g/liter KH2PO4, 1.5 g/liter Na2HPO4·7H2O, 8.0 g/liter NaCl, and 0.2 g/liter KCl). The bacterial suspensions were standardized to a 0.5 McFarland standard (cell concentration of approximately 1.5×10^8 CFU/ml) using a spectrophotometer (600 nm) and Nephelometer (Sensititre, Trek Diagnostics). For the inoculum comprised of a composite of five *E. coli* O157:H7 strains, bacterial suspensions to a 0.5 McFarland standard were initially prepared for each of the five strains separately, before combining the strains. All bacterial suspensions were diluted tenfold in PBS before use.

Lactic acid intervention. The lactic acid treatment was performed in a beef homogenate acidified with 5% lactic acid. The 10% (w/w) homogenate was prepared by 2 min of homogenizing (Masticator, IUL Instruments, Barcelona, Spain) fresh beef with distilled water.

The homogenate was then passed through cheesecloth, and the liquid was sterilized by autoclaving. For each individual strain (non-O157 STEC or *Salmonella*) or strain mixture (*E. coli* O157:H7), 28 ml of the sterile homogenate was pipetted into a sterile 100 ml Erlenmeyer flask containing a sterile magnetic stirrer bar. The flask was placed onto a magnetic stirrer, and while stirring, 0.3 ml of the diluted bacterial suspension was added to the homogenate. The target inoculation level was approximately 6 log CFU/ml. Prior to addition of lactic acid, an aliquot (1 ml) of the inoculated homogenate was removed for microbiological analysis to determine the inoculation level (untreated control). A 1.7 ml volume of lactic acid (88%, Purac America, Lincolnshire, IL) was then added to the stirred inoculated homogenate, giving a final concentration of approximately 5% lactic acid. Immediately after addition of the lactic acid (2 seconds [time 0-min]), and at intervals of 2, 4, 6, and 8 min, 1 ml aliquots were removed from the flask for microbial analyses. Before and after addition of acid, pH (Denver Instrument, Arvada, CO) values of samples were measured using standard laboratory procedures described by Byelashov *et al*. (*2010*).

Microbiological analyses. Aliquots (1 ml) removed from the treated homogenate at each time interval were neutralized in 9 ml D/E neutralizing broth (Difco), and subsequently serially diluted with 0.1% buffered peptone water (Difco). Appropriate dilutions were surface-plated on TSA (for *Salmonella*, and wild-type STEC strains), TSA+rif (for rifampicin-resistant STEC strains) and XLD agar (for *Salmonella* strains). Colonies were counted after incubation of plates at 35°C for 24-48 h. The detection limit of the analysis was 1.0 log CFU/ml.

Statistical analyses. The study was conducted twice (two biologically-independent repetitions), with three acid interventions per strain or strain mixture (for *E. coli* O157:H7) within each repetition (total of six replicates). Each of the two repetitions was considered as a blocking factor

in a complete randomized block design. Mean microbial counts (log CFU/ml) of individual strains (or strain mixture) within each time interval (control, and 0, 2, 4, 6, 8 min) were compared statistically with ANOVA-based procedures followed by Dunnett's-adjusted multiple comparison method for further mean separation using the PROC MIXED command of $SAS_{9.2}$ (SAS Institute, Inc., Cary, NC). Using this procedure, surviving microbial counts of the wild-type non-O157 STEC strains (TSA counts), and *Salmonella* strains (TSA counts) within each time intervals, were compared with surviving counts of the wild-type 5-strain *E. coli* O157:H7 mixture (TSA counts). Similarly, surviving counts of rifampicin-resistant non-O157 STEC strains (TSA+rif counts), and *Salmonella* strains (XLD counts) within each time interval, were compared to surviving counts of the rifampicin-resistant 5-strain *E. coli* O157:H7 mixture (TSA+rif counts). In addition to this analysis, a repeated measures analysis, using the PROC GLM command of SAS, was used to analyze the effect of lactic acid exposure on surviving counts within each strain (i.e., for mean separation of mean values of control, and 0, 2, 4, 6, 8 min for each strain). For both analyses, *P* values less than 0.05 ($P<0.05$) were considered statistically significant.

In order to determine the inactivation rates and time required to achieve a 4D reduction for the strains evaluated in this study, GInaFiT software, a non-log-linear microbial survivor curve were used. This model as described by Geeraerd *et al*. (2005) reports specific inactivation rates of K_{max} expressed in the unit of 1/min, their associated standard error, and time required for 4D reduction. In order to calculate these values, log CFU counts of before exposure and 0, 2, 4, 6, and 8 min after exposure to lactic acid of wild-type (parent) and rifampicin-resistant non-O157 serogroups (i.e., O26, O45, O103, O111, O121, and O145), and the two or three phenotypes of *Salmonella* serovars (i.e., susceptible, MDR, and/or MDR-AmpC) were analyzed with the

GInaFiT software. K_{max} values obtained for each serogroup of non-O157 *E. coli* and two or three phenotypes of *Salmonella* Newport and Typhimurium were compared statistically (Student-best t-test at α=0.05) to those obtained from a five-strain mixture of *E. coli* O157:H7. Furthermore a student based t-test procedure (α=0.05) was used to compare K_{max} values of wild-type (parent) and rifampicin-resistant strains of *E. coli* serotypes and serogroups. Reduction of microbial counts in this study were investigated using the biphasic GInaFiT model and thus K_{max1} values express the inactivation rate of reduction phase while K_{max2} values correspond to the phase at which the tested pathogens counts are at or below the detection limit. Both K_{max} values are in units of 1/min, hence lower values indicate longer times required to achieve a 4D reduction (Geeraerd *et al*., 2005).

RESULTS AND DISCUSSION

Acid resistance of *E. coli* strains. The pH of the beef homogenate ranged from 5.78±0.31 to 5.91±0.30 before addition of lactic acid, and 2.32±0.18 to 2.64±0.70 after addition of lactic acid. Inoculated levels (6.1±0.1 log CFU/ml) of the wild-type *E. coli* O157:H7 5-strain mixture were reduced (P<0.05) to 1.1±0.2 log CFU/ml by the end of the challenge (i.e., 8 min of exposure). For the rifampicin-resistant *E. coli* O157:H7 5-strain mixture, inoculated levels of 5.9±0.2 log CFU/ml reached the detection limit at 6 min of exposure. Overall, for the wild-type non-O157 STEC strains, irrespective of serotype, 85.7% (30 out of 35 strains) reached the detection limit (<1.0 log CFU/ml) within 0 min (i.e., 2 seconds following the addition of lactic acid to the inoculated beef homogenate) to 6 min of exposure (Figure 4.1 and Appendix Tables A7 to A12). Similarly, most (82.9%; 29 out of 35) of the rifampicin-resistant variants of the non-O157 STEC strains reached the detection limit within 0 (2 seconds) to 6 min of exposure (Figure 4.2 and Appendix Tables A7 to A12).

Nearly all strains of *E .coli* O26 tested in this experiment showed lower lactic acid resistance ($P<0.05$) than *E. coli* O157:H7 for times 0 (2 seconds) to 8 min (Figure 4.1, Appendix Table 4A7). Similar results were obtained for both TSA counts (wild-type cells) and TSA+rif counts (rifampicin-resistant cells). Similar to *E. coli* O26, nearly all strains of *E. coli* O111, O103, O45, O121, and O145 showed lower ($P<0.05$) lactic acid resistance than the strain mixture of *E. coli* O157:H7 for the time intervals of control, 0 (2 seconds), 2, 4, 6, and 8 min (Figures 4.1 and 4.2, Appendix Tables A7 to A12).

Results of recent studies show that 0.1 to 43.0% of carcasses in packing plants, 0.01 to 43.3% of sub-primal cuts in packing plants, 0.1 to 4.4% of meat cuts in supermarkets, an average of 2.4% of meat products used in fast food chains, 0.1 to 54.2% of ground beef, and 0.1 to 4.4% of sausages in supermarket may be contaminated with *E. coli* O157:H7 (Hussein and Bollinger, 2005b). Similarly, 1.7 to 58.0% of sub-primal cuts in packing plants, 3.0 to 62.5% in supermarkets, an average of 3.0% of meat products used in fast food chains, 2.4 to 30.0% of ground beef, 17.0 to 49.2% of sausages, and 1.7 to 58.0% of carcasses in packing plants were contaminated with non-O157 STEC (Hussein and Bollinger, 2005b), confirming the concern that control of non-O157 STEC will continue to gain importance for assuring the safety of muscle foods. Overall, results of this acid challenge, conducted in a beef homogenate acidified with 5% lactic acid, showed that in most cases, individual strains of non-O157 STEC (wild-type and rifampicin-resistant) were less ($P<0.05$) acid tolerant than the *E. coli* O157:H7 5-strain mixture (wild-type and rifampicin-resistant) indicating that currently in-place lactic acid interventions in processing of red meat, validated to be efficient against *E. coli* O157:H7 are most probably efficient against the six tested serogroups of non-O157 STEC as well.

Acid resistance of *Salmonella* strains. Of the *Salmonella* isolates, 87.9% (29 out of 33 isolates) reached the detection limit within 0 to 4 min (based on TSA counts) or 0 to 2 min (based on XLD agar counts) of exposure, irrespective of *Salmonell*a serotype or antibiotic resistance phenotype (Figures 4.3 and 4.4, Appendix Tables A13 to A17). Results of this study are in agreement with other investigations of multidrug-resistant salmonellae inoculated onto surface of fresh meat. It was shown that MDR *Salmonella* was reduced at least as effectively as *E. coli* O157:H7 and susceptible *Salmonella* when treated with antimicrobial interventions currently in use at most U.S. beef processing plants (Arthur *et al*., 2004). In a similar study, Hughes *et al*. (2010) showed that 3% lactic acid applied on beef briskets had similar pathogen reduction potential against both MDR and susceptible *Salmonella*. The present study showed the low acid tolerance of nearly all tested *Salmonella* strains as compared to *E. coli* O157:H7. In addition, for the majority of time intervals of strains, MDR and MDR-AmpC phenotypes of *Salmonella* were equally or less resistant to interventions than susceptible *Salmonella* serovars. Lower tolerance of drug-resistant *Salmonella* relative to its drug-susceptible serovars was reported in study of Morosini *et al.* (2000). They showed acquisition, maintenance, and expression of *amp*C and other drug resistant genes may cost *Salmonella* serovars reduction in lifestyle attributes making MDR and MDR-AmpC phenotypes of *Salmonella* potentially less resistant to antimicrobial interventions.

Inactivation rates and time required to achieve 4D reductions. Tables 4.4 and 4.5 show K_{max1} values indicating specific inactivation rates the during reduction phase after exposure to lactic acid. All tested serogroups of *E. coli* (i.e., O26, O45, O103, O111, O121, and O145) showed higher (P<0.05) K_{max1} values relative to those obtained from the five-strain mixture of *E. coli* O157:H7 by both wild-type cells and rifampicin-resistant variants. This indicates that the six

tested serogroups required shorter time for 4D reductions compared to *E. coli* O157:H7 and thus it can be concluded that these six serogroups were less lactic acid tolerant than *E. coli* O157:H7 (Table 4.4). For the six non-O157 serogroups as well as the five-strain mixture of *E. coli* O157:H7, there were no differences ($P \geq 0.05$) between K_{max1} values of wild-type and rifampicin-resistant variants indicating that at the conditions tested in this study, lactic acid resistant of wild-type and rifampicin-resistant variants were not statistically different ($P \geq 0.05$) (Table 4.4). This finding is in agreement with the results of Kaspar and Tamplin (1993), showing wild-type and rifampicin resistant cells of foodborne pathogens behave similarly in response to antimicrobials and therefore can be used interchangeably in food microbiology laboratory challenge research. Use of rifampicin-resistant variants facilitates selective enumeration of the strains from the natural meat microflora (Kaspar and Tamplin, 1993).

Similar to *E. coli* serogroups, all five phenotypes of *Salmonella* serovars tested in this study, showed K_{max1} values higher ($P < 0.05$) than those obtained for the five-strain mixture of *E. coli* O157:H7. This indicates that they were more sensitive to lactic acid than the five-strain mixture of *E. coli* O157:H7. K_{max2} values correspond to the phase at which the tested pathogen counts are at or below the detection limit and thus predictably the differences among K_{max2} values for the *E. coli* O157:H7, the six tested non-O157 *E. coli* serogroups, and the three phenotype for *Salmonella* Newport and Typhimurium were not appreciable (Tables 4.4 and 4.5); they ranged from 0.00±0.28 to 1.34±0.10. Results of non-log-linear microbial survivor curve analyses, indicated that the non-O157 *E. coli* serogroups and drug resistant and susceptible *Salmonella* serovars tested in this study required less time for a 4D reduction compared to *E. coli* O157:H7.

Overall, the results of the this acid intervention conducted in a beef homogenate acidified with 5% lactic acid, nearly all individual strains of antibiotic resistance and susceptible *S.* Newport and *S.* Typhimurium and non-O157 *E. coli* were less ($P<0.05$) acid tolerant than wild-type and rifampicin-resistant *E. coli* O157:H7 5-strain mixtures. In other words, the six tested serogroups of non-O157 *E. coli* required shorter time for 4D reductions compared to *E. coli* O157:H7 and similarly all five phenotypes of *Salmonella* serovars tested in this study *coli* required shorter time for 4D reductions compared to *E. coli* O157:H7 as the result of exposure to lactic acid.

Table 4.1. Sources of non-O157 STEC strains evaluated.

***E. coli* serotype**	**Strain**	**Source**	**Provided by***
O26:H11	hSTEC_03 (O26-1)	human	Dr. Wheeler
O26:H11	imp_113.1 (O26-2)	beef	Dr. Wheeler
O26:H11	81.0211 (A1-006)	antelope	Dr. DebRoy
O26	85.1150 (A1-007)	pig	Dr. DebRoy
O26:H2	93.0494 (A1-008)	human	Dr. DebRoy
O26	0.1302 (A1-009)	cow	Dr. DebRoy
O26:H11	5.2217 (A1-010)	human	Dr. DebRoy
O45	99E_2750 (O45-1)	human	Dr. Wheeler
O45	O45-2	human	Dr. Wheeler
O45:H2	05-6545	human	Dr. Fratamico
O45:H2	96-3285	human	Dr. Fratamico
O103:H2	hSTEC_05 (O103-1)	human	Dr. Wheeler
O103	MDR0089 (O103-2)	beef	Dr. Wheeler
O103:H2	3.2607 (A1-011)	horse	Dr. DebRoy
O103:H11	86.0765 (A1-012)	mouse	Dr. DebRoy
O103:H2	87.1368 (A1-013)	goat	Dr. DebRoy
O103:H2	90.1764 (A1-014)	cow	Dr. DebRoy
O103:H2	92.0084 (A1-015)	human	Dr. DebRoy
O111:H8	hSTEC_08 (O111-1)	human	Dr. Wheeler
O111:NM	18DA (O111-2)	human	Dr. Wheeler
O111	93.0523 (A1-001)	human	Dr. DebRoy
O111	4.0005 (A1-002)	cow	Dr. DebRoy
O111	4.0522 (A1-003)	cow	Dr. DebRoy
O111	85.1154 (A1-004)	pig	Dr. DebRoy
O111	93.0522 (A1-005)	human	Dr. DebRoy
O121	10896 (O121-1)	human	Dr. Wheeler
O121	imp_450 (O121-2)	beef	Dr. Wheeler
O121:H19	97-3068	human	Dr. Fratamico
O121:NM	03-4064	human	Dr. Fratamico
O121:H19	08023	human	Dr. Fratamico
O145:NM	hSTEC_22 (O145-1)	human	Dr. Wheeler
O145	MAY109 (O145-2)	beef	Dr. Wheeler
O145:NM	03-4699	human	Dr. Fratamico
O145:NM	83-75	human	Dr. Fratamico
O145:H28	07865	cow	Dr. Fratamico

* Isolates kindly provided by Dr. Chitrita DebRoy (*E. coli* Reference Center, The Pennsylvania State University, University Park, PA), Dr. Pina Fratamico (Eastern Regional Research Center, USDA-ARS-NAA, Wyndmoor, PA), and Dr. Tommy Wheeler (U.S. Meat Animal Research Center, USDA-ARS-NPA, Clay Center, NE)

Table 4.2. Sources and antibiotic resistance profiles of *Salmonella* Newport strains evaluated.

Strain	Source	Antibiotic resistance[a]	Phenotype:	Provided by
FSL S5-639	human	S	Susceptible	Dr. Wiedmann[1]
CVM N4505	ground turkey	S	Susceptible	Dr. Zhao[2]
CVM N18445	ground beef	S	Susceptible	Dr. Zhao
CVM N1509	ground turkey	S	Susceptible	Dr. Zhao
FSL R6-531	human	AMP, CHL, R, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
FSL R8-0104	human	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
FSL S5-413	human	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
FSL S5-436	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
FSL S5-577	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
FSL S5-920	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
FSL R8-2926	human	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
FSL R8-2350	bovine	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Wiedmann
CVM N635	ground beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT	MDR-AmpC	Dr. Zhao
CVM 22698	pork chop	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT	MDR-AmpC	Dr. Zhao
CVM 29461	ground beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Zhao
CVM 22707	ground beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Zhao
CVM N19852	ground beef	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Zhao

[a] Per results of the Sensititre® antimicrobial susceptibility system CMV2AGNF panel (Trek Diagnostic Systems, Cleveland, OH). Antibiotics included on the panel include ampicillin (AMP), amoxicillin/clavulanic acid (AUG2), cefoxitin (FOX), ceftiofur (XNL), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfisoxazole (FIS), tetracycline (TET), trimethoprim/sulfamethoxazole (SXT). MDR: resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT). MDR-AmpC: resistant to at least ACSSuT, amoxicillin-clavulanic acid and ceftiofur, and a decreased susceptibility to ceftriaxone (MIC $\geq$ 2 μg/ml). S: sensitive. [1]Department of Food Science, Cornell University, Ithaca, NY;[2]Center for Veterinary Medicine, U.S. FDA, Laurel, MD

Table 4.3. Sources and antibiotic resistance profiles of *Salmonella* Typhimurium strains evaluate.

***Salmonella* serotype**	**Strain**	**Antibiotic resistance[a]**	**Phenotype:**	**Provided by**
Typhimurium	FSL S5-536	S	Susceptible	Dr. Wiedmann[1]
	CVM N7300	S	Susceptible	Dr. Zhao[2]
var. O 5- (Copenhagen)	CVM N15788	S	Susceptible	Dr. Zhao
var. O 5- (Copenhagen)	CVM N18534	S	Susceptible	Dr. Zhao
	FSL R6-215	AMP, CHL, STR, FIS, TET	MDR	Dr. Wiedmann
	FSL S9-165	AMP, CHL, STR, FIS, TET	MDR	Dr. Wiedmann
	FSL R8-2540	AMP, CHL, STR, FIS, TET	MDR	Dr. Wiedmann
	CVM N6431	AMP, CHL, STR, FIS, TET	MDR	Dr. Zhao
var. O 5- (Copenhagen)	CVM 30662	AMP, CHL, STR, FIS, TET	MDR	Dr. Zhao
	CVM N497	AMP, CHL, STR, FIS, TET	MDR	Dr. Zhao
var. Copenhagen	FSL S5-786	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
	FSL S5-916	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
var. Copenhagen	FSL S5-385	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, KAN	MDR-AmpC	Dr. Wiedmann
var. O 5- (Copenhagen)	CVM N176	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX	MDR-AmpC	Dr. Zhao
	CVM 33831	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, SXT, KAN	MDR-AmpC	Dr. Zhao
var. O 5- (Copenhagen)	CVM 30034	AMP, CHL, STR, FIS, TET, AUG2, XNL, AXO, FOX, NAL, KAN	MDR-AmpC	Dr. Zhao

[a] Per results of the Sensititre® antimicrobial susceptibility system CMV2AGNF panel (Trek Diagnostic Systems, Cleveland, OH). Antibiotics included on the panel include ampicillin (AMP), amoxicillin/clavulanic acid (AUG2), cefoxitin (FOX), ceftiofur (XNL), ceftriaxone (AXO), chloramphenicol (CHL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), nalidixic acid (NAL), streptomycin (STR), sulfisoxazole (FIS), tetracycline (TET),

trimethoprim/sulfamethoxazole (SXT). MDR: resistant to at least ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (ACSSuT). MDR-AmpC: resistant to at least ACSSuT, amoxicillin-clavulanic acid and ceftiofur, and a decreased susceptibility to ceftriaxone (MIC $\geq$ 2 μg/ml). S: sensitive. [1]Department of Food Science, Cornell University, Ithaca, NY;[2]Center for Veterinary Medicine, U.S. FDA, Laurel, MD

Table 4.4. Non-linear microbial survivor analysis for comparing inactivation rates of seven serotype/serogroups of parent and rifampicin-resistant shiga toxin-producing *E. coli*. K_{max1} and K_{max2} (parameter± SE) values are specific inactivation rates for two phases of the biphasic curves fitted for each serotype/serogroups, expressing the inactivation rate of reduction phase (K_{max1}) and the phase at which the tested pathogens reached the detection limit (K_{max2}). Both values are expresses in 1/min units indicating longer time required for microbial cell reductions associated to smaller K_{max} values.

Serotype/serogroup	Number of strains	Phenotype	K_{max1} (1/min)	K_{max2} (1/min)	4D Reduction (min)
E. coli O157:H7[b]	A five-strain mixture	Parent	1.34±0.36 a	1.34±0.10a	7.20
E. coli O157:H7	A five-strain mixture	Rifampicin resistant	2.32±0.75 a	0.22±0.40b	6.64
E. coli O26	7 single strains	Parent	3.47±0.30 a*	0.03±0.29a*	2.72
E. coli O26	7 single strains	Rifampicin resistant	4.19±0.29 a^	0.11±0.26a	2.24
E. coli O45	4 single strains	Parent	5.47±0.30a*	0.22±0.14a*	2.16
E. coli O45	4 single strains	Rifampicin resistant	4.34±0.39a^	0.36±0.13a	2.34
E. coli O103	7 single strains	Parent	3.49±0.50a*	0.23±0.27a*	2.72
E. coli O103	7 single strains	Rifampicin resistant	5.23±0.60a^	0.47±0.09a	2.26
E. coli O111	7 single strains	Parent	4.70±0.08a*	0.39±0.08a*	2.40
E. coli O111	7 single strains	Rifampicin resistant	4.32±0.40a^	0.16±0.18a	2.16
E. coli O121	5 single strains	Parent	4.93±0.09a*	0.55±0.09a*	3.02
E. coli O121	5 single strains	Rifampicin resistant	5.10±0.08a^	0.36±0.08a	2.64
E. coli O145	5 single strains	Parent	4.37±0.14a*	0.42±0.14a*	2.14
E. coli O145	5 single strains	Rifampicin resistant	3.87±0.26a^	0.00±0.32a	2.40

[a] K_{max} values (parameter± SE, [1/min]) of parent cells of the six serotype of non-O157 *E.coli* followed by * are significantly (student-based t-test, $P< 0.05$) different than K_{max} value of parent cells of *E.coli* O157:H7 mixture and K_{max} values of rifampicin-resistant variants of the six serotype of non-O157 *E.coli* followed by ^ are significantly (student-based t-test, $P<0.05$) different than K_{max} value of rifampicin-resistant *E.coli* O157:H7 mixture.

[b] *P*= parent cells; *R*= Rifampicin-resistant variants; Within each serotype (i.e., O157:H7 mixture, O26. O45, O103. O111. O121, and O145 values of K_{max} values followed by same lowercase letters are not significantly different (student-based t-test, $P\geq 0.05$).

Table 4.5. Non-linear microbial survivor analysis for comparing inactivation rates of drug susceptible and resistant *Salmonella* serovars relative to parent and rifampicin-resistant *E. coli* O157:H7. K_{max1} and K_{max2} (parameter± SE) values are specific inactivation rates for two phases of the biphasic curves fitted for each serotype/serovars, expressing the inactivation rate of reduction phase (K_{max1}) and the phase at which the tested pathogens reached the detection limit (K_{max1}). Both values are expresses in 1/min units indicating longer time required for microbial cell reductions associated to smaller K_{max} values.

Serotype/Serovar	**Number of strains**	**Phenotype**	**K_{max1}** (1/min)	**K_{max2}** (1/min)	**4D Reduction (min)**
E. coli O157:H7 [a]	A five-strain mixture	parent cells	1.34±0.36	1.34±0.10	7.20
E. coli O157:H7	A five-strain mixture	Rifampicin-resistant	2.32±0.75	0.22±0.40	6.64
Salmonella Typhimurium	4 single strains	Susceptible	4.18±0.61*	0.00±0.28*	2.24
Salmonella Typhimurium	6 single strains	MDR	7.67±0.10*^	0.12±0.10*	1.32
Salmonella Typhimurium	6 single strains	MDR-AmpC	7.12±0.08*^	0.14±0.08*	1.20
Salmonella Newport	4 single strains	Susceptible	7.02±0.39*^	0.00±0.29*	1.36
Salmonella Newport	13 single strains	MDR-AmpC	4.55±0.09*^	0.08±0.09*	1.60

[a] K_{max} values (parameter± SE, [1/min]) of *Salmonella* serovars followed by * are significantly (student-based t-test, $P< 0.05$) different than K_{max} value of parent cells of *E.coli* O157:H7 mixture and K_{max} values of *Salmonella* serovars followed by ^ are significantly (student-based t-test, $P<0.05$) different than K_{max} value of rifampicin-resistant *E.coli* O157:H7 mixture.

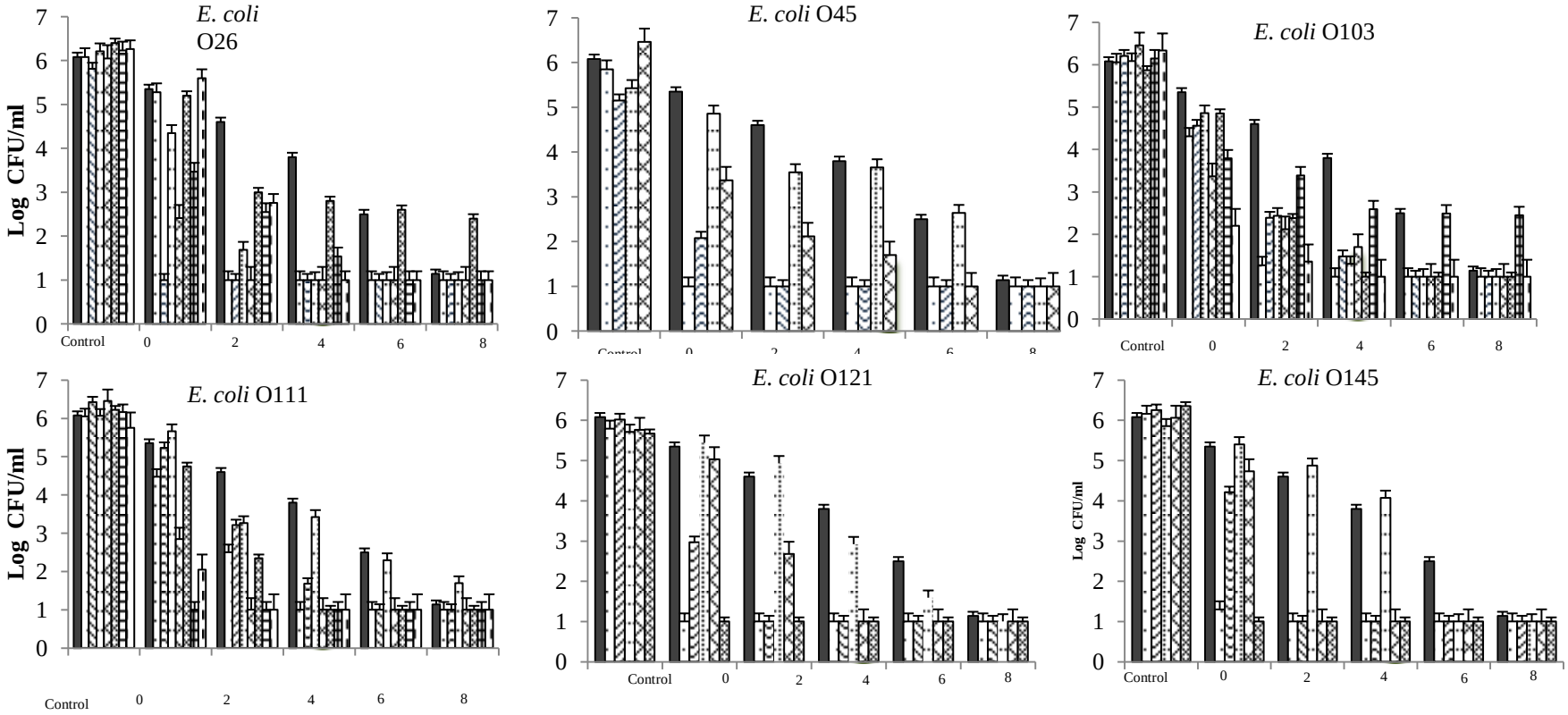
E. coli O26
E. coli O45
E. coli O103
E. coli O111
E. coli O121
E. coli O145
Log CFU/ml
Control
0
2
4
6
8

Figure 4.1 (Data in Appendix Tables A7 to A12). Survival of wild-type non-O157 *E. coli* strains (log CFU/ ml) compared to 5-strain mixture of *E. coli* O157:H7 in beef homogenate as affected by 5% Lactic acid treatment at 25°C (TSA counts). The left bar (solid black) in all figures show counts of *E. coli* O157:H7 5-strain mixture (ATCC 43888, ATCC 43895, C1-057, C1-072, and C1-109), rest of the bars from left to right in for each serogroup represent isolation source (strain identification ID). ***E. coli* O26:** Human (hSTEC_03); Beef (imp_113.1); Antelope (81.0211); Pig (85.1150); Human (93.0494); Cow (0.1302); Human (5.2217). ***E. coli* O45:** Human (99E_2750); Human (O45-2); Human (05-6545); Human (96-3285); ***E. coli* O103:** Human (hSTEC_05); Beef (MDR0089); Horse (3.2607); Mouse (86.0865); Goat (87.1368); Cow (90.1764); Human (92.0084). ***E. coli* O111:** Human (93.0523); Cow (4.0005); Cow (4.0522); Pig (85.1154); Human (93.522); Human (hSTEC_08); Human (18DA). ***E. coli* O121:** Human (10896); Beef (imp_450); Human (97-3068); Human (03-4064); Human (08023). ***E. coli* O145:** Human (hSTEC_22); Beef (MAY109); Human (03-4699); Human (83-75); Cow (07865).

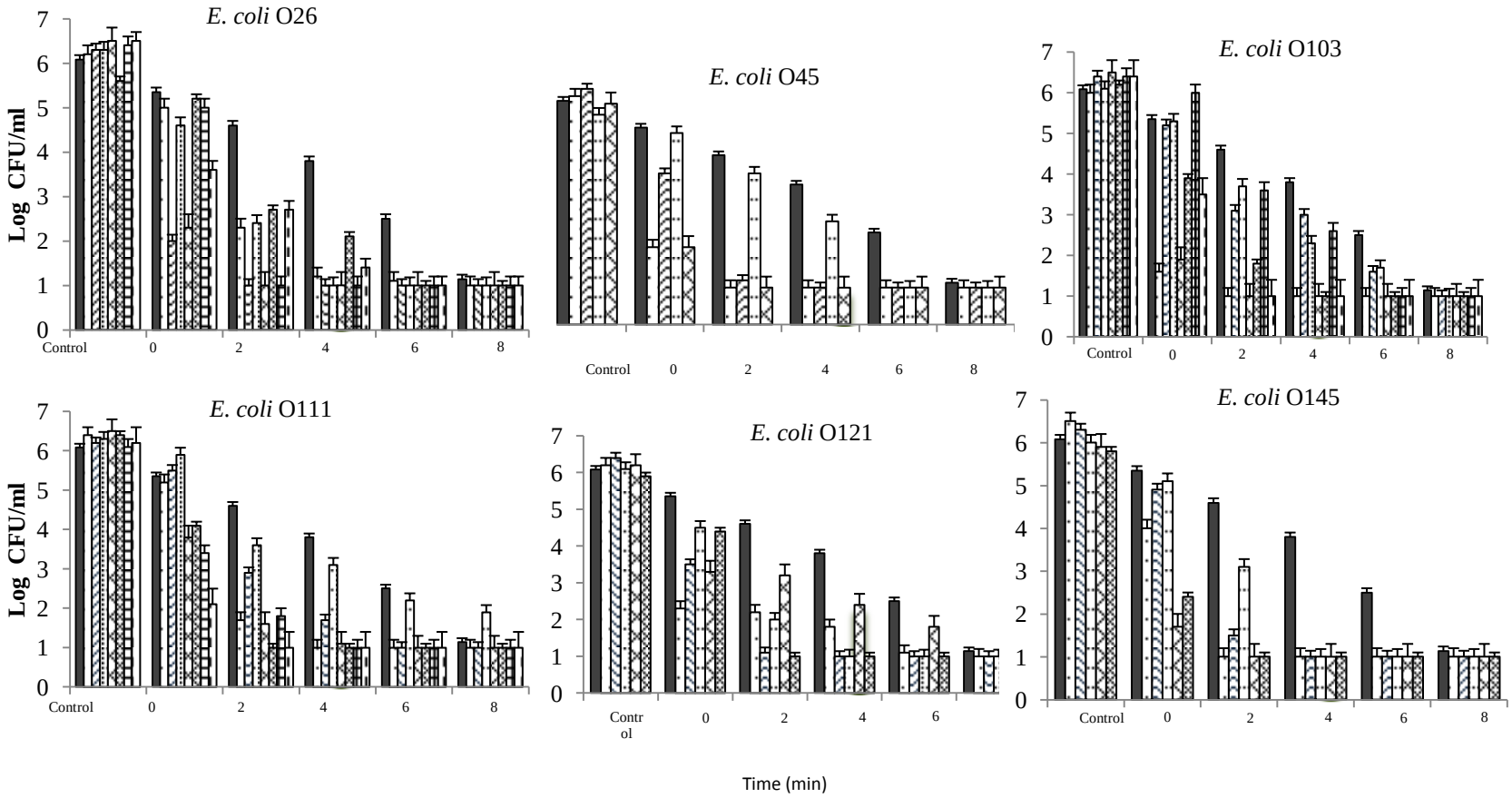
E. coli O26
E. coli O45
E. coli O103
E. coli O111
E. coli O121
E. coli O145
Log CFU/ml
Control
0
2
4
6
8
Time (min)

Figure 4.2 (Data in Appendix Tables A7 to A12). Survival of rifampicin-resistant non-O157 *E. coli* strains (log CFU/ ml) compared to 5-strain mixture of *E. coli* O157:H7 in beef homogenate as affected by 5% Lactic acid treatment at 25°C (TSA+rif counts). The left bar (solid balck) in all figures show counts of *E. coli* O157:H7 5-strain mixture (ATCC 43888, ATCC 43895, C1-057, C1-072, and C1-109), rest of the bars from left to right in for each serogroup represent isolation source (strain identification ID). ***E. coli* O26:** Human (hSTEC_03); Beef (imp_113.1); Antelope (81.0211); Pig (85.1150); Human (93.0494); Cow (0.1302); Human (5.2217). ***E. coli* O45:** Human (99E_2750); Human (O45-2); Human (05-6545); Human (96-3285); ***E. coli* O103:** Human (hSTEC_05); Beef (MDR0089); Horse (3.2607); Mouse (86.0865); Goat (87.1368); Cow (90.1764); Human (92.0084). ***E. coli* O111:** Human (93.0523); Cow (4.0005); Cow (4.0522); Pig (85.1154); Human (93.522); Human (hSTEC_08); Human (18DA). ***E. coli* O121:** Human (10896); Beef (imp_450); Human (97-3068); Human (03-4064); Human (08023). ***E. coli* O145:** Human (hSTEC_22); Beef (MAY109); Human (03-4699); Human (83-75); Cow (07865).

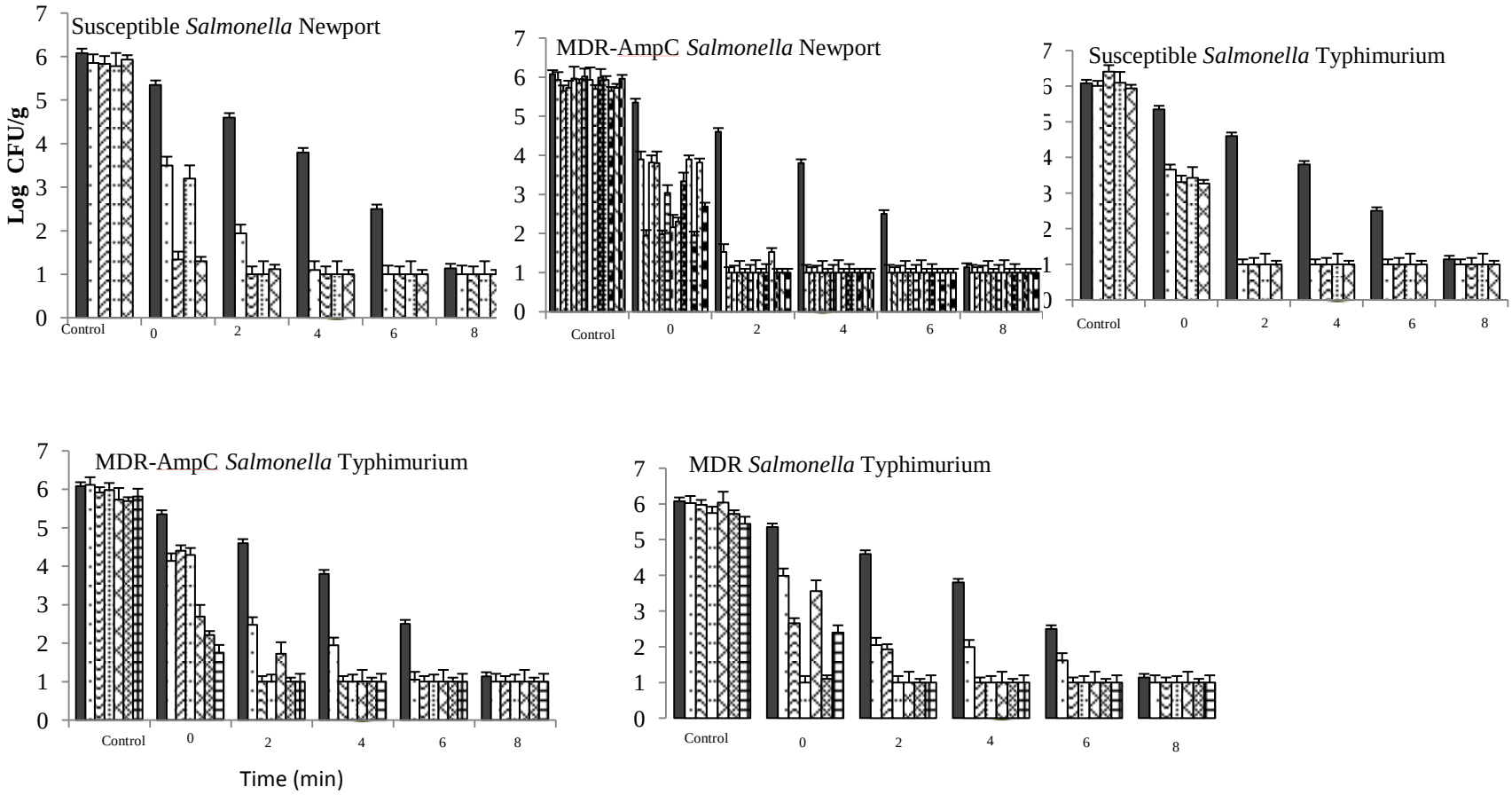
Susceptible *Salmonella* Newport
MDR-AmpC *Salmonella* Newport
Susceptible *Salmonella* Typhimurium
MDR-AmpC *Salmonella* Typhimurium
MDR *Salmonella* Typhimurium
Log CFU/g
Control
0
2
4
6
8
Time (min)

Figure 4.3 (Data in Appendix Tables A13 to A17). Survival of susceptible, MDR and/or MDR-AmpC *Salmonella* Newport and Typhimurium strains (log CFU/ ml) compared to 5-strain mixture of *E. coli* O157:H7 in beef homogenate as affected by 5% Lactic acid treatment at 25°C (TSA counts). **Susceptible *Salmonella* Newport:** Turkey (CVM N4505); Beef (CVM N18445); Turkey (CVM N1509); Human (FSL S5-639). **MDR-AmpC Salmonella Newport:** Human (FSL R6-531); Human (FSL R8- 0104); Human (FSL S5-413); Bovine (FSL S5- 436); Bovine (FSL S5-577); Bovine (FSL S5-920); Human (FSL R8-2926); Bovine (FSL R8-2350); Beef (CVM N635); Pork (CVM 22698); Beef (CVM 29461); Beef (CVM 22707); Beef (CVM N19852). **Susceptible *Salmonella* Typhimurium:** Chicken (CVM N7300); Beef (CVM N15788); Chicken (CVM N18534); Human (FSL S5-536). **MDR *Salmonella* Typhimurium:** Human (FSL R6-215); Bovine (FSL S9-165); Human (FSL R8-2540); Chicken (CVM N6431); Chicken (CVM 30662); Pork (CVM N497). **MDR-AmpC *Salmonella* Typhimurium:** Bovine (FSL S5-786); Bovine (FSL S5-786); Bovine (FSL S5-916); Chicken (CVM N176); Cattle (CVM 33831); Turkey (CVM 30034).

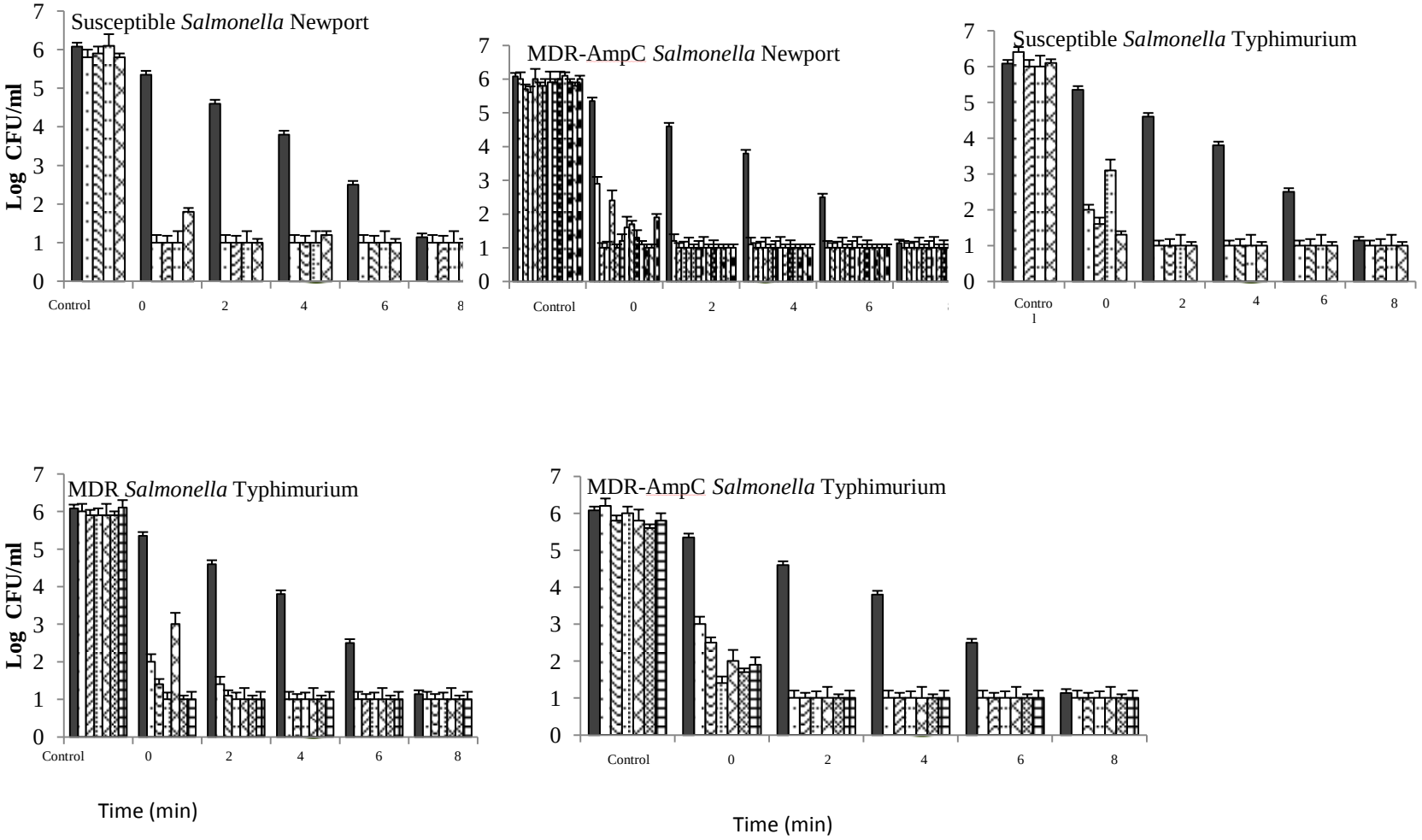
Susceptible *Salmonella* Newport
MDR-AmpC *Salmonella* Newport
Susceptible *Salmonella* Typhimurium
MDR *Salmonella* Typhimurium
MDR-AmpC *Salmonella* Typhimurium
Log CFU/ml
Control
0
2
4
6
8
Time (min)

Figure 4.4 (Data in Appendix Tables A13 to A17). Survival of susceptible, MDR and/or MDR-AmpC *Salmonella* Newport and Typhimurium strains (log CFU/ ml) compared to 5-strain mixture of *E. coli* O157:H7 in beef homogenate as affected by 5% Lactic acid treatment at 25°C (XLD counts). **Susceptible *Salmonella* Newport:** Turkey (CVM N4505); Beef (CVM N18445); Turkey (CVM N1509); Human (FSL S5-639). **MDR-AmpC Salmonella Newport:** Human (FSL R6-531); Human (FSL R8- 0104); Human (FSL S5-413); Bovine (FSL S5- 436); Bovine (FSL S5-577); Bovine (FSL S5-920); Human (FSL R8-2926); Bovine (FSL R8-2350); Beef (CVM N635); Pork (CVM 22698); Beef (CVM 29461); Beef (CVM 22707); Beef (CVM N19852). **Susceptible *Salmonella* Typhimurium:** Chicken (CVM N7300); Beef (CVM N15788); Chicken (CVM N18534); Human (FSL S5-536). **MDR *Salmonella* Typhimurium:** Human (FSL R6-215); Bovine (FSL S9-165); Human (FSL R8-2540); Chicken (CVM N6431); Chicken (CVM 30662); Pork (CVM N497). **MDR-AmpC *Salmonella* Typhimurium:** Bovine (FSL S5-786); Bovine (FSL S5-786); Bovine (FSL S5-916); Chicken (CVM N176); Cattle (CVM 33831); Turkey (CVM 30034).

CHAPTER V

Sensitivity to lactic acid of shiga toxin-producing *Escherichia coli* and susceptible and multidrug-resistant *Salmonella* Newport and Typhimurium inoculated on beef trimmings

SUMMARY

Shiga toxin-producing *Escherichia coli* and *Salmonella* are of concern in meat and there is interest as to whether lactic acid decontamination treatments are adequate for their reduction in beef trimmings. The efficacy of lactic acid (LA) decontamination of beef trimmings was evaluated against (i) six non-O157 Shiga toxin-producing *E. coli* (nSTEC) serogroups and (ii) antibiotic susceptible and multidrug resistant *S.* Newport and *S.* Typhimurium. The antimicrobial effects against these pathogens were compared to those obtained against *E. coli* O157:H7. Four-strain mixture inocula of rifampicin-resistant *E. coli* serogroups O157:H7, O26, O45, O103, O111, O121 and O145, and antibiotic susceptible and multidrug resistant (MDR and/or MDR-AmpC) *S.* Newport and *S.* Typhimurium were evaluated on beef trimmings (100-g pieces). The inoculated (3 log CFU/cm^2) trimmings were immersed (30 s) in solutions of LA (5%, 25 and 55°C). Pathogen populations on untreated and treated samples were enumerated (two or three repetitions, three samples each), and data were analyzed as a complete randomized block design. Initial levels (3.1-3.3 log CFU/cm^2) of *E. coli* O157:H7 and nSTEC serogroups were reduced (P<0.05) by 0.7 (*E. coli* O157:H7) and 0.4-0.9 (nSTEC) log CFU/cm^2 in 25°C LA-treated samples, and 1.4 (*E. coli* O157:H7) and 1.0-1.3 (nSTEC) log CFU/cm^2 in 55°C LA-treated samples. No differences (P≥0.05) were obtained between surviving counts of the six nSTEC

serogroups and those of *E. coli* O157:H7. LA at 25°C and 55°C reduced ($P<0.05$) *Salmonella* counts (3.0-3.3 log CFU/ cm^2) by 1.2-1.5 and 1.5-1.9 log CFU/cm^2, respectively, while corresponding *E. coli* O157:H7 reductions were 0.5 and 1.2 log CFU/cm^2, respectively. Reductions of *Salmonella* counts were not influenced by serovar or antibiotic resistance phenotype, and were similar ($P\geq0.05$) or higher ($P<0.05$) than reductions of *E. coli* O157:H7. The results indicated that LA decontamination of beef trimmings can be as effective against the six nSTEC serogroups and antibiotic susceptible and multidrug resistant *S.* Newport and *S.* Typhimurium as it is against *E. coli* O157:H7.

INTRODUCTION

Shiga toxin-producing *E. coli* and *Salmonella* are of major concern in primary and further processing of meat and meat products (Jay *et al*., 2005). Due to prevalence of shiga toxin-producing *E. coli* (STEC) and *Salmonella* serovars in muscle foods (Barkocy-Gallagher *et al*., 2003; Bosilevac *et al.*, 2009), inactivation of these pathogens has been the subject of many recent academic and industrial investigations in pre- and post-harvest processing of muscle foods (Sofos, 2009; Oliver *et al*., 2009). Consumption of undercooked ground beef, roast beef, smoked meat products, sausages and nonintact steaks contaminated with O157 and non-O157 serotypes of *E. coli* is associated with reported outbreaks and sporadic cases of human illness (Hussein, 2007; Hussein and Bollinger, 2005a, b). *E. coli* related foodborne illness symptoms range from mild diarrhea, abdominal pain, vomiting, and bloody diarrhea to hemorrhagic colitis (HC) and Hemolytic Uremic Syndrome (HUS) (Nataro and Kaper, 1998). Although rare in occurrence, HUS can affect infected hosts, especially infants, children and the elderly, and is characterized by thrombocytopenia, microangiopathic hemolytic anemia, and acute renal failure (Hussein, 2007). From 1984 to 2007, 146 STEC outbreaks and sporadic cases of human illness had been

traced to consumption of beef contaminated with O157 and non-O157 serotypes of *E. coli,* with 88% of the cases traced back directly to ground and nonintact beef (Hussein and Bollinger, 2005a). Along with *E. coli* O157:H7, in recent years, other serotypes of EHEC have been involved in foodborne episodes. A recall of ground beef associated with *E. coli* O26 by a Pennsylvania company (USDA-FSIS, 2011), a recall of ground beef contaminated with *E. coli* O111 in Japan (USDA-FSIS, 2011), and an outbreak of beef sausage contaminated with *E. coli* O26 in Denmark (Ethelberg *et al.,* 2009) are some of the most recent examples. *E. coli* O26, O45, O103, O111, O121, and O145 serotypes are responsible for the majority of episodes of foodborne illness and HUS, associated with non-O157 shiga toxin-producing *E. coli* in the United States (Bethelheim, 2007; Mathusa *et al.,*2010; Grant *et al.,* 2011). Most recent epidemiological investigations estimate every year in the United States, *E. coli* O157:H7 is responsible for 3,268 illness episodes with 46.2% and 0.5% hospitalization and death rates, respectively (Scallan *et al*., 2011). Similarly, it is estimated that 1,579 illness episodes, with 12.8% hospitalization rate and 0.3% death rate are caused by non-O157 STEC strains every year (Scallan *et al*., 2011).

As one of the most researched pathogens, inactivation of *salmonella* by various interventions has been subject of research for more than 100 years (FDA, 2009). More than 30 Salmonellosis outbreaks in the United States and around the world have been associated with fresh meat as well as processed low-moisture food products in recent years (FDA, 2009). In addition multidrug resistant (MDR) and multidrug resistant *Salmonella* serovars with acquired *AmpC* gene (MDR-Amp*C*) exist in the food chain and cause crucial challenges in antibiotic chemotherapy of foodborne Salmonellosis (Bosilevac *et al.,* 2009; Arthur *et al*., 2008). Recent investigations indicate that 0.6% of ground meat samples may harbor drug-resistant *Salmonella*

(Bosilevac *et al*., 2009) with approximately 7% of them displaying a MDR-AmpC phenotype (Zhao *et al*., 2009). Most recent epidemiological investigations estimate that every year in the United States, nontyphoidal *Salmonella* serovars are responsible for 1,229,007 illness episodes with 27.2% and 0.5% hospitalization and death rates, respectively (Scallan *et al*., 2011). A multi-state outbreak linked to fresh ground beef contaminated with MDR strains of *S.* Typhimurium in 2012 is a recent example of the involvement of MDR *Salmonella* serovars in foodborne episodes (Food Safety News, 2012).

Due to involvement of *E. coli* O157:H7 in numerous national and international foodborne episodes in the last few decades, this pathogen had been the primary target of validation plans and food safety management systems (Sofos, 2009; Oliver *et al*., 2009). The purpose of this experiment was to determine whether lactic acid resistance of CDC's top-six non-O157 STEC as well as multidrug resistant and susceptible *Salmonella* Newport and Typhimurium is different than *E. coli* O157:H7 inoculated on beef trimming.

MATERIALS AND METHODS

Preparation of bacterial strains and inoculation. In experiment one, 4-strain mixtures of food and human-disease associated *E. coli* O157:H7, and of each of O26, O45, O103, O111, O121, and O145 serogroups kindly provided by Drs. Wheeler , DebRoy, and Fratamico (Table 5.1), and in experiment two 4-strain mixtures for each of MDR, MDR-AmpC, and susceptible *S.* Typhimurium, and MDR, MDR-AmpC *S.* Newport, kindly provided by Drs. Zhao and Wiedmann (Table 5.2), were used.

In the experiment of non-O157 STEC, in order to facilitate selective enumeration of the STEC inocula from the natural meat microflora of beef trimmings, rifampicin-resistant (100

μg/ml) variants of the STEC strains were selected as described by Kaspar and Tamplin (1993). Use of strains with a selective marker, like antibiotic resistance, also allows recovery of cells injured by exposure to chemical decontamination treatments, by plating the cells on a non-selective medium (tryptic soy agar, in our studies) supplemented with the antibiotic (100 μg/ml rifampicin, in this studies). For both experiments, rifampicin-resistant variants of *E. coli* O157:H7 strains were used as controls and were available at the Pathogen Reduction Laboratory of the Center for Meat Safety and Quality of Colorado State University, and included ATCC 43888, ATCC 43895, C1-057, and C1-072 (Carlson *et al.,* 2009).

Each strain was activated separately from a single colony of the stored stock as described by Yang *et al.* (2009b). Each strain was then washed and centrifuged at 4,629 *g* for 15 min with 10 ml of sterile saline (0.85% NaCl) and resuspended in 10 ml Phosphate-buffered Saline (PBS, 0.2 g KH_2PO_4, 1.5 g $Na_2Hpo_4.7H_2O$, 8.0 g NaCl, and 0.2 g KCl per liter) (Yang *et al.,* 2009a). After suspension, four strains of each serotype were combined and tenfold serially diluted. The surface of the meat samples (area approximately 100 cm^2) was then inoculated with 100 μL per side of the diluted composite of each pathogen serotype, with a 15 min interval between inoculation of each side, to achieve an initial inoculation level of approximately 3-4 Log CFU/g.

Application of lactic acid and microbiological analyses. Inoculated meat samples were inserted into a bag (Whirl-Pak, Modesto, CA) with 150 mL of 5% lactic acid (Purac America INC., Lincolnshire, IL) at 25 or 55°C for 30 seconds, removed with sterile forceps and drained for 60 seconds on a sterile plastic strainer. Treated samples were then stored for 1 hour in a 4°C refrigerator to simulate the testing condition from an industrial setting to a testing laboratory. Prior and after lactic acid intervention, weights of the samples were recorded to determine the moisture uptake due to the intervention. Before and after acid intervention and 24 hour after

intervention, pH (Denver Instrument, Arvada, CO) values of samples were measured following standard procedures described by Byelashov *et al*. (2010).

In order to stop action of lactic acid on cells, after acid intervention and exposure period, 100 ml of D/E neutralizing broth (Becton, Dickinson and Company, Sparks, MD) was added to each sample. Samples were then homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min (6 strokes per s), and serially diluted (10-fold) with 0.1% buffered peptone water (Difco, Becton Dickinson, Franklin Lakes, NJ) and spread-plated onto surface of Trypticase Soy Agar (TSA, Becton, Dickinson and Company, Sparks, MD), TSA with 5 mg/liter Rifampicin (TSA+Rif), and modified SMAC (during *Salmonella* experiment only; mSMAC, Mc Conkey Sorbitol Agar with 20 mg/l novobiocin, and 2.5 mg/L Potasium Tellurite, Becton, Dickinson and Company, Sparks, MD) for rifampicin-resistant STEC, and TSA and Xylose lysine deoxycholate (XLD) medium (Neogen Corporation, Lansing, MI) for *Salmonella* serovars, and were counted after a incubation period of 24-48 hours at and 30°C.

Statistical analysis. The experiment with the non-O157 STEC serotypes was conducted separately from the study with the *S.* Newport/Typhimurium antibiotic resistance profiles. These studies were conducted twice (for *S.* Newport/Typhimurium) and three times (for the non O157 STEC serotypes) with three samples analyzed per repetition (total of six and nine replicates, respectively). Each repetition was considered as a blocking factor in a complete randomized block design. Microbial counts, transformed into log CFU/cm^2, were statistically compared with ANOVA-based procedures followed by Tukey-adjusted multiple comparison methods for further mean separation using the PROC MIXED command of SAS (v9.2). In addition to this analysis, a Tukey-adjusted ANOVA test, using the PROC GLM command of SAS, was used to compare counts of samples before and after antimicrobial treatment within each *E. coli* serotypes or *S.*

Newport/Typhimurium antibiotic resistance phenotypes. The pH values were statistically analyzed with a student-based t-test by PROC GLM. In all cases, *P* values less than 0.05 ($P<0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

It should be noted that the study with the non-O157 STEC serotypes was conducted separately from the study with the S. Newport and S. Typhimurium antibiotic resistance profiles. Thus, results are presented and discussed separately.

In the experiment of non-O157 STEC, initial levels of inoculated *E. coli* O157:H7 and the six non-O157 STEC serogroups on beef trimmings ranged from 3.1 to 3.3 log CFU/cm^2 (Figure 5.1 and Appendix Table A18). Treatment of samples with lactic acid applied at 25 or 55°C reduced ($P<0.05$) *E. coli* O157:H7 counts by 0.7 and 1.4 log CFU/cm^2, respectively. Corresponding reductions of the six non-O157 STEC serogroups were 0.4 to 0.9 and 1.0 to 1.3 log CFU/cm^2, respectively (Figure 5.1 and Appendix Table A18). Trends in counts were similar for background microflora and inoculated pathogen as expressed by TSA counts (Figure 5.2 and Appendix Table A19). Overall, initial counts of total bacterial populations were 3.5 to 3.6 log CFU/cm^2 on all inoculated trimmings. Total bacteria counts were reduced ($P<0.05$) by 0.7 to 1.1 and 1.0 to 1.4 log CFU/cm2 following treatment with lactic acid at 25 or 55°C, respectively (Figures 5.1 and 5.2 and Appendix Tables A18 and A19).

The pH of untreated beef trimmings was 5.41±0.31, and was reduced ($P<0.05$) to 4.12±0.32 and 4.03±0.24 after treatment with 25 or 55°C lactic acid solutions, respectively. Following storage of samples at 4°C for 24 h, pH values of 5.44±0.33, 4.36±0.31, and 4.23±0.22 were obtained for untreated trimmings, and trimmings treated with 25 or 55°C lactic acid,

respectively. The moisture uptake of samples decontaminated with lactic acid ranged from 4.7±1.2 to 6.71±1.7% for the 25°C solution, and 5.4±1.9 to 6.5±1.3% for the 55°C solution, with no difference among samples inoculated with different serogroups ($P \geq 0.05$).

Six serogroups of non-O157 STEC and the *E. coli* O157:H7 strain mixture tested in this experiment showed similar ($P \geq 0.05$) resistance to lactic acid at 25°C; microbial counts (TSA+rif) after treatment of non-O157 STEC were less than 0.3 log different than *E. coli* O157:H7 counts ($P < 0.05$) (Figure 5.1 and Appendix Table A18). Similar to the above-mentioned counts of TSA+rif, microbial counts on TSA media (representing both inoculated pathogen and background microflora) for all seven *E. coli* serogroups were not statistically different ($P \geq 0.05$) for lactic acid treatment at 25°C (Figure 5.2 and Appendix Table A19). As expected, the lactic acid treatment at 55°C was more effective ($P < 0.05$) for decontamination of meat samples than 25°C and with no appreciable difference among the seven STEC serogroups (Figures 5.1 and 5.2 and Appendix Tables A18 and A19). Overall, irrespective of lactic acid treatment (25 or 55°C), there were no appreciable differences in surviving counts among all six non-O157 STEC serogroups relative to surviving counts of *E. coli* O157:H7, indicating that the six non-O157 STEC can be controlled as effectively as *E. coli* O157:H7 by lactic acid intervention.

In the experiment with *Salmonella* serovars, counts of inoculated populations of *E. coli* O157:H7 and *Salmonella* serovars on untreated beef trimmings were 3.0 to 3.3 log CFU/cm^2 (Figures 5.3 and 5.4 and Appendix Tables A20 and A21). *E. coli* O157:H7 counts were reduced ($P < 0.05$) by 0.5 (TSA+rif) and 0.8 (mSMAC) log CFU/cm^2 after treatment of samples with 25°C lactic acid, and 1.2 (TSA+rif) and 1.5 (mSMAC) log CFU/cm2 after treatment with 55°C lactic acid (Figures 5.3 and 5.4 and Appendix Tables A20 and A21). Overall, decontamination of trimmings with 25 or 55°C lactic acid solutions reduced *Salmonella* counts by 1.2 to 1.5 and 1.5

to1.9 log CFU/cm^2, respectively (Figures 5.3 and 5.4 and Appendix Tables A20 and A21). Lactic acid decontamination of trimmings reduced (P<0.05) the pH of samples from 4.90±0.23 (untreated control) to 4.22±0.21 (25°C solution) and 4.23±0.11 (55°C solution). The pH values of treated samples were 4.53±0.21 (25°C solution) and 4.60±0.11 (55°C solution) after 24 h at 4°C. The moisture uptake of samples treated with 25 or 55°C solutions of lactic acid was 4.6±0.7 to 6.6±1.8 % and 4.7±2.1 to 8.0±3.5 %, respectively, with no difference among the serogroups (P≥0.05).

MDR *S.* Typhimurium, MDR-AmpC *S.* Typhimurium and Newport, and susceptible *S.* Typhimurium and Newport after lactic acid treatment at 25°C showed lower (P<0.05) counts (TSA+rif) than *E. coli* O157:H7 and had with similar trends when treated at 55°C (Figures 5.3 and 5.4 and Appendix Tables A20 and A21). TSA counts, representing both inoculated pathogen and background microflora, of samples inoculated with the five serovars of *Salmonella* were, however, not significantly (P≥0.05) different than *E. coli* O157:H7 in most cases (Figures 5.5 and Appendix Tables A22). *Salmonella* counts, irrespective of serogroup or antibiotic resistance profile, were similar (P≥0.05) or lower (P<0.05; by 0.7-1.1 and 0.4-0.6 log CFU/cm2 compared with TSA+rif and mSMAC counts of *E. coli* O157:H7, respectively) than counts of *E. coli* O157:H7 following decontamination of samples with 25 or 55°C lactic acid solutions (Figures 5.3 and 5.4 and Appendix Tables A20 and A21).

Multidrug resistant (MDR) and MDR *Salmonella* serovars with acquired *AmpC* gene (MDR-Amp*C*) appearance in the food chain causes crucial challenges in antibiotic chemotherapy of foodborne salmonellosis (Bosilevac *et al.*, 2009; Arthur *et al*., 2008). Recent investigations indicate that 0.6% of ground meat samples may harbor drug-resistant *Salmonella* (Bosilevac *et al*., 2009), with approximately 7% of them displaying a MDR-AmpC phenotype (Zhao *et al*.,

2009). Results of recent studies also show the wide-spread presence of STEC in muscle foods. It is estimated that 0.1 to 43.0% of carcasses in packing plants, 0.01 to 43.3% sub-primal cuts in packing plants, 0.1 to 4.4% of meat cuts in supermarkets, average of 2.4% meat products used in fast food chains, 0.1 to 54.2% of ground beef, and 0.1 to 4.4% of sausages in supermarket can be contaminated with O157 STEC (Hussein and Bollinger, 2005b). Similarly, 1.7 to 58.0% of sub-primal cuts in packing plants, 3.0 to 62.5% in supermarkets, average of 3.0% of meat products used in fast food chains, 2.4 to 30.0% of ground beef, 17.0 to 49.2% of sausages, and 1.7 to 58.0% of carcasses in packing plants were contaminated with non-O157 STEC (Hussein and Bollinger, 2005b). Although due to involvement of *E. coli* O157:H7 in numerous national and international foodborne episodes in last few decades, this pathogen had been the primary target of validation plans and food safety management systems (Sofos, 2009; Oliver *et al*., 2009), but results of this study show that these emerging pathogens are less or as acid tolerant as *E. coli* O157:H7 indicating that currently in-place lactic acid interventions in primary processing of red meat is most probably efficient against these emerging pathogens as well.

Table 5.1. Shiga toxin-producing *Escherichia coli* strains used in the experiment.

***E. coli* serogroup**	**Strain (alternate ID given by providing institution)**	**Source**
O26:H11	hSTEC_03 (O26-1)	Human[1]
O26:H2	93.0494 (A1-008)	Human[2]
O26	0.1302 (A1-009)	Cow[2]
O26:H11	5.2217 (A1-010)	Human[2]
O45	99E_2750 (O45-1)	Human[1]
O45	O45-2	Human[1]
O45:H2	05-6545	Human[3]
O45:H2	96-3285	Human[3]
O103	MDR0089 (O103-2)	Beef[1]
O103:H2	87.1368 (A1-013)	Goat[2]
O103:H2	90.1764 (A1-014)	Cow[2]
O103:H2	92.0084 (A1-015)	Human[2]
O111:H8	hSTEC_08 (O111-1)	Human[1]
O111	93.0523 (A1-001)	Human[2]
O111	4.0005 (A1-002)	Cow[2]
O111	4.0522 (A1-003)	Cow[2]
O121	10896 (O121-1)	Human[1]
O121	imp_450 (O121-2)	Beef[1]
O121:H19	97-3068	Human[3]
O121:NM	03-4064	Human[3]
O145:NM	hSTEC_22 (O145-1)	Human[1]
O145	MAY109 (O145-2)	Beef[1]
O145:NM	03-4699	Human[3]
O145:H28	07865	Cow[3]
O157:H7	ATCC 43895	Raw meat[4]
O157:H7	C1-057	Bovine fecal[4]
O157:H7	C1-072	Bovine fecal[4]
O157:H7	C1-109	Bovine fecal[4]

Isolates kindly provided by (1) Dr. Tommy Wheeler (U.S. Meat Animal Research Center, USDA-ARS-NPA, Clay Center, NE), (2) Dr. Chitrita DebRoy (*E. coli* Reference Center, The Pennsylvania State University, University Park, PA), (3) Dr. Pina Fratamico (Eastern Regional Research Center, USDA-ARS-NAA, Wyndmoor, PA). (4) Isolates were available in center for red meat safety and quality of Colorado State University.

Table 5.2. *Salmonella* strains used in the experiment.

***Salmonella* serotype**	**Strain (alternate ID given by providing institution)**	**Strain**	**Source**
Newport	Susceptible	FSL S5-639	Human[1]
Newport	Susceptible	CVM N4505	Ground turkey[2]
Newport	Susceptible	CVM N18445	Ground beef[2]
Newport	Susceptible	CVM N1509	Ground turkey[2]
Newport	MDR-AmpC	FSL S5-436	Bovine[1]
Newport	MDR-AmpC	FSL S5-920	Bovine[1]
Newport	MDR-AmpC	CVM 22698	Pork chop[2]
Newport	MDR-AmpC	CVM N19852	Ground beef[2]
Typhimurium	Susceptible	FSL S5-536	Human[1]
Typhimurium	Susceptible	CVM N7300	Chicken breast[2]
Typhimurium	Susceptible	CVM N15788	Ground beef[2]
Typhimurium	Susceptible	CVM N18534	Chicken breast[2]
Typhimurium	MDR	FSL R6-215	Human[1]
Typhimurium	MDR	FSL R8-2540	Human[1]
Typhimurium	MDR	CVM N6431	Chicken breast[2]
Typhimurium	MDR	CVM 30662	Chicken breast[2]
Typhimurium	MDR-AmpC	FSL S5-786	Bovine[1]
Typhimurium	MDR-AmpC	CVM N176	Chicken breast[2]
Typhimurium	MDR-AmpC	CVM 33831	Cattle[2]
Typhimurium	MDR-AmpC	CVM 30034	Ground turkey[2]

Isolates kindly provided by (1)Dr. Martin Wiedmann (Department of Food Science, Cornell University, Ithaca, NY) and (2) Dr. Shaohua Zhao (Center for Veterinary Medicine, U.S. FDA, Laurel, MD).

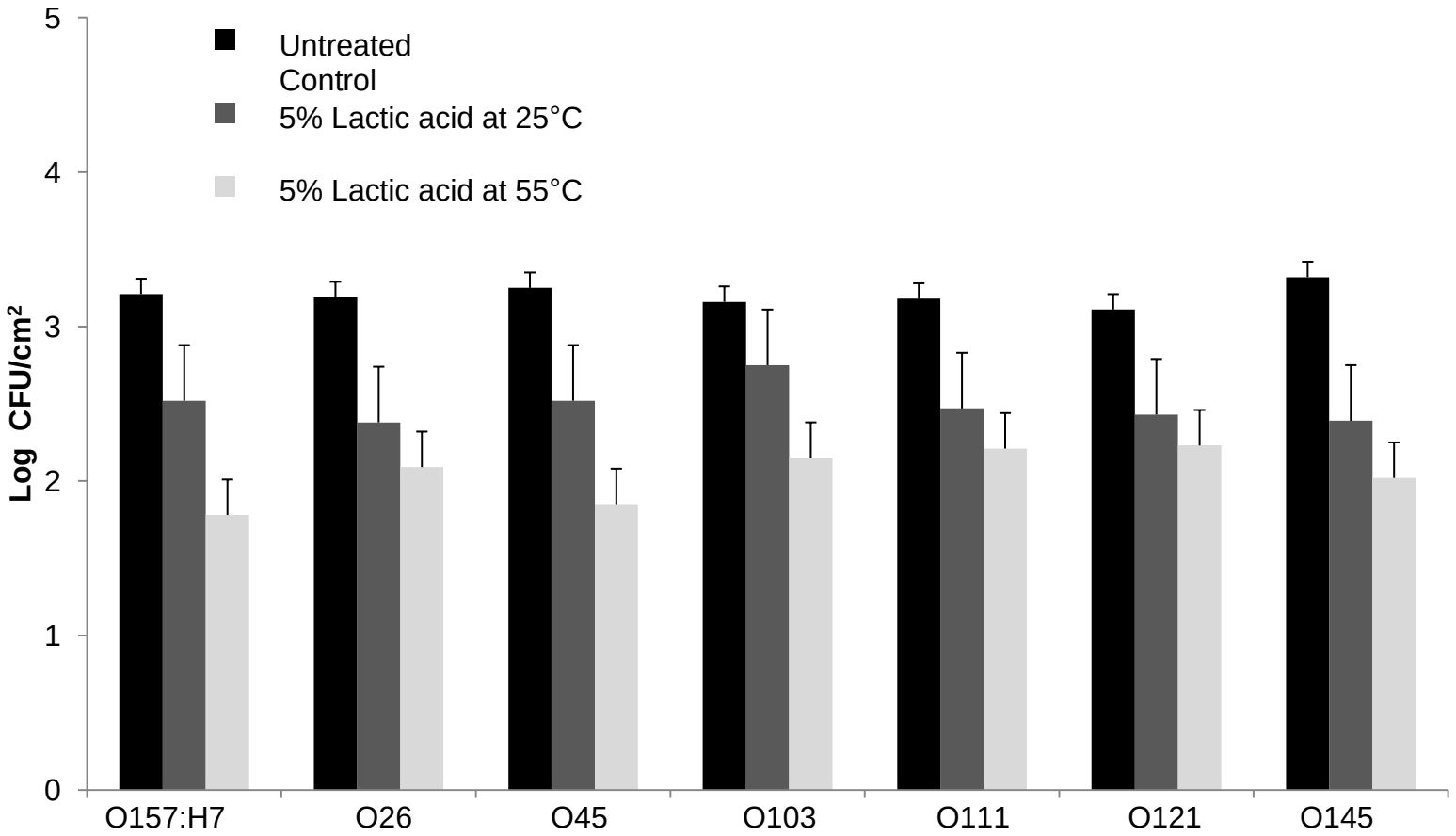

Figure 5.1 (Data in Appendix Table A18). Effects of lactic acid immersion interventions at 25°C and 55°C against 7 inoculated serogroups/serogroups of Shiga toxin-producing *E. coli* (TSA+Rif counts) on beef trimmings.

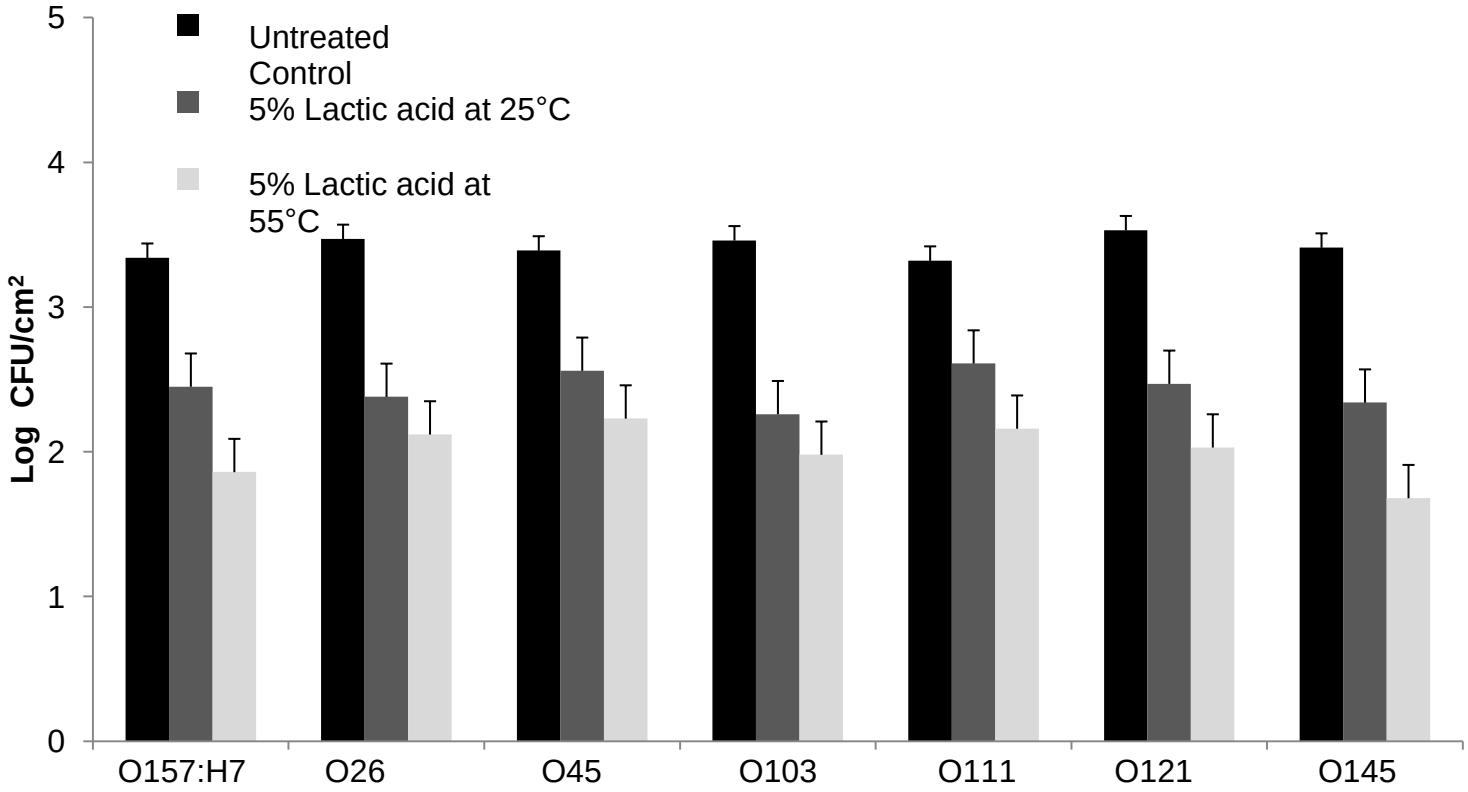

Figure 5.2 (Data in Appendix Table A19). Effects of lactic acid immersion interventions at 25°C and 55°C against total bacterial populations (TSA counts) on beef trimmings.

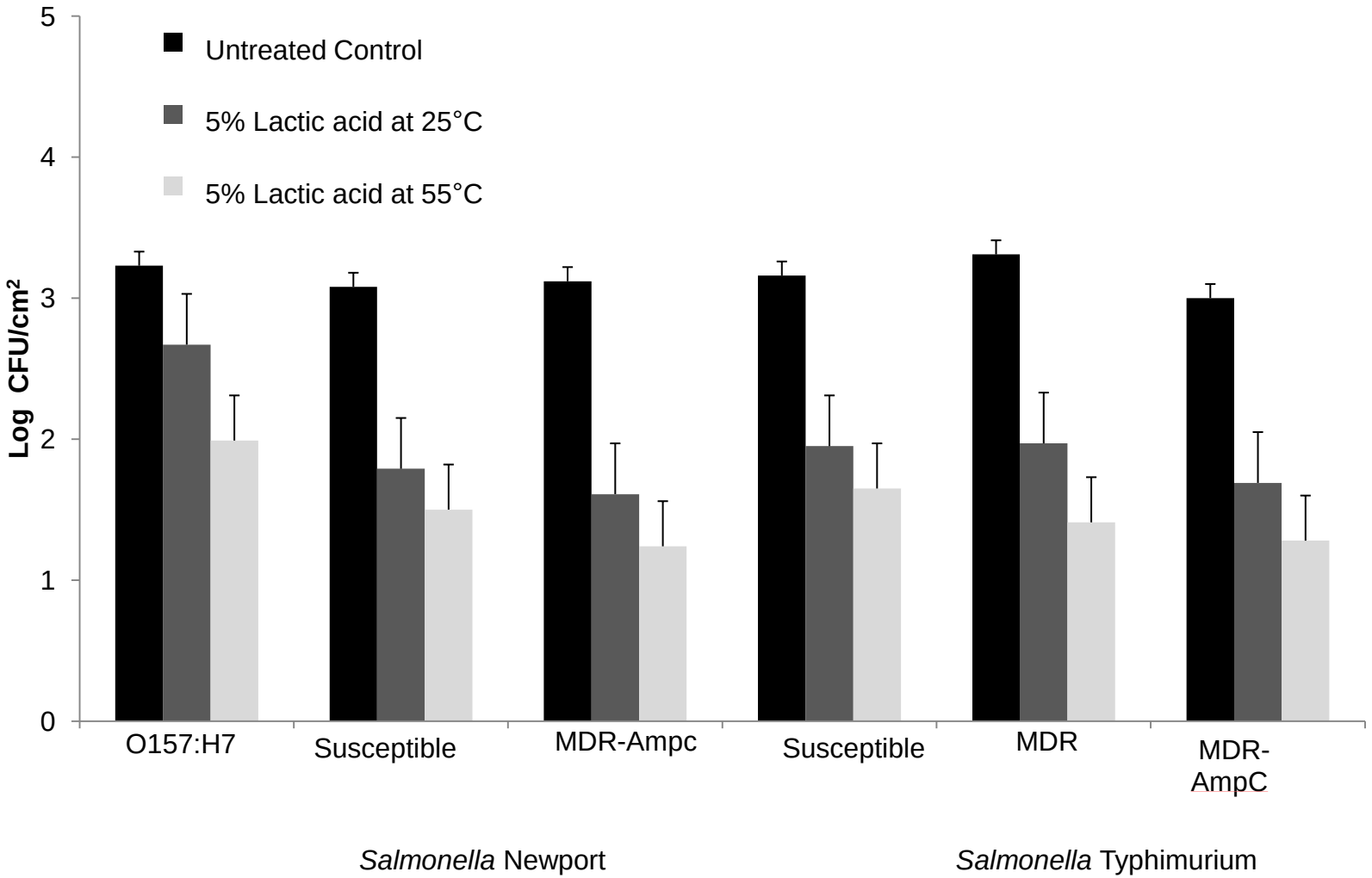

Figure 5.3 (Data in Appendix Table A20). Effects of lactic acid immersion interventions at 25°C and 55°C against inoculated *E. coli* O157:H7 (TSA+ Rif counts) and antibiotic susceptible and multidrug resistant *S.* Newport/Typhimurium (XLD counts) on beef trimmings.

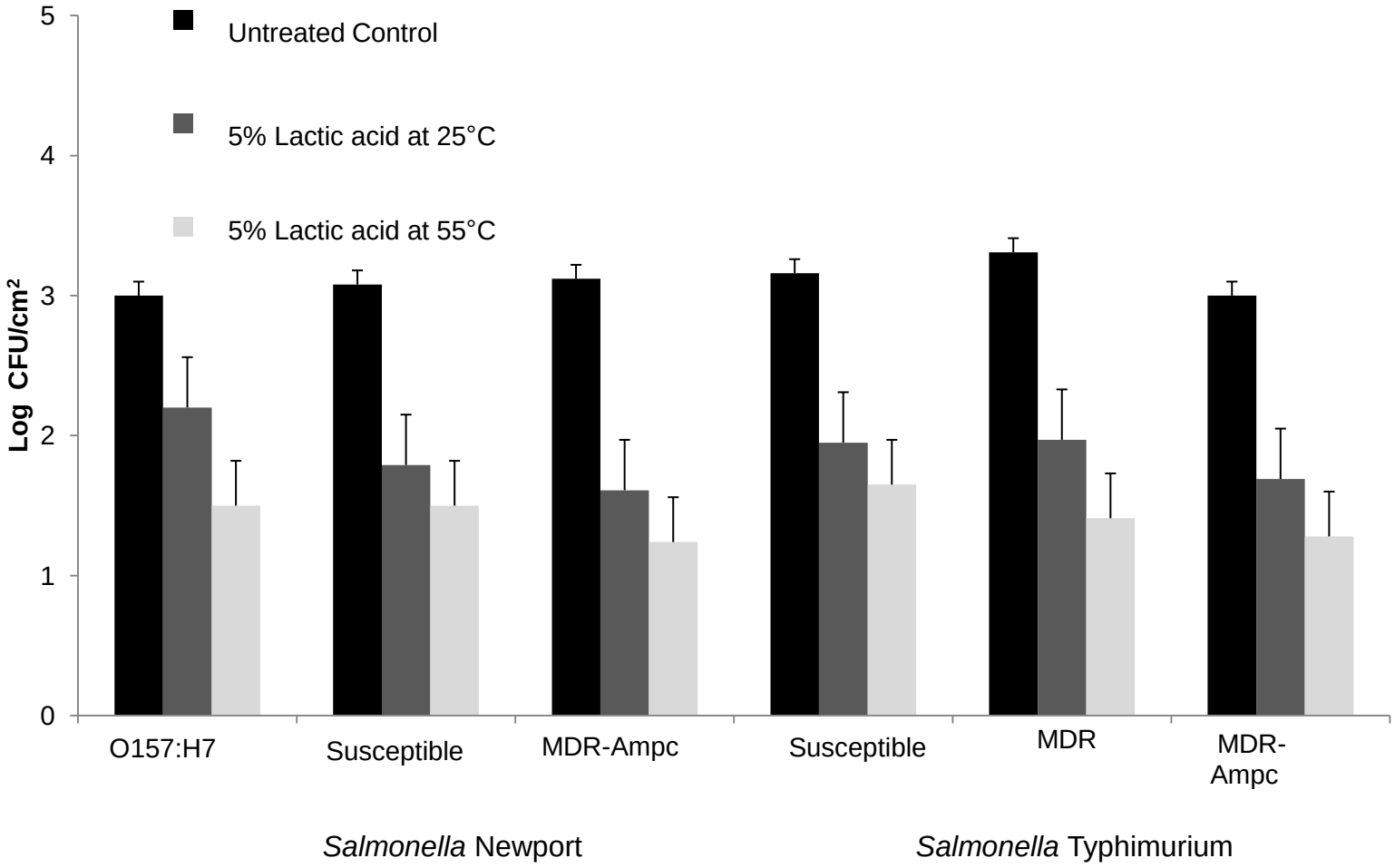

Figure 5.4 (Data in Appendix Table A21). Effects of lactic acid immersion interventions at 25°C and 55°C against inoculated *E. coli* O157:H7 (mSMAC counts) and antibiotic susceptible and multidrug resistant *S.* Newport/Typhimurium (XLD counts) on beef trimmings.

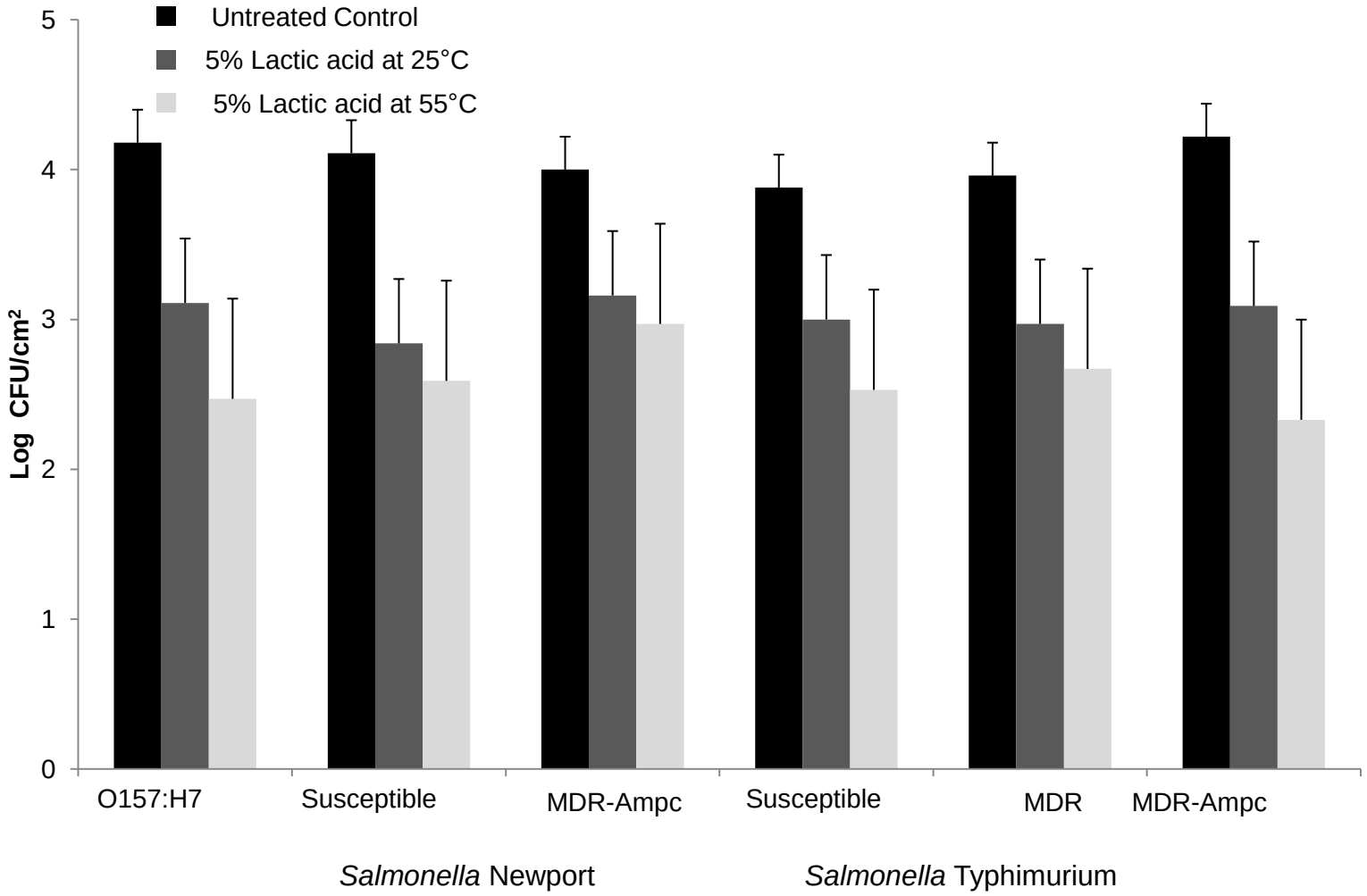

Figure 5.5 (Data in Appendix Table A22). Effects of lactic acid immersion interventions at 25°C and 55°C against total bacterial populations (TSA counts) on beef trimmings.

CHAPTER VI

Effects of reheating against *Listeria monocytogenes* inoculated on cooked chicken breast meat stored aerobically at 7°C

SUMMARY

Presence of *Listeria monocytogenes* in domestic and foodservice environments has increased attention to handling practices during food preparation and storage. The objective of the present study was to evaluate survival and multiplication of *L. monocytogenes* inoculated on cooked chicken breasts which were stored aerobically at 7°C for 7 days. Reduction of pathogen cells by microwave, domestic oven, and stove top reheating was also evaluated. *L. monocytogenes* populations increased from 3.7±0.1 to 7.8±0.2 log CFU/g after 7 days. Microwave oven reheating for 90 seconds, and stove-top and oven-reheating to 70°C internal temperature reduced pathogen populations to <0.4-2.6, <0.4-4.8, and 1.4-5.9 log CFU/g, respectively; numbers of survivors after reheating were higher (P<0.05) in products stored for increasing length of time up to 7 days. At shorter microwaving times and lower product internal temperatures (stove-top and oven-reheating), similar reduction trends were observed but with higher levels of survivors after treatment. Although reheating methods in this study reduced *L. monocytogenes* contamination by 2-5 log CFU/g, growth of the pathogen during previous storage allowed high numbers of survivors after reheating, especially after 2 days of storage. This indicates that storage period, and type and intensity of reheating need to be considered for safe consumption of leftovers.

INTRODUCTION

Cooked leftover food, both in the domestic environment and at foodservice establishments, has reduced levels of background microflora. In case of cross-contamination during refrigerated storage, leftover foods may harbor and support growth of foodborne pathogens (Murphy *et al*., 2001), especially psychotropic pathogens such as *Listeria monocytogenes* and *Yersinia enterocolitica* (Jay *et al.,* 2005; Juneja and Sofos, 2010). Many consumers tend to link foodborne illness to food consumed outside the home (Kennedy *et al.,* 2005), but recent epidemiological investigations indicate that poor hygienic practices in the domestic environment is also a major contributing factor to foodborne disease episodes (Redmond *et al.,* 2003). Infrequent hand-washing, poor hand-washing technique, lack of hand-washing prior to food preparation, inadequate cleaning of kitchen surfaces, involvement of pets in the kitchen, touching of face, mouth, nose and/or hair during preparation of food (Jay *et al.,* 1999), as well as improper storage, and inadequate cooking and reheating (James *et al.,* 1992; Griffth *et al.,* 1994; Marklinder *et al.,* 2004*)* are some of the practices that could potentially result in introduction of pathogens of public health significance into food products, and allow their survival and multiplication to levels of concern. Contamination in kitchen environment can be transferred to a food product during preparation and storage. It has been shown that the microbiological profile of cooked food stored in a kitchen refrigerator is very similar to microbial profiles of swabs taken from the same kitchen environments (Toule and Murphy, 1978). In recent years, various studies have detected foodborne pathogens, and specifically *L. monocytogenes*, on kitchen surfaces, dish cloths, sinks, drains, and refrigerators (Gandhi *et al*., 2007; Jackson *et al.,* 2007; Kilonzo-Nthenge *et al.,* 2008) making leftovers potentially hazardous foods for contamination with *L. monocytogenes.*

Listeriosis caused by *L. monocytogenes* is a severe illness, being responsible for 19% of foodborne disease associated deaths in the United States (Scallan *et al.,* 2011). The very young, elderly, pregnant women and the immunocompromized are the most susceptible groups (Ryser and Marth, 2007). Due to the presence of *L. monocytogenes* in a wide array of environments, its halophilic nature, its potential to form biofilms, and its ability to survive and multiply at refrigeration temperatures, it has been of special interest in academic and industrial research in recent years (Sofos 2008; Sofos 2009; Sofos and Geornaras, 2010).

Chicken meat is gaining more popularity, both among domestic consumers and in foodservice establishments; while 30 years ago poultry accounted for approximately 21 % of meat consumption in the United States, it was recently estimated that it accounts for at least 37%, higher than the consumption of beef, pork, or lamb (AMIF 2009; Harley 2000). White muscle tissue (i.e., breast meat) of chicken is considered as one of the most popular cuts of meat for both domestic uses and commercial processing in the United States (AMIF 2009; Harley 2000), and has been involved in many foodborne outbreaks and recalls including a recall of 10,878 kg of cooked chicken breast contaminated with *L. monocytogenes* (FDA 2010).

While many studies have targeted inactivation of *L. monocytogenes* in ready-to eat products (Murphy *et al.,* 2001; Rodriguez-Marval *et al.,* 2009; Shen *et al.,* 2009) limited work has designed to investigate inactivation of *L. monocytogenes* during storage of leftover foods using domestically available appliances. Thus, the objective of this study was to investigate survival and multiplication of background microflora and inoculated *L. monocytogenes* on cooked boneless skinless chicken breasts stored aerobically at 7 °C. The effects of three reheating methods, applied at 0, 1, 2, 4, and 7 days of storage, against the pathogen and background microflora were also investigated.

MATERIALS AND METHODS

Preparation of bacterial inoculum. Five food and human-disease originated strains of *L. monocytogenes*, kindly provided by Dr. Martin Wiedmann (Department of Food Science, Cornel University, Ithaca, NY), representing diverse ribotypes, PFGE patterns, lineages, and serotypes were used in this study (Fugett *et al.*, 2006). These strains were J1-177 (lineage I, Serotype 1/2b), C1-056 (lineage II, Serotype 1/2a), N3-013 (lineage I, Serotype 4b), R2-499 (lineage II, Serotype 1/2a), and N1-227 (lineage I, Serotype 4b), and were kept on PALCAM agar (Difco, Becton Dickinson, Franklin Lakes, NJ) at 4°C prior to the study. Strains were activated separately from a single colony of the stored stock as described by Yang *et al.* (2009a). Each strain was then washed and centrifuged at 4,629 *g* for 15 min with 10 ml sterile saline (0.85% NaCl), resuspended in 10 ml of ham homogenate (Yang *et al*., 2009b), and habituated separately for two days at 7°C before inoculation, to allow acclimatization of *L. monocytogenes* cells to the food environment and low-temperature. Before inoculation, the suspensions of the five habituated stains were composited and serially, 10-fold diluted, in phosphate-buffered saline (PBS, pH 7.4; 0.2 g/liter KH_2PO_4, 1.5 g/liter $Na_2HPO_4 \cdot 7H_2O$, 8.0 g/liter NaCl, and 0.2 g/liter KCl) to achieve an initial concentration of 3-4 log CFU per gram of sample. The *L. monocytogenes* counts of the composite inoculum after habituation were 9.2±0.2 CFU/ml.

Preparation of chicken samples, inoculation, and storage. Fresh boneless skinless chicken breast muscles purchased from a local processor were aseptically cut into approximately 100-g pieces and stored in sealed plastic bags (approximately 20 samples in each bag) at -20°C for less than two weeks prior to use. Chicken samples were thawed at refrigerated temperature (4°C) for approximately 48 h and cooked (16-in electrical skillet, National Presto Industries, Inc., Eau Claire., WI) to the target internal temperature of 73.8°C.The temperature was recorded every 5 s

with k-type thermocouples connected to PicoLog data acquisition software (Pico Technology, Ltd., Cambridge, UK). Cooked samples were stored aerobically at 7°C in Pyrex dishes (25 by 35 cm, 5 cm deep) covered with cling paper for no more than 2 h. The surfaces of the cooked and cooled-to-4°C samples were then inoculated with 100 µL per side of the above-mentioned diluted habituated composite of *L. monocytogenes* strains, with a 15 min interval between inoculations of each side, to achieve an initial inoculation level of approximately 3-4 log CFU/g. The inoculated samples were placed in Pyrex dishes (15 samples in each dish) covered with cling paper, and stored aerobically in a 7°C incubator. During storage, samples were reheated and analyzed microbiologically and for physiochemical parameters on days 0, 1, 2, 4, and 7.

Product reheating. Cooked stored inoculated samples were individually placed in a microwave safe dish (22 cm dia, 4 cm deep) and subjected to 30, 60 or 90 s of microwave heating at the 100% power level in a domestic microwave oven (Amana, Model Radarange AMC5243, Newton, IA) with 1100 W power output. At the end of the intervention, the surface temperature of each sample was measured and recorded manually by a noncontact infrared thermometer (Oaklon TemoTestr IR, Lane Cove, Australia) from a distance of approximately 15 cm (to cover a reading area of 2.75 cm^2, based on manufacturer's recommendation). Immediately after microwaving and recording of surface temperature, each sample was aseptically transferred in a sterile filter bag (Whirl-Pak, Modesto, CA), placed into ice-water slush, and prepared for microbiological analyses.

For oven reheating, each sample was placed onto a sterile stainless steel tray with a k-type thermocouple, aseptically inserted into its geometrical center. A domestic oven (Magic Chef Standard Kitchen Oven, Maytag Group, Newton, IA) was preheated to 148°C (300°F) for approximately 30 min and samples were then reheated to internal temperatures of 50, 60 or 70

°C. The temperature of the cooking chamber of the oven was also monitored with a k-type thermocouple suspended approximately in the center of the oven, without any contact with the surroundings.

For reheating by the stove-top method, each sample with inserted thermocouple in its geometrical center was placed onto the sterile surface of a non-stick skillet (diameter approximately 30 cm [Farberware Licensing Company, LLC. Berwick PA]) preheated on a domestic oven stove top (Magic Chef Standard Kitchen Oven, Maytag Group, Newton, IA). Every two min samples were flipped over for exposure of both sides to the skillet surface. The samples were reheated to the internal temperatures of 50, 60, or 70 °C, with collection of time/temperature profile data every 5 s as described for oven reheating. Surface temperature of the skillet was also measured by suspending a thermocouple in approximately 5 ml of vegetable oil in a 20 ml glass container placed onto the frying skillet during the reheating procedure. For both stove top and oven, similar to microwave treatment, immediately after reaching the designated internal temperature, samples were placed in sterile filter bags (Whirl-Pak, Modesto, CA), cooled in ice-water slush, and analyzed microbiologically. Selection of the internal temperatures, reheating methods, and times for microwave intervention was based on a preliminary experiment (data not shown).

Microbiological and physiochemical analyses. As indicated, for microbiological analyses, each sample was placed in a sterile filter bag (Whirl-Pak, Modesto, CA), with an equal amount of maximum recovery diluent (0.85% NaCl and 0.1% peptone [Difco, Becton Dicknison]), added immediately after reheating and cooling the samples in ice, homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min (6 strokes per s), and serially diluted (10-fold) with 0.1% buffered peptone water (Difco, Becton Dickinson, Franklin Lakes, NJ). Samples were

spread-plated onto tryptic soy agar (Acumedia, Lansing, MI) with 0.6% yeast extract (Difco, Becton Dicknison) and PALCAM agar (Difco, Becton, Dicknison), for enumeration of total aerobic bacteria and *L. monocytogenes* counts after incubation at 25 and 30°C for 72 and 48 h, respectively. The detection limit for these microbiological analyses was 0.4 CFU log per g of sample. Samples with no detected colonies by plating at the detection level were enriched to evaluate presence/absence of the pathogen by a procedure modified form the USDA-FSIS (2008) method, as described by Rodriguez-Marval *et al*. (2009). For all the samples with below the detection limit pathogen counts, no pathogen was detected after the above-mentioned enrichment stage. Water activity (AquaLab Instrument, Decagon Devices, Inc., Pullman, WA) and pH (Denver Instrument, Arvada, CO) of samples were measured as described by Byelashov *et al*. (2010).

Experimental design and statistical analyses. The experiment was repeated twice with different ingredients with three replicates within each of these two repetitions. Mean microbial counts of each treatment or storage period, after log transformation, as well as cooking time, pH, and water activity values, were compared statistically with ANOVA-based procedures followed by Tukey-adjusted multiple comparison methods for further mean separation at type one error level of 0.05 (α=0.05). The dataset was analyzed as a randomized complete block design, with each of the two repetition trials considered as a blocking factor, using Proc GLM and Proc Mixed commands of SAS 9.2 (SAS, Inc., Chicago, IL). Additionally, in order to compare the inactivation rates of *L. monocytogenes* by stove-top and oven reheating methods at three internal temperatures of 50, 60, and 70ºC, GInaFiT software, a non-log-linear microbial survivor curve were used, comparing counts of days 7, 4, 2, 1, and 0 (after inoculation). This model as described by Geeraerd *et al.* (2005) and reports specific inactivation rates of K_{max} and adjusted-R^2. K_{max} is

specific inactivation rate for log-linear monophasic curves fitted for each internal temperature with unit of 1/time and thus longer time required for microbial cell reductions is associated to smaller K_{max} values while adjusted-R^2 values show proportion of the data described by the model. K_{max} values obtained for each internal temperature of stove-top reheating were compared statistically to those obtained from oven reheating using student-best t-test procedure at α=0.05.

RESULTS AND DISCUSSION

Water activity, pH and temperature measurements. Water activity and pH of reheated stored samples ranged from 0.982± 0.001 to 0.992±0.001, and 6.03±0.13 to 6.12 ±0.12, respectively with no difference ($P \geq 0.05$) among samples of different days of storage and reheating methods. Surface temperatures of the microwave treated samples ranged from 48.8±2.5 to 57.6±4.0, 64.5±4.0 to 69.5±4.0, and 75.7±4.7 to 82.6±3.1°C, for samples reheated for 30, 60, and 90 s, respectively. The values of pH for these samples ranged from 6.05±0.07 to 6.13±0.08, 5.88±0.08 to 6.14±0.11, and 5.99±0.18 to 6.19±0.16, for samples reheated for 30, 60, and 90 s, respectively, with no statistical difference ($P \geq 0.05$).

The temperatures of the oven cooking chamber for samples reheated to target internal temperatures of 50, 60 and 70 °C were 145.9 ±11.0, 147.6 ±9.9, and 143.5 ±1.2 °C, respectively. Surface temperatures of the cooking area during stove top reheating were 142.7 ±12.4, 140.9 ±9.9, and 148.3 ±11.3°C for samples reheated to internal temperatures of 50, 60, or 70 °C, respectively. The values of pH for samples reheated in the oven were in the ranges of 6.05±0.07 to 6.13±0.10, 6.02±0.09 to 6.13±0.05, and 6.03±0.04 to 6.20±0.10 for samples reheated to internal temperatures of 50, 60, and 70 °C, respectively, and 6.07±0.07 to 6.13±0.12, 6.09±0.06 to 6.16±0.03, and 5.98±0.12 to 6.10±0.06, for samples reheated on stove top to internal

temperatures of 50, 60, and 70 °C, respectively, without significant differences ($P \geq 0.05$) among samples reheated to different internal temperatures.

Survival and growth during storage. Many previous studies, have isolated *L. monocytogenes* from domestic and industrial refrigerators as well as food preparation and processing environments (Sergelidis *et al.,* 1997; Walker *et al.,* 1990), making leftovers potentially hazardous foods due to contamination with psychotropic pathogens including *L. monocytogenes***.** In the present study, initial counts of *L. monocytogenes* on the day of inoculation were 3.7±0.1 log CFU/g and increased to 7.8±0.2 log CFU/g by day-7 of storage at 7°C (Figure 6.1 and Appendix Table A23) confirming the concern that if cross-contamination occurs after preparation of foods, *L. monocytogenes* can multiply extensively during the refrigerated storage. Similar results were observed for aerobic plate counts with more than a 4-log increase from the first day to day-7 (Figure 6.2 and Appendix Table A24). The extensive multiplication of *L. monocytogenes* in this study could be explained by increased hydrolysis of macromolecules and bioavailability of nutrients on the surface of chicken samples as a result of cooking (Damodaran *et al.,* 2008) that can enhance multiplication of the pathogen as well as the background microflora during storage.

Inactivation by reheating. Microwave oven reheating showed a high potential for reduction of *L. monocytogenes* counts. On day-0, microwaving for 30, 60, and 90 s was responsible for reductions of 0.3, 1.1, and more than 3.4 log CFU/g of *L. monocytogenes*, respectively. Similar trends were observed for samples reheated on days 1, 2, 4, and 7 (Figure 6.1 and Appendix Table A23). For example on day-4, counts of the untreated control were 6.2±0.1 and were reduced ($P<0.05$) to 4.8±0.1, 4.2±0.1 and 1.8±0.8 as the result of microwaving treatment for 30, 60, and 90 s, respectively. As storage days increased, higher number of survivors after reheating was

observed. In other words, storage time that affected the initial microbial counts and each day of storage had a major impact on subsequent death due to reheating. On day-4, as an example, although 90 s of microwaving caused more than a 4-log reduction of the pathogen, the 1.8 log CFU/g of survivors could still be of concern from a food safety standpoint but the same (i.e., microwaving for 90 s) intervention on day-0 was able to reduce the *L. monocytogenes* counts to undetectable levels. This indicates that intensity of reheating needs to be adjusted based on the storage period (that affects the initial bacterial load) of food in order to assure reduction of potential pathogen counts to acceptable levels. In general, the microwave treatment, as compared to other reheating methods evaluated in this study, had high potential for reduction of microbial loads with 3.4, 3.5, 4.2, 4.4, and 5.2 log CFU/g reductions in 90 s at 0, 1, 2, 4, and 7 days of storage, respectively (Figures 6.1 and 6.2 and Appendix Tables A23 and A24).

The effectiveness of microwave treatments against surface inoculated *L. monocytogenes* is in agreement with previous studies. Rodriguez-Marval *et al*. (2009) demonstrated that *L. monocytogenes* inoculated on surface of frankfurters could be reduced by 3.7 log CFU/g after 75 s of microwaving. Similarly, Shen *et al*. (2009) showed that 30 s of microwaving resulted in 0.8 to 1.3 log CFU/g reduction of inoculated *L. monocytogenes.* It is noteworthy that although microwave treatments showed high pathogen reduction effectiveness, it has been reported that their performance can be considerably affected by size and position of the food, and age and power output of microwave (Swain *et al.,* 2008) that need to be considered before development of recommendations for safe microwave reheating of leftover food by consumers.

Oven reheating on day-0 reduced the pathogen by 2.9, 2.7, and 1.4 log CFU/g after reheating to internal temperatures of 50, 60, and 70 °C, respectively (Figures 6.3 and Appendix Table A25). Similar to the microwave treatment, as storage time between inoculation and

reheating increased, microbial multiplication increased and more pathogen cells survived on the reheated samples (Figures 6.3 and 6.4 and Appendix Table A25 and A26). In other words, storage time that affected the initial microbial counts on each day of storage had a major impact on subsequent death due to reheating. For stove-top reheating, on days 0 and 1, *L. monocytogenes* loads of samples reheated to the internal temperature of 70 °C were less than one log CFU/g but like other treatments more survivors were detected as the time interval between inoculation and reheating increased. Similar results were observed by other investigators for reduction of inoculated *L. monocytogenes* on pork Scrapple reheated by pan-frying methods during storage (Adenkule *et al.,* 2009).

Comparing the domestic oven and stove-top reheating, the two methods required considerably different lengths of time to reach the same internal temperatures (i.e., 50, 60, and 70°C) in the geometric center of the samples (Figure 6.7 and Appendix Table A27), and, thus, exhibited different pathogen reduction potential. On day-7, as an example, the oven and stove top reheating to internal temperatures of 70°C required 24.2 and 17.1 min (Figure 6.7 and Appendix Table A27), and were responsible for reductions of 2.0 and 3.0 log CFU/g of the inoculated pathogen, respectively (Figures 6.3 and 6.4 and Appendix Tables A25 and A26). These differences could be explained by the different heat transfer principles involved in each method: oven reheating involves convection heat transfer whereas stove top reheating involves conduction as the primary mode of heat transfer (Adler *et al.,* 2009).The other characteristic of oven and stove reheating methods investigated in this study was the relatively high standard deviation of reheating time to reach designated internal temperatures. The high variation in performance of domestic cooking appliances were also reported by other investigators (Gupta *et*

al., 2010; Shen *et al.,* 2010), and it appears that these variations need to be considered for preparation of safe reheating recommendations for consumers.

Table 6.1 provides inactivation rates of *L. monocytogenes* throughout days 7, 4, 2, 1, and 0 by stove-top and oven reheating methods to internal temperatures of 50, 60, and 70ºC. Based on adjusted R^2 values, 87% to 89% of samples reheated by stove-top, and 92 to 97% of oven-reheated samples were described by the utilized GInaFiT model, a log-linear monophasic curve fitted for each internal temperature. At internal temperatures of 50, 60 ºC, stove-top reheating had higher ($P<0.05$) K_{max} values (K_{max} parameter±SE), indicating that this method required lower time to reach the internal temperature of 50 and 60 ºC compared to oven reheating. For internal temperature of 70ºC, K_{max} values of stove-top and oven reheating were 2.57±0.18 and 2.58±0.14, respectively with no statistical difference ($P\geq0.05$).

In summary, due to different heat transfer mechanisms (i.e., primarily convection-based transfer for oven and conduction-based transfer for stove-top reheating) these two methods demonstrated different pathogen reduction potentials and times for reaching the same designated internal temperature indicating a need for method-specific recommendations for assuring the safety of leftover reheated in domestic and foodservice environments by different heating methods.

Along with increasing evidence of *L. monocytogenes* presence in domestic and food service environments in recent years, it appears that safe management of leftover food, especially for people at-risk for listeriosis, is of particular importance. In our study, initial counts of *L. monocytogenes* increased ($P<0.05$) by over 4.0 log CFU/g during 7 days of storage at 7°C, confirming the concern that if cross-contamination occurs after preparation of foods, *L.*

monocytogenes can multiply extensively during refrigerated storage. Although the reheating methods investigated in this study reduced *L. monocytogenes* contamination by 2-5 log CFU/g, growth of the pathogen during storage allowed high numbers of survivors after reheating, especially after two days of storage. In other words, storage time that affected the initial microbial counts had a major impact on subsequent death due to reheating. This indicates that storage period, and type and intensity of reheating need to be considered for safe consumption of leftover food. Regardless of reheating method, high numbers of survivors after reheating, especially after two days of storage indicated a need of consideration for utilization of leftover food, especially by at-risk populations.

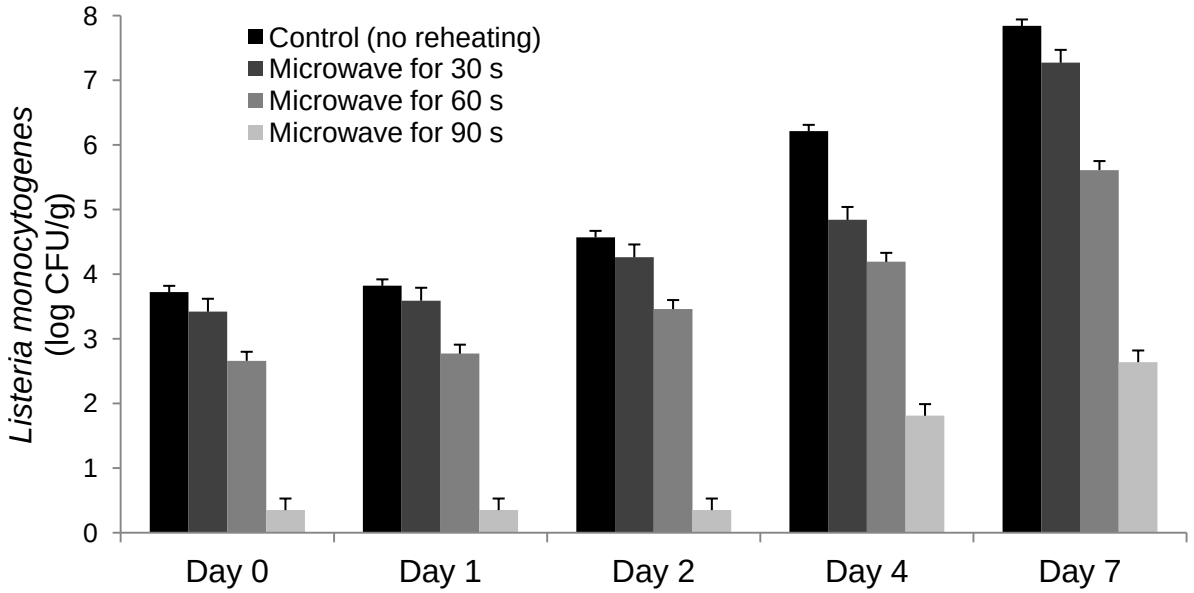

Figure 6.1 (Data in Appendix Table A23). Effects of domestic microwave oven reheating against inoculated *Listeria monocytogenes* (log CFU/g) of cooked chicken during a 7 day aerobic storage at 7°C.

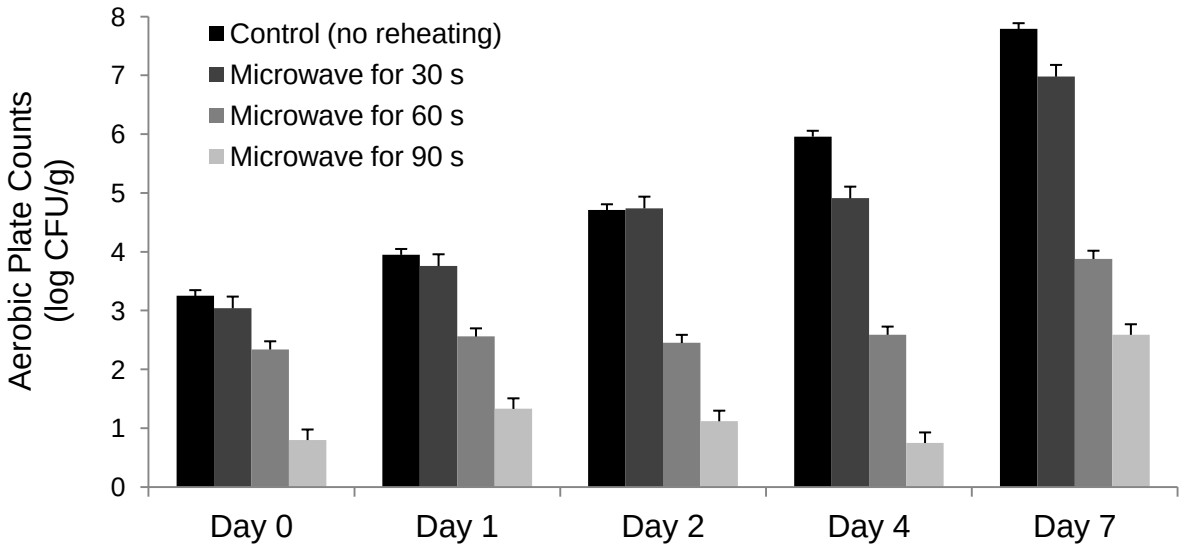

Figure 6.2 (Data in Appendix Table A24). Effects of domestic microwave oven reheating against aerobic bacterial counts (log CFU/g) of cooked chicken during a 7 day aerobic storage at 7°C.

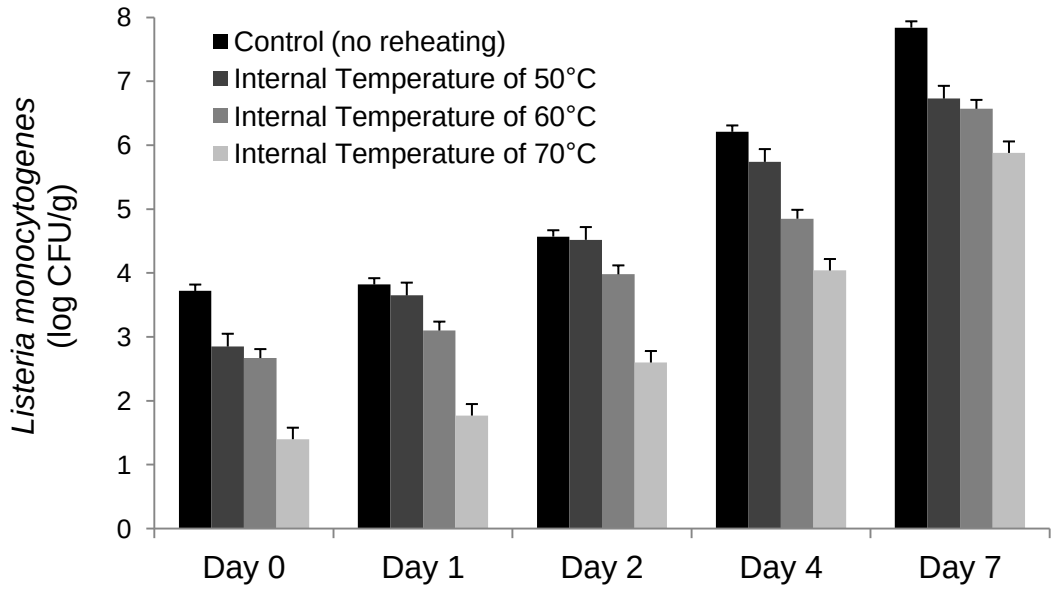

Figure 6.3 (Data in Appendix Table A25). Effects of domestic oven reheating against inoculated *Listeria monocytogenes* (log CFU/g) of cooked chicken during a 7 day aerobic storage at 7°C.

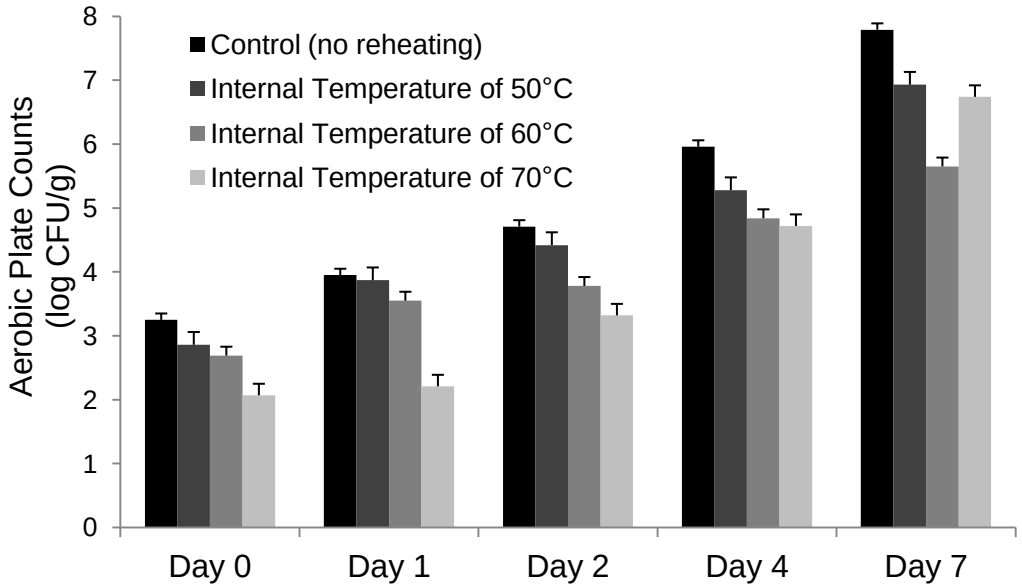

Figure 6.4 (Data in Appendix Table A26). Effects of domestic oven reheating against aerobic (log CFU/g) bacteria of cooked chicken during a 7 day aerobic storage at 7°C.

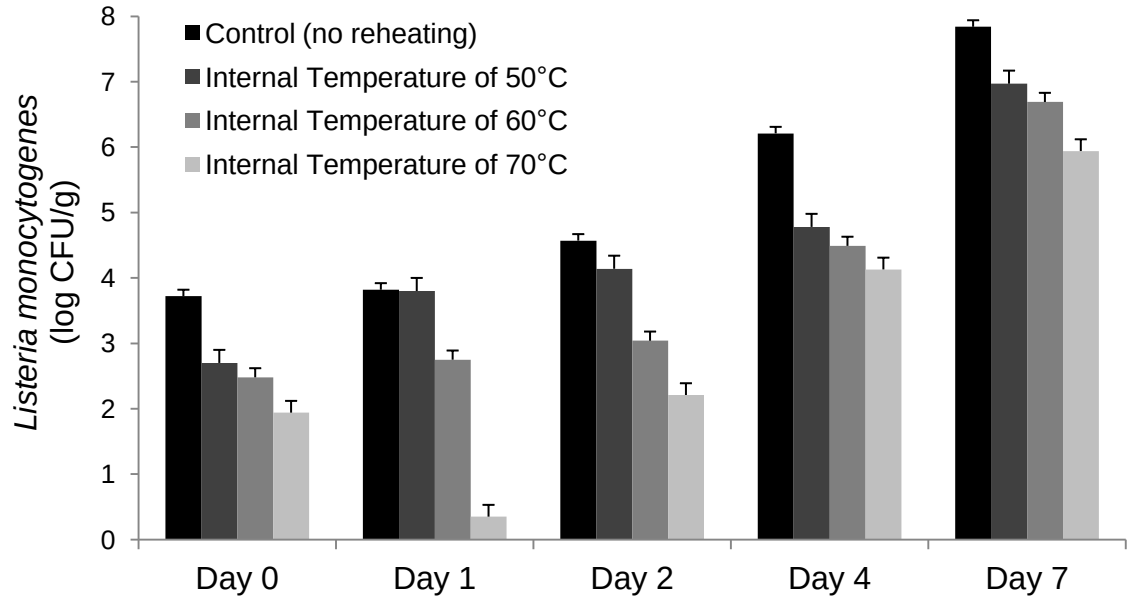

Figure 6.5 (Data in Appendix Table A25). Effects of domestic stove top reheating against inoculated *Listeria monocytogenes* (log CFU/g) of cooked chicken during a 7 day aerobic storage at 7°C.

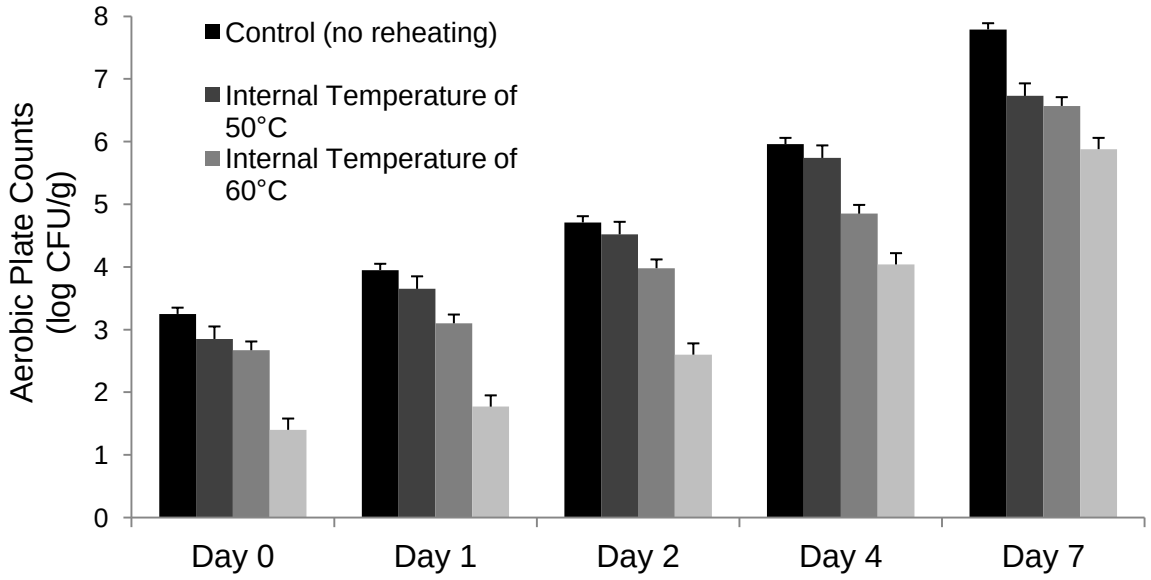

Figure 6.6 (Data in Appendix Table A26). Effects of domestic stove top reheating methods against aerobic bacteria (log CFU/g) of cooked chicken during a 7 day aerobic storage at 7°C.

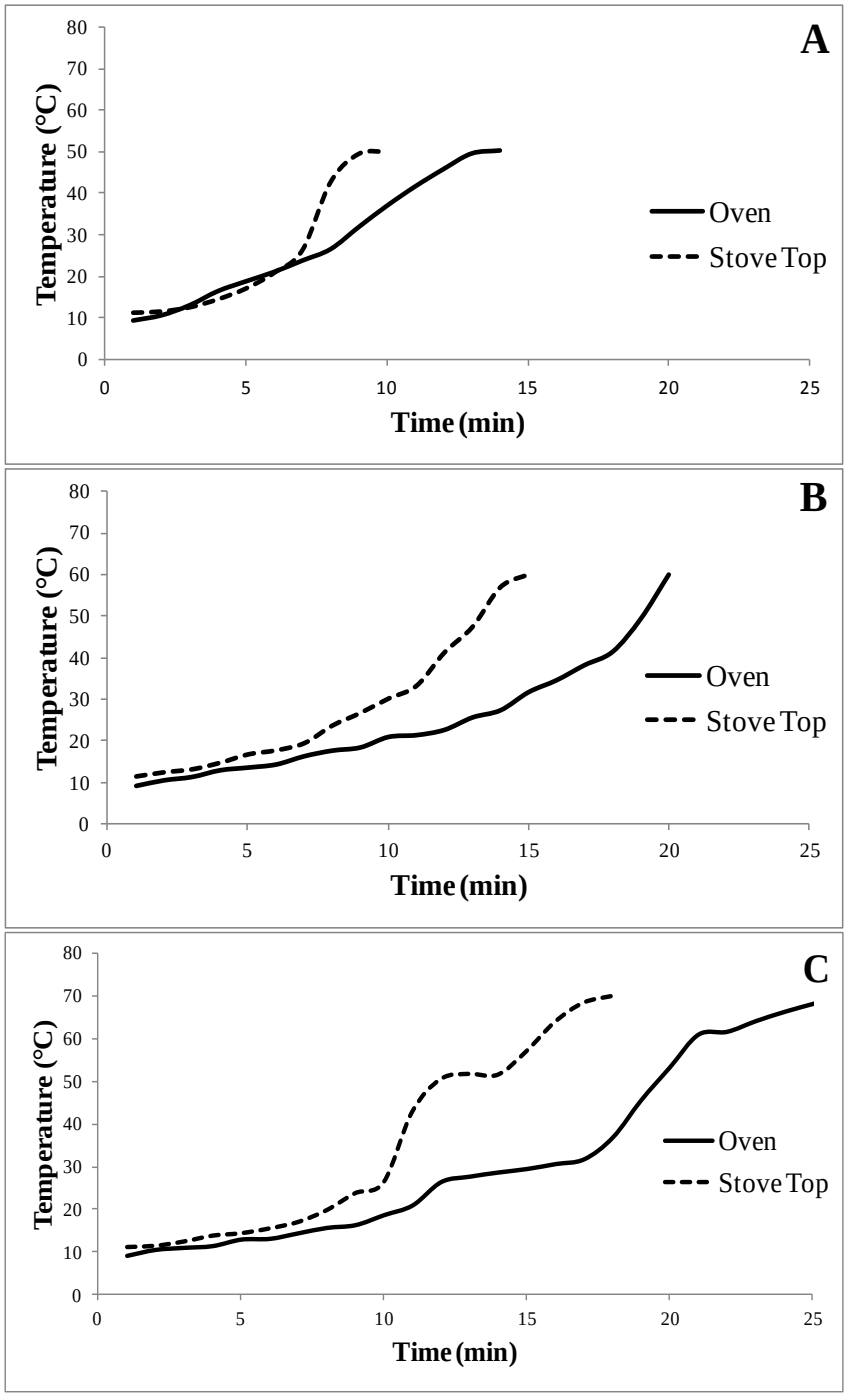

Figure 6.7 (Data in Appendix Table A27). Time and temperature profiles of samples reheated by domestic oven and stove top methods. (A) samples reheated to internal temperature of 50 °C, (B) samples reheated to internal temperature of 60 °C, and (C) samples reheated to internal temperature of 70 °C.

Table 6.1. Non-linear microbial survivor analysis for comparing inactivation rates of *L. monocytogenes* by stove-top and oven reheating, comparing counts of days 7, 4, 2, 1, and 0 (after inoculation).

		L. monocytogenes inactivation	
Reheating Method	Internal Temperature (°C)	K max	R^2
Stove-top[a]	70	2.57±0.18	0.87
	60	2.65±0.17*	0.89
	50	3.04±0.20*	0.89
Domestic Oven	70	2.58±0.14	0.92
	60	2.20±0.11	0.93
	50	2.27±0.08	0.97

[a] K_{max} values associated to stove-top reheating followed by a * is significantly larger (P<0.05) than values of oven reheating for each internal temperature. K_{max} values (parameter± standard error) are specific inactivation rates for log-linear monophasic curves fitted for each internal temperatures with unit of 1/time and thus longer time required for microbial cell reductions is associated to smaller K_{max} values.

CHAPTER VII

Microwave reheating for reduction of *Listeria monocytogenes* during refrigerated storage of post-cooking inoculated chicken breast meat treated with different marinades

SUMMARY

Listeria monocytogenes has been isolated from various raw and processed food products, as well as domestic and commercial food preparation areas, having the potential to serve as source of cross-contamination of ready-to-eat foods. This study evaluated growth of *L. monocytogenes* inoculated on cooked chicken meat with different marinades and survival of the pathogen as affected by microwave oven reheating during aerobic storage at 7°C. Raw chicken breast meat samples were treated with three commercially-formulated and three domestically-available marinades, and then cooked (74.4°C internal temperature), cooled to 4°C, and surface-inoculated with *L. monocytogenes.* During storage at 7°C, on days 0, 1, 2, 4, and 7, samples were reheated by microwave oven (1100 W) for 45 or 90 s and analyzed microbiologically. *L. monocytogenes* counts on non-marinated control samples increased ($P<0.05$) from 2.7±0.1 to 6.9±0.1 log CFU/g during storage. At day-7 of storage, pathogen levels on samples marinated with tomato juice were not different ($P\geq0.05$; 6.9±0.1 log CFU/g) from those of the control, whereas for samples treated with the remaining marinades, pathogen counts were 0.7 to 2.0 log CFU/g lower ($P<0.05$) than those of control samples. Microwave reheating reduced *L. monocytogenes* by 1.9 to 4.1 (at 45 s) and 2.1 to 5.0 (at 90 s) log CFU/g. With similar trends across different marinates, the high levels of *L. monocytogenes* survivors found after microwave reheating, especially after two days

of storage, indicate that length of storage and reheating time need to be considered for safe consumption of leftover cooked chicken.

INTRODUCTION

As one of the oldest further processing methods of muscle foods, marinating is a common approach for meat preparation in commercial food processing as well as domestic and foodservice operations (Maguelonne, 2009). In addition to adding value and improving palatability, marinating ingredients may provide improvement in shelf-life through their antimicrobial properties.

As an ubiquitous microorganism, *Listeria monocytogenes* has been isolated from a variety of environments, including the domestic home environment and commercial food preparation areas as well as different processed and raw food products, having the potential to serve as source of cross-contamination of ready-to-eat foods. (Gandhi and Chikindas, 2007). Presence of *L. monocytogenes* in various environments, its halophilic nature, its capability to form biofilms, and its ability to multiply and proliferate at refrigeration temperatures have allowed this pathogen to be linked with many foodborne outbreak episodes (Ryser and Marth, 2007). Listeriosis caused by *L. monocytogenes* is primarily a foodborne severe illness with the very young and infants, the elderly, and immunocompromized patients among the most susceptible groups (Ryser and Marth, 2007). Most recent epidemiological investigations of the Centers for Disease Control and Prevention show that *L. monocytogenes* is responsible for 1,662, 1,520, and 266 episodes of illness, hospitalization, and death every year in the United States, with over 99% of the cases associated to contaminated food products (Scallan *et al.*, 2011a, 2011b, Morris, 2011).

Chicken meat accounts for 37% of meat consumption in the United States which is higher than the consumption of beef, pork, or lamb (Harley 2000; AMI 2009). White chicken muscle tissue (i.e., breast meat) is among the most popular cuts of meat in market, and have been associated with many foodborne outbreak episodes (Harley 2000; AMI 2009). A nationwide recall of cooked chicken breast in 2007 is an example of survival and multiplication of *L. monocytogenes* during storage of cooked chicken breasts (FDA 2010).

While some marinating formulations have been shown to have little to no effects in reducing microbial load of poultry products during storage (Perko-Makela *et al.,* 2000), some studies suggest marinating ingredients can reduce foodborne pathogens after application and during storage. Bjorkroth *et al.* (2000) suggested use of tomato-based marination to inhibit multiplication of lactic acid bacteria associated with gaseous spoilage of modified-atmosphere-packaged raw chicken meat. In two studies, Pathania *et al.* (2010a, 2010b) also showed that teriyaki and lemon pepper marinades could both reduce the *Salmonella* load on chicken skin and red meat during aerobic storage.

This study evaluated the effect of three commercially formulated (tomato-based, soy sauce-based, and lemon based), and three domestically available (tomato juice, soy sauce, and lemon juice) marinades against post-cooking inoculated *L. monocytogenes* on chicken breasts, during a 7-day aerobic storage period at 7°C. Pathogen survival after microwave oven reheating of stored marinated chicken was also investigated.

MATERIALS AND METHODS

Preparation procedure of strains. A mixture of food and human-disease derived *L. monocytogenes* strains, provided by Dr. Martin Wiedman (Department of Food Science, Cornel

University, Ithaca, NY) was used in this study (Fugett *et al.*, 2006). These strains are J1-177 (lineage I, Serotype 1/2b), C1-056 (lineage II, Serotype 1/2a), N3-013 (lineage I, Serotype 4b), R2-499 (lineage II, Serotype 1/2a), and N1-227 (lineage I, Serotype 4b). Each strain was stored on PALCAM agar (Difco, Becton Dickinson, Franklin Lakes, NJ) at 4°C prior to the study. Individual strains were activated separately from a single colony of the stored stock cultures as described by Yang *et al.* (2009a). The culture of each strain was then washed and centrifuged at 4,629 *g* for 15 min with 10 ml sterile saline (0.85% NaCl), resuspended in 10 ml ham homogenate (Yang *et al.*,2009b), and habituated separately for two days at 7°C product inoculation to allow acclimatization of *L. monocytogenes* cells to the food environment and low-temperature. Before inoculation, the cell suspension of all five habituated stains were composited and 10-fold serially diluted, in phosphate-buffered saline (PBS, pH 7.4; 0.2 g/liter KH2PO4, 1.5 g/liter Na2HPO4·7H2O, 8.0 g/liter NaCl, and 0.2 g/liter KCl) to achieve an initial concentration of 3-4 log CFU of pathogen per gram of sample.

Sample preparation, inoculation, and storage. Fresh boneless skinless chicken breast meat purchased from a local processor was aseptically cut into approximately 100-g pieces and stored in sealed plastic bags (approximately 20 samples in each bag) at -20°C for less than two weeks prior to use. Chicken samples were thawed at refrigerated temperature (4°C) for approximately 48 h and marinated with one of the marinades presented in Table 7.1 for 30 minutes (1:1 meat and marinade ratio) at refrigerated temperature (4°C). Marinated samples were then cooked (16-in electrical skillet, National Presto Industries, Inc., Eau Claire., WI) to the internal temperature of 74.4±0.4°C. The temperature was recorded every 5 s with k-type thermocouples connected to PicoLog data acquisition software (Pico Technology, Ltd., Cambridge, UK). Cooked samples were aerobically stored at 7°C in Pyrex dishes (25 by 35 cm, 5 cm deep) covered with cling

paper for no more than 2 h. The surfaces of the cooked and cooled samples were then inoculated with 100 µL per side of the above-mentioned diluted habituated composite of *L. monocytogenes* strains, with a 15 min interval between inoculations of each side, to achieve an initial inoculation level of approximately 2-3 log CFU/g. The inoculated samples were then placed in Pyrex dishes (15 samples in each dish) covered with cling paper, and stored in a 7°C incubator for reheating interventions and microbiological and physiochemical analyses.

Reheating of inoculated samples. During storage (i.e., days 0, 1, 2, 4, and 7) samples were reheated in a domestic microwave oven (Amana, Model Radarange AMC5243, Newton, IA) with 1100 W power output. Cooked and stored inoculated samples were individually placed in a microwave safe dish (22 cm dia, 4 cm deep) and were treated in the microwave oven for 45 or 90 s at the 100% power level. At the end of the intervention, the surface temperature of each sample was recorded by a noncontact infrared thermometer (Oaklon TemoTestr IR, Lane Cove, Australia) from a distance of approximately 15 cm (to cover a reading area of 2.75 cm^2, based on manufacturer's recommendation). Immediately after microwaving and recording of surface temperature, each sample was aseptically transferred in a sterile filter bag (Whirl-Pak, Modesto, CA), placed into ice-water slush, and prepared for microbiological analyses.

Microbiological, pH, and water activity analyses. For microbiological analyses, each sample was diluted with an equal weight of maximum recovery diluent (0.85% NaCl and 0.1% peptone [Difco, Becton Dicknison]), homogenized (Masticator, IUL Instruments, Barcelona, Spain) for 2 min (6 strokes per s), and serially diluted (10-fold) with 0.1% buffered peptone water (Difco, Becton Dickinson, Franklin Lakes, NJ). Samples were spread-plated onto tryptic soy agar (Acumedia, Lansing, MI) with 0.6% yeast extract (Difco, Becton Dicknison) and PALCAM agar (Difco, Becton, Dicknison), respectively, for enumeration of aerobic plate counts (APC) and *L.*

monocytogenes counts after incubation at 25°C and 30°C for 72 and 48 h. The detection limit for these microbiological analyses was 0.3 log CFU/g. Water activity (AquaLab Instrument, Decagon Devices, Inc., Pullman, WA) and pH (Denver Instrument, Arvada, CO) of samples and marinades were measured based on procedures described by Byelashov *et al.* (2010).

Statistical analyses. The experiment was repeated twice (as biologically independent trials) with different ingredients with three replicates within each of the two repetitions. Each repetition experiment was considered as a blocking factor and thus the experiment was analyzed as a randomized complete blocked design using Proc Mixed command of SAS (SAS, Inc., Chicago, IL). Mean microbial counts of each treatment or storage period, after log transformation were compared statistically using a ANOVA-based procedures followed by Tukey-adjusted multiple comparison methods for further mean separation at type one error level of 0.05 (α=0.05).

RESULTS AND DISCUSSION

Physical and chemical analyses of samples and marinades. Water activity of samples were ranging from 0.976±0.014 to 0.989±0.027 with no significant difference among non-marinated control and marinated samples across the 7-day storage period ($P \geq 0.05$). The pH of non-marinated control and samples marinated with commercial tomato-based, commercial soy-based, commercial lemon-based, tomato juice, soy sauce, and lemon juice ranged from (mean±SD) 6.03±0.14 to 6.11±0.12, 5.19±0.89 to 5.55±0.19, 5.91±0.18 to 6.18±0.39, 5.76±0.18 to 5.94±0.14, 5.83±0.24 to 5.86±0.14, 5.85±0.16 to 6.00±0.32, and 5.48±0.28 to 5.50±0.22, respectively. Surface temperatures (after microwave reheating) of samples for the above-mentioned order of marinades were 59.7±4.2 to 64.3±4.3, 60.3±3.9 to 62.2±4.0, 60.6±0.4.0 to 63.2±4.2, 59.0±3.9 to 64.7±4.3, 60.5±1.2 to 63.3±3.4, 59.5±1.2 to 79.1±2.1, and 58.6±1.6 to

63.9±2.0°C for samples reheated for 45 seconds and 75.1±5.0 to 77.9±4.2, 76.3±3.5 to 77.0±4.0, 77.1±4.2 to 77.7±4.5, 73.8±1.3 to 79.5±1.15, 74.8±1.2 to 79.6±2.6, 75.3±2.3 to 79.1±2.4, and 77.0±2.8 to 78.4±3.2°C for samples reheated for 90 seconds in microwave oven. As presented in Table 7.1, pH and water activity of marinades ranged from 2.71±0.42 to 4.59±0.01 and 0.851±0.001 to 0.989±0.002 respectively.

Survival and multiplication during storage. On the day of inoculation (day 0) *L. monocytogenes* counts of non-marinated control, and samples marinated with commercial tomato-based, commercial soy-based, commercial lemon-based, tomato juice, soy sauce, and lemon juice were 2.7±0.1, 2.6±0.2, 2.7±0.1, 2.4±0.3, 2.5±0.1, 2.7±0.1, and 2.6±0.1 log CFU/g, respectively, with no statistical differences among the samples ($P \geq 0.05$). On day-7 of storage, counts were increased to 4.9±0.1 to 6.9±0.1, with pathogen counts of non-marinated control> tomato juice> soy sauce> commercial soy-based> commercial tomato-based> commercial lemon-based> lemon juice (Figure 7.1 and Appendix Table A32). Similar trends were observed for survival and multiplication of aerobic plate counts (Figure 7.2 and Appendix Table A32). Extensive multiplication of *L. monocytogenes* during the 7-day storage at 7°C could be explained by increased hydrolysis of macronutrients on the surface of samples due to cooking, resulting in increased bioavailability of micro- and macro-nutrients for inoculated pathogen and background microflora (Damodaran *et al.,* 2007).

Microbial reduction by microwaving. On day-0 (<2 h after inoculation), treatment in the microwave oven at 100% power level for 45 s reduced *L. monocytogenes* counts to 0.6 and less than 0.7 log CFU/g, respectively for non-marinated control and marinated samples (Figure 7.3 and Appendix Tables A28). Similarly, treatment on day-0 for 45 s reduced aerobic plate counts to 0.7 and 0.9 log CFU/g, respectively, for non-marinated control and marinated samples (Figure

7.4 and Appendix Table A29). Microwaving for 90 s on day-0 reduced the *L. monocytogenes* counts to less than 0.3 and 0.4 log CFU/g for non-marinated control and marinated samples, respectively (Figure 7.5 and Appendix Table A30) and reduced aerobic plate counts to 0.4 or less than 0.4 log CFU/g for non-marinated control and marinated samples, respectively (Figure 7.6 and Appendix Table A31). As storage days increased, multiplication of *L. monocytogenes* and aerobic plate counts resulted in more survivors after both microwave treatments. As an example, on day-4 of storage, 2.3±0.3 and 1.9±0.3 log CFU/g of *L. monocytogenes* were detected after microwaving of non-marinated control for 45 and 90 s (Figures 7.3 and 7.5 and Appendix Tables A28 and A30). Marinated samples with lemon juice, commercial lemon-based, and tomato-based had consistently lower ($P<0.05$) counts and survivors after microwave treatments across the storage period than non-marinated samples, while marinated samples with tomato juice, soy sauce, and commercial soy-based had higher ($P<0.05$) or as higher ($P\geq0.05$) *L. monocytogenes* counts as non-marinated samples (Figures 7.3 and 7.5 and Appendix Tables A28 and A30). Similar trends were observed for aerobic plate counts for 45- and 90-second microwave treatments (Figures 7.4 and 7.6 and Appendix Tables A29 and A31). The effectiveness of microwave treatments against surface inoculated *L. monocytogenes* has been investigated in some recent studies. Rodriguez-Marval *et al.* (2009) demonstrated that inoculated *L. monocytogenes* on surface of frankfurters could be reduced by 3.7 log CFU/g in 75 s of microwaving. Similarly, Shen *et al.* (2009) showed 30 s of microwaving can result in 0.8-1.3 log CFU/g reduction of inoculated *L. monocytogenes.*

In Conclusion, although microwave reheating was responsible for 1.9 to 4.1 and 2.1 to 5.0 log CFU/g reduction of *L. monocytogenes* counts during storage, for treatments at 45 S and 90 S, respectively, numbers of pathogen survivors after reheating increased as populations on stored

(7°C) product increased. With similar trends across different marinated samples, high levels of *L. monocytogenes* survivors after microwave oven reheating, especially after two days of storage, indicates that length of storage and reheating time need to be considered for safe consumption of leftover cooked chicken.

Table 7.1. Marinating ingredients, and their pH (mean±SD) and water activity (mean±SD).

	Ingredients	pH	Water activity
Commercial marinades			
Tomato puree based commercial marinade (A1® Classic Marinade)	Tomato Paste, vinegar, corn syrup, high fructose corn syrup, salt, raisin juice, dried onion, modified food starch, less than 1% of thyme, dried red bell pepper, sodium benzoate.	3.10±0.12	0.962± 0.025
Lemon based commercial marinade (Lawry's® Lemon Pepper)	Water, lemon juice, high fructose corn syrup, salt, distilled vinegar, soybean oil, modified food starch, black pepper, xanthan gum, fried minced onion, distilled potassium sorbate, sodium benzoate, natural flavors, sodium bisulfate.	2.71±0.42	0.946± 0.006
Soy sauce based commercial marinade (Kikkoman® Teriyaki marinade)	Soy Sauce (water, wheat, soy bean, salt), wine, high fructose corn syrup, water, vinegar, salt, spices, onion powder, succinic acid, garlic powder, sodium benzoate .	3.29±0.11	0.884±0.008
Domestic marinades			
Lemon Juice (100%) (Kroger® company)	Lemon juice (water, lemon juice concentrate) sodium bisulfate, lemon oil, sodium benzoate.	2.58±0.03	0.989± 0.001
Soy Sauce (Kikkoman® company)	Water, wheat, soybean, salt, sodium benzoate.	4.59±0.01	0.851±0.001
Tomato Juice (100%) (Campbell's® company)	Tomato juice (water, tomato concentrate), salt, ascorbic acid.	4.03±0.01	0.994±0.001

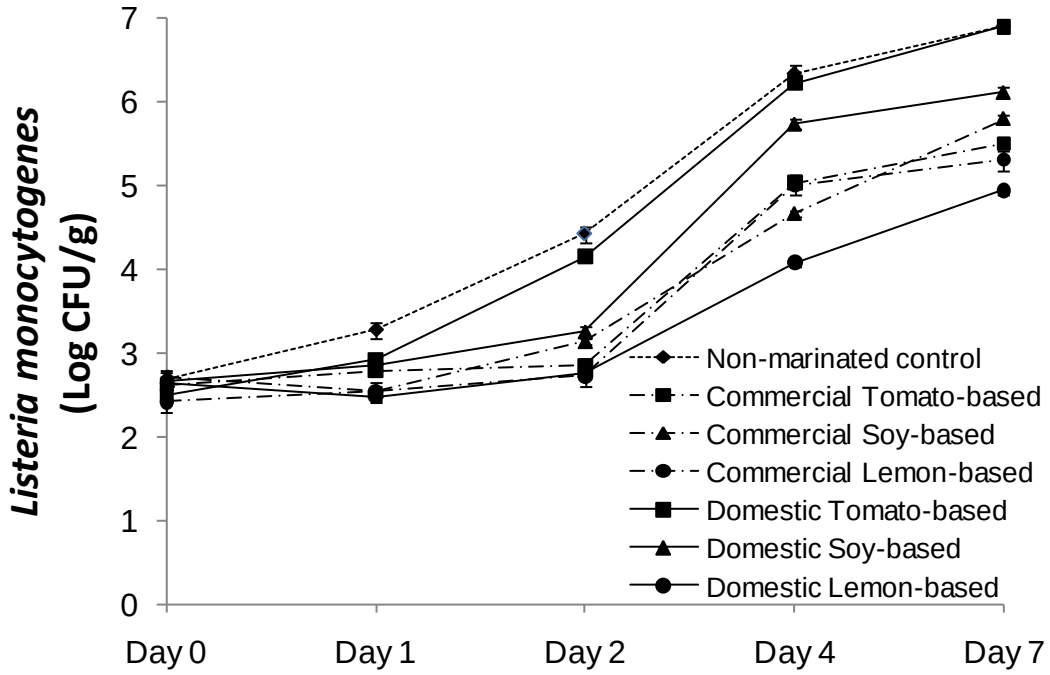

Figure 7.1 (Data in Appendix Table A32). *Survival and multiplication of L. monocytogenes on marinated cooked chicken stored aerobically at 7°C.*

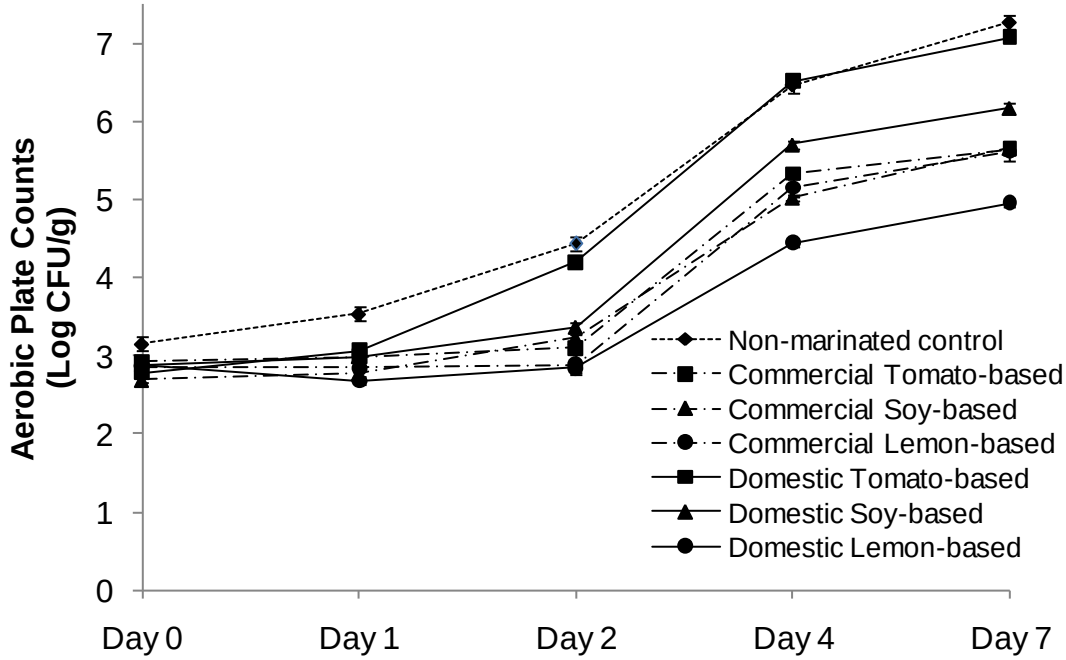

Figure 7.2 (Data in Appendix Table A32). *Survival and multiplication of aerobic plate counts on marinated cooked chicken stored aerobically at 7°C.*

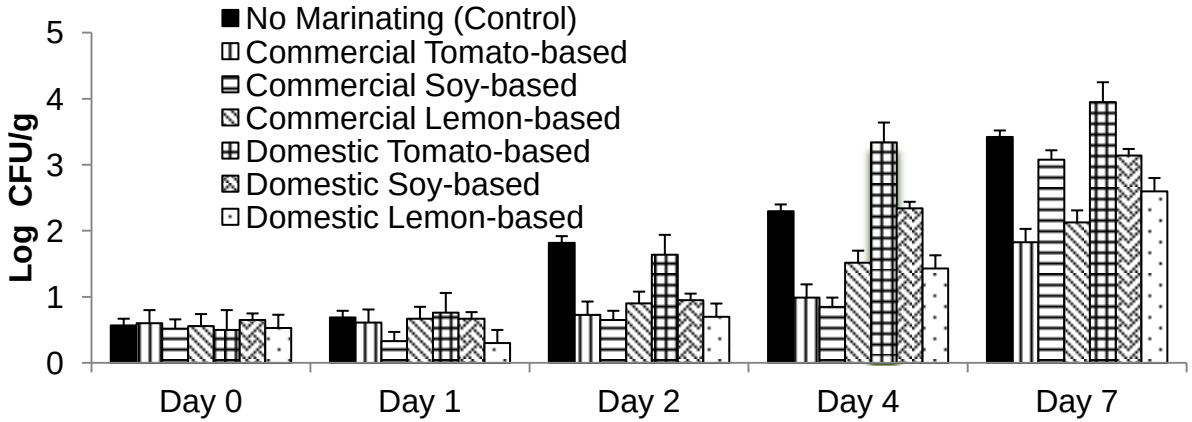

Figure 7.3 (Data in Appendix Table A28). Effects of microwave reheating for 45 seconds on reduction of inoculated *Listeria monocytogenes* (log CFU/g) during a 7 day aerobic storage at 7°C.

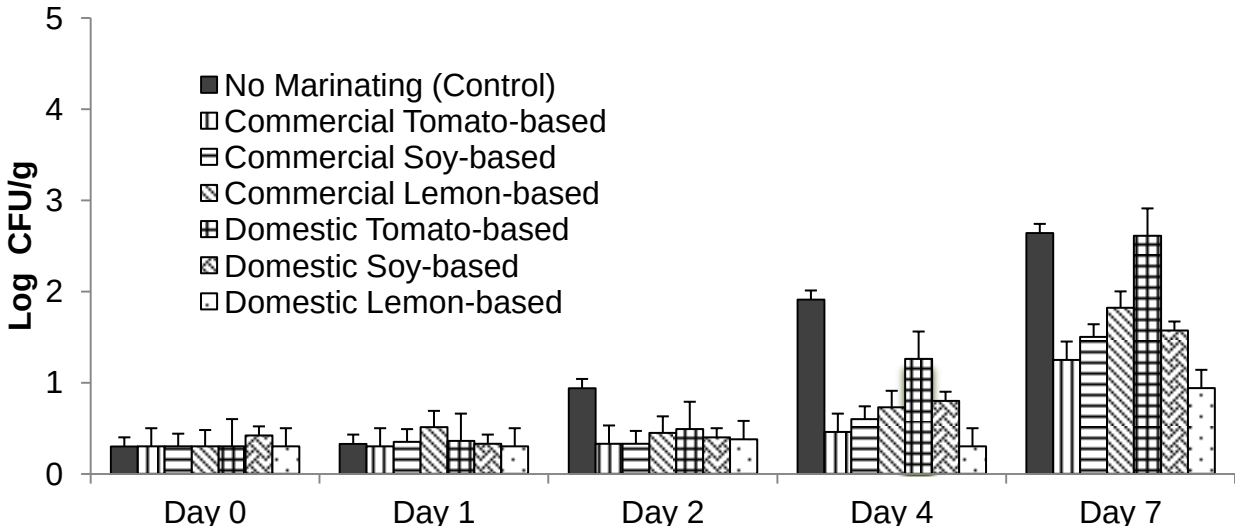

Figure 7.4 (Data in Appendix Table A29). Effects of microwave reheating at 45 seconds on reduction of inoculated aerobic plate counts during a 7 day aerobic storage at 7°C.

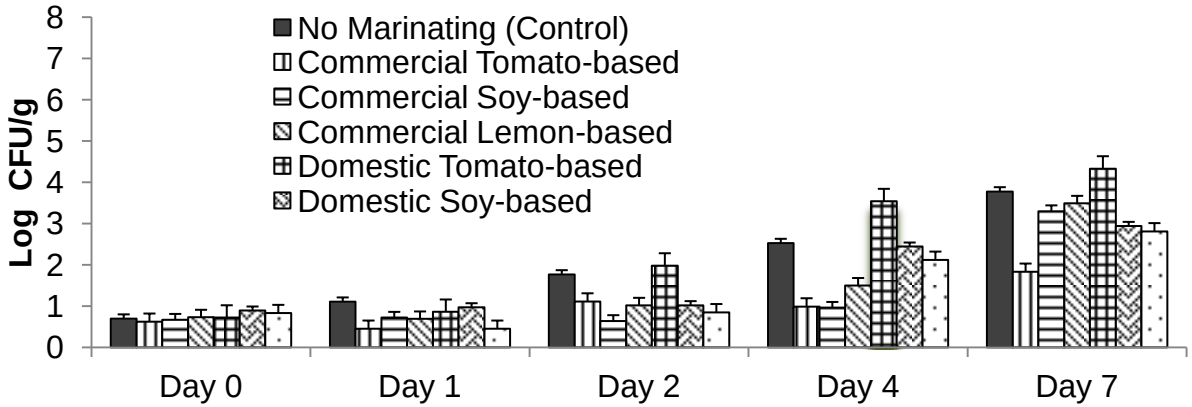

Figure 7.5 (Data in Appendix Table A30). Effects of microwave reheating for 90 seconds on reduction of inoculated *Listeria monocytogenes* during a 7 day aerobic storage at 7°C.

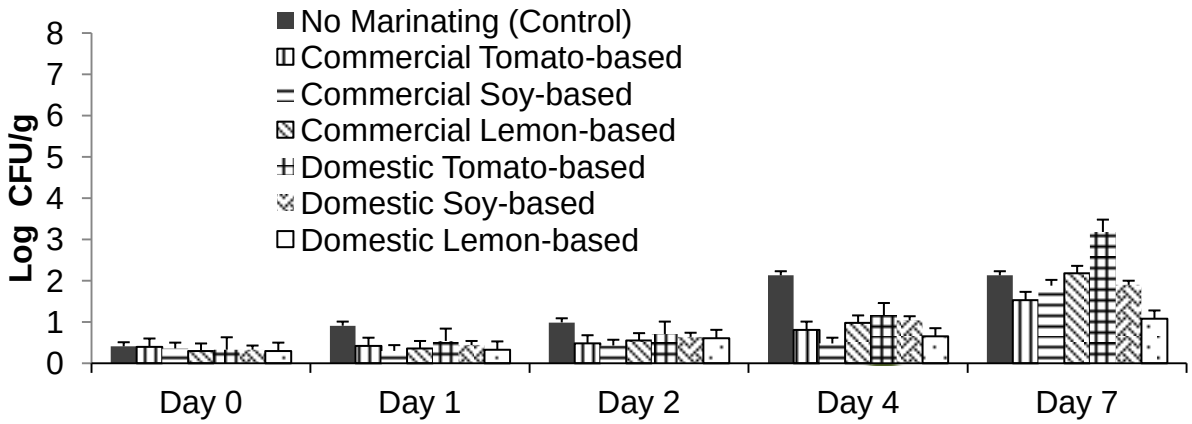

Figure 7.6 (Data in Appendix Table A31). Effects of microwave reheating at 90 seconds on reduction of inoculated aerobic plate counts during a 7 day aerobic storage at 7°C.

CHAPTER VIII

CONCLUSIONS

Formation and decontamination of biofilms of foodborne pathogenic *E. coli* and *Salmonella* serovars. In this study, at vast majority of time intervals, temperatures and sanitation treatments, MDR and MDR-AmpC phenotypes of *Salmonella* were equally or less resistant to interventions than susceptible *Salmonella* serovars, indicating that validating a cleaning and sanitation procedure against susceptible *Salmonella* serovars will most probably be equally effective against the control of drug-resistant phenotypes as well. This study also indicated control of non-O157 STEC serogroups on stainless steel material can be achieved with similar interventions validated against *E. coli* O157:H7.

Under the conditions of this study, it was observed that efficacy of the tested sanitizers was reduced as a result of higher temperatures used in biofilm formations. Treatments with a quaternary ammonium compound-based sanitizer, were able to reduce biofilm mass of one-week mature biofilms to less than the detection limit for most pathogen groups when tested at 4°C, while the same treatments at 25°C, allowed survival of over 100 pathogenic cell/cm^2 on the surface of stainless steel.

In this study, it was observed that sanitizer efficacy against one-week mature biofilms at three tested temperatures was low. Nearly for all the seven tested pathogen groups stored at 4, 15, and 25°C, it was observed that efficacy of the peroxyacetic-based sanitizer for inactivation of one-week mature biofilm, at the highest manufacturers recommended concentration for inactivation of food contact surfaces, was low. The quaternary ammonium compound-based sanitizer tested

in this study, especially at higher temperatures of biofilm formation, was not capable of complete sanitation of the surfaces as well, when tested at the maximum recommended concentration for general disinfection. This confirms and reinforces the importance and need for incorporating proper cleaning including physical scrubbing before applying sanitizers for effective removal of biofilms in processing areas.

This study was designed to compare serogroups of pathogens for ability to survive and form biofilm and for their sensitivity AB and QAC sanitizers. Overall, for decontamination of stainless steel coupons from biofilm of foodborne pathogenic bacteria, we observed low efficacy of quaternary ammonium compound-based and peroxyacetic-based chemical decontamination which were previously validated to be effective in removal of planktonic cells. This decrease in efficacy was more notable as a result of increase in temperature of biofilm environment. Seven tested pathogen groups in this study had similar biofilm formation and susceptibility to chemical decontamination indicating that a successful cleaning and sanitation program validated against *E. coli* O157:H7 will most probably be effective against the other tested non-O157 *E. coli* and *Salmonella* serovars as well.

Lactic acid resistance of shiga toxin-producing *E. coli* and multidrug-resistant and susceptible *Salmonella* serovars in meat homogenate. Results showed that in most cases, individual strains of non-O157 STEC (wild-type and rifampicin-resistant) were less acid tolerant than the *E. coli* O157:H7 5-strain mixture (wild-type and rifampicin-resistant). This indicates that currently in-place lactic acid interventions in processing of red meat, validated to be efficient against *E. coli* O157:H7 are most probably efficient against the six tested serogroups of non-O157 STEC as well. Similarly, the present study showed the low acid tolerance of nearly all tested *Salmonella* strains as compared to *E. coli* O157:H7. In addition, for the majority of time

intervals of strains, MDR and MDR-AmpC phenotypes of *Salmonella* were equally or less resistant to interventions than susceptible *Salmonella* serovars.

All tested serogroups of *E. coli* (i.e., O26, O45, O103, O111, O121, and O145) showed higher K_{max1} values relative than those obtained for the five-strain mixtures of wild-type cells and rifampicin-resistant variants of *E. coli* O157:H7. This indicates that the six tested serogroups required shorter time for 4D reductions compared to *E. coli* O157:H7. Thus it can be concluded that these six serogroups were less lactic acid tolerant than *E. coli* O157:H7. For the six non-O157 serogroups as well as the five-strain mixture of *E. coli* O157:H7, there were no differences between K_{max1} values of wild-type and rifampicin-resistant variants. This indicates that under the conditions tested in this study, lactic acid resistance of wild-type and rifampicin-resistant variants were not statistically different, showing that wild-type and rifampicin resistant cells of foodborne pathogens behave similarly in response to antimicrobials and therefore can be used interchangeably in food microbiology laboratory challenge research.

Overall, the results of this acid intervention conducted in a beef homogenate acidified with 5% lactic acid, indicated that nearly all individual strains of antibiotic resistant and susceptible *S.* Newport and *S.* Typhimurium and non-O157 *E. coli* were less acid tolerant than both the wild-type and the rifampicin-resistant *E. coli* O157:H7 5-strain mixtures.

Decontamination of beef trimmings inoculated with shiga toxin-producing *E. coli* and multidrug-resistant and susceptible *Salmonella* serovars by lactic acid. Six serotypes of non-O157 STEC and *E. coli* O157:H7 strain mixture tested in this experiment showed similar resistance to lactic acid during the intervention at 25°C; microbial counts after treatment of non-O157 STEC were less than 0.3 log different than *E. coli* O157:H7 counts with no statistical

difference. As expected, lactic acid treatments at 55°C were more effective ($P<0.05$) for decontamination of meat samples with no appreciable differences among the seven STEC serotypes. Overall, irrespective of lactic acid treatment (i.e., 25 or 55°C), there were no appreciable differences in surviving counts of all six non-O157 STEC serotypes on treated samples compared to surviving counts of *E. coli* O157:H7 indicating that the six non-O157 STEC can be controlled as effectively as *E. coli* O157:H7 by lactic acid interventions.

Salmonella serovars, including MDR *S.* Typhimurium, MDR-AmpC *S.* Typhimurium and Newport, and susceptible *S.* Typhimurium and Newport, when exposed to lactic acid treatment at 25°C showed lower surviving counts than *E. coli* O157:H7 with similar trends for treatments at 55°C. *Salmonella* counts, irrespective of serotype or antibiotic resistance profile, were similar or lower than counts of *E. coli* O157:H7 following decontamination of samples with 25 or 55°C lactic acid solutions.

Results of this study showed that the above-mentioned emerging pathogens are less or as acid tolerant as *E. coli* O157:H7 indicating that currently in-place lactic acid interventions in primary processing of red meat are most probably efficient against these emerging pathogens as well.

Reheating against *Listeria monocytogenes* inoculated on cooked chicken. In summary, due to different heat transfer mechanisms (i.e., primarily convection-based transfer for oven and conduction-based transfer for stove-top reheating) different investigated reheating methods demonstrated different pathogen reduction potentials and times for reaching the same designated internal temperature. This indicates a need for method-specific recommendations for assuring the safety of leftovers reheated in domestic and foodservice environments by different heating methods. Along with increasing evidence of *L. monocytogenes* presence in domestic and food

service environments in recent years, it appears that safe management of leftover food, especially for people at-risk for listeriosis, is of particular importance. In this study, initial counts of *L. monocytogenes* increased by over 4.0 log CFU/g during 7 days of storage at 7°C, confirming the concern that if cross-contamination occurs after preparation of foods, *L. monocytogenes* can multiply extensively during refrigerated storage. Although the reheating methods investigated in this study reduced *L. monocytogenes* contamination by 2-5 log CFU/g, growth of the pathogen during storage allowed high numbers of survivors after reheating, especially after two days of storage. In other words, storage time affected the initial microbial counts and had a major impact on subsequent death due to reheating. This indicates that storage period, and type and intensity of reheating need to be considered for safe consumption of leftover food. Regardless of reheating method, high numbers of survivors after reheating, especially after two days of storage indicated a need of consideration for utilization of leftover food, especially by at-risk populations.

Microwave reheating and marinating for reduction of *Listeria monocytogenes.* Although microwave reheating was responsible for 1.9 to 4.1 and 2.1 to 5.0 log CFU/g reduction of *L. monocytogenes* counts during storage, for treatments at 45 seconds and 90 seconds, respectively, numbers of pathogen survivors after reheating increased as populations on stored (7°C) product increased. With similar trends across different marinated samples, high levels of *L. monocytogenes* survivors after microwave oven reheating, especially after two days of storage, indicates that length of storage and reheating time need to be considered for safe consumption of leftover cooked chicken.

CHAPTER IX

REFERENCES

Adekunle, A. O., A. C. S. Porto-Fett, J. E. Call, B. Shoyer, K. Gartner, L. Tufft, and J. B. Luchansky. 2009. Effect of storage and subsequent reheating on viability of *Listeria monocytogenes* on pork scrapple. *J. Food Prot.* 72:2530-2537.

Adler, J. M., I. Geornaras, K. E. Belk, G. C. Smith, and J. N. Sofos. 2009. Thermal inactivation of *Escherichia coli* O157:H7 at different depth of panbroiled and roasted non-intact steak. 96 th annual meeting of the International Association for Food Protection, Grapevine, TX. Abstract No. T8-02.

Ahn, C. K., N. J. Holt, and P. I. Tarr. 2009. Shiga Toxin-Producing *Escherichia coli* and the Hemolytic Uremic Syndrome: What Have We Learned in the Past 25 Years? *J. Ad. Ex. Med. Bio.* 634:1-17.

AMI (American Meat Institute). 2009. U.S. Meat and Poultry Production and Consumption: an overview. Available at: http://www.meatami.com/ht/a/GetDocumentAction/i/48781. Accessed January 26 2012.

Anonymous. 2012. If Farmers do not rein in the use of antibiotics for livestock, people will be severely affected. Editorial article. *Nature* 486:440.

Arthur, T. M., J. M. Bosilevac, X. Nou, S. D. Shackelford, T. L.Wheeler, M. P. Kent, D. Jaroni, B., Pauling, D. M. Allen, and M. Koohmaraie. 2004. *Escherichia coli* O157 prevalence and enumeration of aerobic bacteria, *Enterobacteriaceae*, and *Escherichia coli* O157 at various steps in commercial beef processing plants. *J. Food Prot.* 67:1080-1086.

Arthur, T. M., N. Kalchayanand, J. M. Bosilevac, D. M. Brichta-Harhay, S. D. Shackelford, J. L. Bono, T. L. Wheeler, and M. Koohmaraie. 2008. Comparison of effects of antimicrobial interventions on multidrug-resistant *Salmonella*, susceptible *Salmonella*, and *Escherichia coli* O157:H7. *J. Food Prot.* 71:2177-2181.

Barkocy-Gallagher, G. A., T. M. Arthur, M. Rivera-Betancourt, X. Nou, S. D. Shackelford, T. L., Wheeler, and M. Koohmaraie. 2003. Seasonal prevalence of shiga toxin-producing *Escherichia coli*, including O157:H7 and non-O157 serotypes and *Salmonella* in commercial beef processing plants. *J. Food Control* 66:1978-1986.

Beauchamp, C. S., Dourou, D., Geornaras, I., Yoon, Y., Scanga, J.A., Belk, K. E. Smith, G. C., Nychas, G. E., and J. N. Sofos. 2012. Transfer, attachment, and formation of biofilms by *Escherichia coli* O157 on meat-contact surface material. *J. Food. Sci.* 77:343-247.

Bell, B.P., Goldoft, M., Griffin, P.M., Davis, M.A., Gordon, D.C., and P. I. Tarr. A multistate outbreak of *Escherichia coli* O157:H7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers. The Washington experience. *J. Am Med Assoc*. 272:1349–1353

Bettelheim, K. A. 2007. The Non-O157 shiga-toxigenic (Verocytotoxigenic) *Escherichia coli*; Under-rated pathogens. *Crit. Rev. Microbiol.* 33:67-87.

Beumer, R. R., M. C. te Giffel, E. Spoorenberg, and F. M. Rombouts. 1996. *Listeria* species in domestic environment. *Epidemiol. Infect.* 117:437-442.

Beutin, L., Geier, D., Steinruck, H., Zimmermann, S., and F. Scheutz. 1993. Prevalence and some properties of verotoxin (shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. *J. Clin. Microbiol*. 31:2483-2488.

Beutin, L., Geier, D., Zimmermann, S., and H. Karch. 1995. Virulence markers of shiga-like toxin producing *Escherichia coli* strains originated from healthy domestic animals of different species. *J. Clin. Microbiol.* 33:631-635.

Bielaszewska, M., Herbert, S., Almut, L., Rita, P., Wolfang, R., Helmut, T. Alois, C. Jan, J., Kveta, B. and K. Helge. 2000. Cattle can be a reservoir of sorbitol-fermnentating shiga toxin-producing *Escherichia coli* O157:H7 strains and a source of human disease. *J. Clin. Microbiol.* 38:3470-3473.

Bjorkroth, K.J., Geisen, R., Schillinger, U., Weiss, N., De Vos. P., Holzapfel. W.H., Korkeala, H.J., and P. Vandamme. 2000. Characterization of Leuconostoc gasicomitatum sp. Nov., associated spoiled raw tomato-marinated broiler meat strips packaged under modified-atmosphere condition. *Appl. Environ. Microbiol.* 66:3764-3772.

Bodur, T., and A. Cagri-Mehmetoglu. 2012.Removal of *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* O157:H7 biofilms on stainless steel using scallop shell powder. *J. Food Control* 25:1-9.

Bosilevac, J. M., T. M. Arthur, J. L. Bono, D. M. Brichta-Harhay, N. Kalchayanad, D. A. King, S. D. Shackelford, M. L. Wheeler, and M. Koohmaraie. 2009. Prevalence and enumeration of *Escherichia coli* O157:H7 and *Salmonella* in U.S. Abattoirs that process fewer than 1,000 head of cattle per day. *J. Food Prot.* 72:1272-1278.

Boyle, E. C., Bishop, J. L., Grassl, G. A., and B. B. Finlay. 2007. *Salmonella*: from pathogenesis to therapeutics. *J. Bacteriol.* 189: 1489-1495.

Byelashov, O. A., J. M. Adler, I. Geornaras, K. Y. Ko, K. E. Belk, G. C. Smith, and J. N. Sofos. 2010. Evaluation of brining Ingredients and antimicrobials for effects on thermal destruction of *Escherichia coli* O157:H7 in a meat model system. *J. Food Sci.* 75: 209-217.

Carlson, B. A., K. K. Nightingale, G. L. Mason, J. R. Ruby, W. T. Choat, G. H. Loneragan, G. C. Smith, J. N. Sofos, and K. E. Belk. 2009. *Escherichia coli* O157:H7 Strains That Persist in Feedlot Cattle Are Genetically Related and Demonstrate an Enhanced Ability To Adhere to Intestinal Epithelial Cells. Appl. *Environ. Microbiol.* 75: 5927-5978.

Chmielewski, R.A.N., and J. F. Frank. 2003. Biofilm formation and control in food processing facilities. *Compr. Rev. Food Sci. F.* 2:22-32.

Castillo, A., L. M. Lucia, D. B. Roberson, T. H. Stevenson, I. Mercado, and G. R. Acuff. 2001. Lactic acid sprays reduce bacterial pathogens on cold beef carcass surfaces and in subsequently produced ground beef. *J. Food Prot.* 64:58-62.

CDC (Centers for Disease Control and Prevention). 2009. National Antimicrobial Resistance Monitoring System Enteric Bacteria: Human Isolates Final Report, 2006. Atlanta, GA: U.S. Department of Health and Human Services. Available at: http://www.cdc.gov/narms/annual/2006/NARMSAnnualReport2006.pdf. Accessed June 18 2012.

Cizek, A., Alexa, P., Literak, I., Hamrik, J. Noval, P. and J. Smola. 1999. Shiga toxin-producing *Escherichia coli* O157 in feedlot cattle and Norwegian rats from a large-scale farm. *Lett. Appl. Microbiol*. 28:435-439.

Cobbold, R. N., Rice, D. H., Szymanski, M., Call, D. R., and D. D. Hancock. 2004. Comparison of shiga-toxigenic *Escherichia coli* prevalences among dairy, feedlot, and cow-calf herds in Washington State. *Appl. Environ. Microbiol*. 70:4375-4378.

Cutter, C. N., and M. Rivera-Betancourt. 2000. Interventions for the reduction of *Salmonella* Typhimurium DT 104 and non-O157:H7 enterohemorrhagic *Escherichia coli* on beef surfaces. *J. Food Prot.* 63:1326-1332.

Damodaran, S., K. L. Parkin, and O. R. Fennema (ed.). 2008. Fennema's Food Chemistry Fourth Edition. CRC Press Taylor and Francis Group, New York.

Davidson, P. A., Sofos, J. N., and A. L. Branen. 2005. Antimicrobials in Food. Taylor, and Fransis Group LLC. PP: 8-9.

Elder R. O., Keen J. E, SiragusaG. R., Barkocy-Gallagher G. A., Koohmaraie M., and W. W. Laegreid. 2000. Correlation of enterohemorrhagic *Escherichia coli* O157 prevalence in feces, hides, and carcasses of beef cattle during processing. *The National Academy of Sciences* 97: 2999-3003.

Ellebracht, J. W., King, D. A., Castillo, A., Lucia, L. M., Acuff, G. R., Hariis, K. B., and J. W. Savell.2005. Evaluation of peroxyacetic acid as a potential pre-grinding treatment for control of *Escherichia coli* O157:H7 and Salmonella Typhimurium on beef trimming. *J. Meat Sci*. 70:197-203.

Ethelberg, S., B. Smith, M. Torpdahl, M. Lisby, J. Boel, T. Jensen, E. M. Nielsen, and K. Molbak. 2009. Outbreak of non-O157 shiga toxin-producing *Escherichia coli* infection from consumption of beef sausage. *Clin. Infect. Dis.* 48:78-81.

Ezawa, A. F., Gocho, F., Kawata, K., Takahashi, T., and N. Kikuchi. 2004. High prevalence of Enterohemorrhagic *Escherichia coli* (EHEC) O157 from cattle in selected regions of Japan. *J.Vet. Med. Sci.* 66:585-587.

FDA (Food and Drug Administration). 2010. Cooked chicken breasts recalled for *Listeria* contamination. Available at: http://www.fda.gov/Safety/Recalls/ArchiveRecalls/2007/default.htm?fragment25_NextRow=51. Accessed January 26 2012.

FDA (Food and Drug Administration). 2009. Measures to address the risk for contamination by *Salmonella* species in food containing a peanut-derived product as ingredient: Guidance for Industry. Available at: http://www.fda.gov/Food/GuidanceComplianceRegulatoryInformation/GuidanceDocuments/ProduceandPlanProducts/ucm115386.htm Accessed: June 18 2012.

Food Safety News. 2012. 19 ill in Drug-Resistant Salmonella Ground Beef Outbreak. Available at: http://www.foodsafetynews.com/2012/01/19-ill-in-drug-resistant-salmonella-ground-beef-outbreak/ Accessed: February 2012.

Farell, B.L., Ronner, AB., Lee, W. and C. Amy. 1998. Attachment of *Escherichia coli* O157:H7 in ground beef to meat grinders and survival after sanitation with chlorine and peroxyacetic acid. *J. Food Prot. 61*:817-822.

Fratamico, P. M., Schultz, F. J., Benedict, R. C., Buchanan, R. L., and P. H. Cooke. 1996. Factors influencing attachment of *Escherichia coli* O157:H7 to beef tissues and removal using selected sanitizing rinses. *J. Food Prot. 59*:453-457.

Fugett, E., E. Fortes, C. Nnoka, and M. Wiedmann. 2006. International life sciences institute North America *Listeria monocytogenes* strains collection: Development of standard *Listeria monocytogenes* strain sets for research and validation studies. *J. Food Prot.* 69:2929-2938.

Gandhi, M., and M. L. Chikindas. 2007. *Listeria*: A foodborne pathogen that knows how to survive. *Int. J. Food Microbiol*. 113:1-15.

Geeraerd., A.H., V. P. Valdramidis, and J. F. Van Impe. 2005. GInaFiT, a freeware tool to assess non-log-linear microbial survivor curves. *Int. J. Food Microbiol.* 102:95-105.

Georgnaras, I., H. Yang, N. Andritsosn, A. Fouladkhah, S. Manios, G. D. Moschonas, M. C. Nunnelly, K. E. Belk., K. K. Nightingale, D. R. Woerner, G. C. Smith, and J. N. Sofos. 2011. Evaluation of chemical decontamination treatments for beef trimmings against *Escherichia coli* O157:H7, non-O157 shiga toxin-producing, *E. coli* and antibiotic resistant and susceptible *Salmonella* Typhimurium and *Salmonella* Newport. Available at: http://www.amif.org/research/10-119/. Accessed, June 18, 2012.

Giaouris, E., Chorianopoulous, N., Nychas G. E. 2005. Effect of temperature, pH, and water activity on biofilm formation of *Salmonella enteric* Enteritidis PT4 on stainless steel surfaces as indicated by the bead vortexing method and conductance measurement. . *J. Food Prot.* 68:2149-54.

Grant, M. A., C. Hedberg, R. Johnson, J. Harris, C. E. Logue, J. Meng, J. N. Sofos, and J. S. Dickson. 2011. The significance of non-O157 shiga toxin-producing *Escherichia coli* in *food. Food Prot.* Trends. *Food Prot. Trends* 31:33-45.

Greene, S. K., A. M. Stuart, F. M. Medalla, J. M. Whichard, R. M. Hoekstra, and T. M. Chiller. 2008. Distribution of multidrug-resistant human isolates of MDR-ACSSuT *Salmonella* Typhimurium and MDR-AmpC *Salmonella* Newport in the United States, 2003-2005. *Foodborne Pathog. Dis.* 5:669-680.

Griffth, C. J., K. A. Mathias, and P. E. Price. 1994. The mass media and food hygiene. *Brit. Food J.* 96:16-21.

Gupta, S., I. Geornaras, K. E. Belk, G. C. Smith, and J. N. Sofos. 2010. Thermal inactivation of *Escherichia coli* O157:H7 in different parts of beef roasts moisture-enhanced with different brining formulations and cooked to rare and very-rare degrees of doneness. 70th Annual Meeting of the Institute of Food Technologists, Chicago, IL, Abstract No. 038-57.

Gyles, C. L. 2007. Shiga toxin-producing *Escherichia coli*: and overview. J. Anim. Sci. 85:E45-E62.

Harley, M. M. 2000. Changing consumer demand for meat: The U.S. example, 1970-2000. The U.S. Dept. of Agriculture Economic Research center. Available at: http://www.ers.usda.gov/publications/wrs011/wrs011g.pdf . Accessed January 26 2012.

Harris, K., M. F. Miller, G. H. Loneragan, and M. M. Brashears. 2006. Validation of the use of organic acids and acidified sodium chlorite to reduce *Escherichia coli* O157 and *Salmonella* Typhimurium in beef trim and ground beef in a simulated processing environment. *J. Food Prot.* 69:1802-1807.

Hegstad, K., Langsrud, S., Lunestad, B. T., Scheie, A., A., Sunde, M., and S. P. Yazdankhah. 2012. Does the wide use of quaternary ammonium compounds enhance the selection and

spread of antimicrobial resistance and thus threaten our health? *J. Microb Drug Resist.* 16: 91-103.

Hood, S. K., and E. A. Zottola. 1997. Adherence to stainless steel by foodborne microorganisms during growth in model food systems. *Int. J. Food Microbiol.*37:145-153.

Hughes, M.K, S. Yanamala, M. San Francisco, G. H. Loneragan, M. F. Miller, and M. M. Brashears. 2010. Reduction of Multidrug-Resistant and Drug-Susceptible Salmonella in Ground Beef and Freshly Harvested Beef Briskets after Exposure to Commonly Used Industry Antimicrobial Interventions. *J. Food. Protec*. 73: 1231-1237.

Hussein, H. S. 2007. Prevalence and pathogenicity of shiga toxin-producing *Escherichia coli* in beef cattle and their products. *J. Anim. Sci.* 85:E-63-E72.

Hussein, H. S., and L. M. Bollinger. 2005a. Prevalence of shiga toxin-producing *Escherichia coli* in beef cattle. *J. Food Prot*. 68:2224-2241.

Hussein, H. S., and L. M. Bollinger. 2005b. Prevalence of shiga toxin-producing *Escherichia coli* in beef. *Meat Sci.* 71:676-689.

Hussein, H. S., Thran, B. H., Hall, M. R., Kvansnicka, W. G., and R. C. Torell. 2003. Verotoxin-producing *Escherichia coli* in culled beef cows grazing rangeland forages. *Exp. Biol. Med.* 228:352-357.

Jahanner, L., and W. Romer. 2010. Shiga toxins—from cell biology to biomedical applications. *Nature Reviews*| *Microbiol*. 8:105-116.

Jackson, V., I.S. Blai, D. A. McDowell, J. Kennedy, and D. J. Bolton. 2007. The incidence of significant foodborne pathogens in domestic refrigerators. *J. Food Control* 18:346-351.

James, S. J., and J. Evans. 1992. Consumer handling of chilled foods: Temperature performance. *Int. J. Refrig*. 15:299-306.

Jay, M. J., M. J. Loessner, and D. A. Golden. 2005. Modern Food Microbiology. Springer Inc., New York, NY.

Jay, L. S., D. Comar, and L. D. Govenlock. 1999. A video study of Australian domestic food-handling practices. *J. Food Prot.* 62:1285-1296.

Juneja, V. K., and J. N. Sofos (ed.). 2010. Pathogens and toxins in foods: challenges and interventions. ASM Press, Washington, D.C.

Joseph, B., Otta, S. K., Karunasagar, I., and I. Karunasagar. 2001. Biofilm formation by *Salmonella* ssp. on food contact surfaces and their sensitivity to sanitizers. *Int. J. Food Microbiol.* 64:367-372.

Kennedy, J., V. Jackson, I. S. Blair, D. A. McDowell, C. Cowan, and D. J. Bolton. 2005. Food Safety knowledge of consumers and the microbiological and temperature status of their refrigerators. *J. Food Prot.* 68:1421-1430.

Kaspar, C. W., and M. L. Tamplin. 1993. Effects of temperature and salinity on the survival of Vibrio vulnificus in seawater and shellfish. *Appl. Environ. Microbiol.* 59: 2425-2429.

Kilonzo-Nthenge, A., F. Chen, and S. Godwin. 2008. Occurrence of *Listeria* and *Enterobacteriaceae* in Domestic Refrigerators. *J. Food Prot.* 71:608-612.

Maguelonne, T., [Translated by Beel, A.]. 2009 (2nd edition). A History of Food. John Wiley and Sons, Ltd. West Sussex, United Kingdom. PP:83-95.

Marklinder, I. M., M. Lindblad, L. M. Erikson, A. M. Finnson, and R. Lindqvist. 2004. Home storage temperatures and consumer handling of refrigerated foods in Sweden. *J. Food Prot.* 67:2570-2577.

Mathusa, E. C., Y. Chen, E. Enache, and L. Hontz. 2010. Non-O157 Shiga toxin-producing *Escherichia coli* in Foods. *J. Food Prot.* 73(9):1721-1736.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Bresee, J. S., Shapiro, C., Griffin, P. M., and R. R. V. Tauxe. 1999. Food-related illness and death in the United States. *J. Emerg. Infect. Dis.* 5:607-625.

Morosini, M. I., J. A. Ayala, F. Baquero, J. L. Martinez, and J. Blazquez. 2000. Biological Cost of AmpC Production for *Salmonella enteric* Serotype Typhimurium. *Antimicrob. Agents Chemother.* 44:3137-3143.

Morris, J.G. 2011. How safe is our food? *J. Emerg. Infect. Dis.* 17:126-128.

Murphy, R. Y., L. K. Dumcan, E. R. Johnson, M. D. Davis, R. E. Wolfe, and H. G. Brown. 2001. Thermal Lethality of *Salmonella* Senftenberg and *Listeria innocua* in fully cooked and packaged chicken breast strips via steam pasteurization. *J. Food Prot.* 64:2083-2087.

Nataro, J. P., and J. B. Kapper. 1998. Diarrheagenic *Escherichia coli*. *J. Clin. Microbiol. Rev.* 11:142-201.

Oliver, S. P., T. R. Patel, T. R. Callaway, and M. E. Torrence. 2009. ASAS Centennial paper: Developments and future outlook for preharvest food safety. *J. Anim. Sci.* 87:419-437.

Padola, N. L., Sanz, M. E., Blanco, J. E., Blance, M., Blanco, J., Etcheverria, A. I., Arroyo, G. H., Usera, M. A., and A. E. Parma. 2004. Serotypes and virulence genes of bovine

shigatoxigenic *Escherichia coli* (STEC) isolated from a feedlot in Argentina. *Vet. Microbiol.* 100:3-9.

Pathania, A., Mckee, S.R., Bilgili, S.F., and M. Sigh. 2010a. Antomicrobial activity of commercial marinades against multiple strains of *Salmonella* spp. *Int. J. Food Microbiol.* 139:214-217.

Pathania, A., Mckee, S.R., Bilgili, S.F., and M. Sigh. 2010b. Inhibition of Nalidixic acid-resistant *Salmonella* on marinated chicken skin. *J. Food Prot.* 73:2072-2078.

Perko-Makela, P., Koljonen, M., Miettinen, M., and M. Hanninen. 2000. Survival of *campylobacter jejuni* in marinated and nonmarinated chicken products. *J. Food Safety* 20:209-216.

Redmond, E. C., and C. J. Griffith. 2003. Consumer food handling in the home: a review of food safety studies. *J. Food Prot.* 66:130-161.

Rodriguez-Marval, M., I. Geornaras, P. A. Kendall, J. A. Scanga, K. E. Belk, and J. N. Sofos. 2009. Microwave oven heating for inactivation of *Listeria monocytogenes* on frankfurters before consumption. *J. Food Microbiol.* 74: 453-460.

Ryser, E. T., and E. H. Marth. 2007. Listeria, Listeriosis, and food safety (ed.). 2007. CRC Press Taylor and Francis Group, New York.

Ryu, J., Kim, H., and L. R. Beuchat. Attachment and Biofilm Formation by *Escherichia coli* O157:H7 on Stainless Steel as Influenced by Exopolysaccharide Production, Nutrient Availability, and Temperature. *J. Food Prot.* 67:2123-2131.

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States—major pathogens. *J. Emerg Infect Dis.* 17:7-15.

Scallan, E., Griffin, P.M., Angulo, F.J., Tauxe, R.V., and R. M. Hoekstra. 2011b. Foodborne Illness Acquired in the United States—Unspecified Agents. *Emerg Infect Dis* 17:16-22.

Silagyi, K., Kim, S., Lo, Y. M., and C. Wei. 2009. Production of biofilm and quorum sensing by *Escherichia coli* O157:H7 and its transfer from contact surfaces to meat, poultry, ready-to-eat, and produce products. *J. Food Microbiol.* 26:514-519.

Sergelidis, D., Abrahim, A. Sarimvei., C. Panoulis, Pr. Karaioannoglou, and C. Genigeorgis. 1997. Temperature distribution and prevalence of *Listeria* spp. in domestic, retail and industrial refrigirators in Greece. *Int. J. Food Microbiol*. 34:171-177.

Shen, C., Alder, J. M., Geornaras, I., Belk, K. E., Smith G. C., and J. N. Sofos. 2010. Inactivation of *Escherichia coli* O157:H7 in nonintact beefsteaks of different thicknesses cooked by pan broiling, double pan broiling, or roasting using five types of cooking appliances. *J. Food Prot.* 73:461-469.

Shen, C., Geornaras, I., Kendall, P. A., and J. N. Sofos. 2009. Antilisterial activities of salad dressing, without or with prior microwave oven heating, on frankfurters during simulated home storage. *Int. J. Food Microbiol*. 132:9-13.

Shi, X., and X. Zhu. 2009. Biofilm formation and food safety in food industry. *Trends Food Sci. Technol.* 20:407-413.

Simoes, M., Somoes, L. C., M. J. Vieira. 2010. A review of current and emergent biofilm control strategies. *LWT Food Sci. Technol.* 43:573-583.

Smith, D. R., Gray, J. T., Moxley, R. A., Younts-Dahl, S. M., Blackford, M. P., Hinkley, S., Hungerford, L. L.,Milton, C. T., and T. J. Klopfenstein. 2004. A diagnostic strategy to determine the shiga toxin-producing *Escherichia coli* O157 status of pens of feedlot cattle. *Epidemiol. Infec*. 132:297-302.

Sofos, J. N. 2008. Challenges to meat safety in the 21st century. *Meat Sci.* 78:3-13.

Sofos, J. N. 2009. Developments and future outlook for postslaughter food safety. J. *Anim. Sci.* 87:2448-2457.

Sofos, J. N., and I. Geornaras. 2010. Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of *Escherichia coli* O157:H7 in nonintact, and *Listeria monocytogenes* in ready-to-eat, meat products. *Meat Sci.* 86:2-14.

Stopforth, J.D., Samelis., J., Sofos, J.N., Kendall P. A., and G. C. Smith. 2002. Biofilm formation by acid-adapted and nonadapted *Listeria monocytogenes* in fresh beef decontamination washings and its subsequent inactivation with sanitizers. *J. Food Prot.* 65:1212-1227.

Stopforth, J. D., Samelis, J., Sofos, J. N., Kendall, P. A., and G. C. Smith. 2003. Influence of extended acid stressing in fresh beef decontamination runoff fluids on sanitizer resistance to acid-adapted *Escherichia coli* O157:H7 in biofilms. *J. Food Prot.* 66:2258-2266.

Sundheim, G. Langsrud, S., Heir, E., and A. L. Holck. 1998. Bacterial resistance to disinfectants containing quaternary ammonium compounds. *J. Int. Biodeterior. Biodegrad.* 41:235-239.

Swain, M. J., James, S. J., and M.V.L. Swain. 2008. Effect of power output reduction of domestic microwave ovens after continuous (intermittent) use on food temperature after reheating. *J. Food. Eng.* 87:11-15.

Toule, G., and O. Murphy. 1978. A study of bacteria contaminating refrigerated cooked chicken; their spoilage potential and possible origin. *J. Hyg. Camb.* 81:161-169.

Uhlich, G. A., Cooke, P. H., Solomon, E. B. 2006. Analyses of the Red-dry-rough phenptype of an *Escherichia coli* O157:H7 strain and its role in biofilm formation and resistance to *antibacterial agents. Appl. Environ. Microbiol.* 72:2564-2572.

USDA-FSIS (United States Department of Agriculture- Food Safety Inspection Service). 2011. Public Health Importance of Non-O157 Shiga Toxin-Producing Escherichia coli (non-O157 STEC) in the US Food Supply. Available at: http://www.fsis.usda.gov/PDF/STEC_101207.pdf. Accessed June18, 2012.

USDA-FSIS (United States Department of Agriculture- Food Safety Inspection Service). 2010. Pennsylvania Firm Recalls Ground Beef Products Due to Possible E. coli O26 Contamination. Available at: http://www.fsis.usda.gov/News/Recall_050_2010_Release/index.asp. Accessed July 11, 2012.

USDA-FSIS (United States Department of Agriculture- Food Safety Inspection Service). 2008. Laboratory Guidebook notice of Change. Isolation and Identification of *Listeria* Monocytogenes from red meat, poultry, eggs, and environmental samples. Available at: http://www.fsis.usda.gov/Science/Microbiological_Lab_Guidebook/index.asp. Accessed Accessed January 26 2012.

[USDA-FSIS] United States Department of Agriculture, Food Safety and Inspection Service. 1999. FSIS Policy on Non-intact Raw Beef Products Contaminated with E. coli O157:H7. Available at : http://www.fsis.usda.gov/oa/background/O157policy.htm. Accessed November 20, 2012.

Wang, R., Bono, J. L., Kalchayanand, N., Shackelford, S., and D. M. Harhay. 2012. Biofilm formation by shiga toxin-producing *Escherichia coli* O157:H7 and non-O157 strains and their tolerance to sanitizers commonly used in the food processing environment. *J. Food Prot.* 75:1418-1428.

Walker, S. J., Archer, P., and J. G. Banks. 1990. Growth of *Listeria monocytogenes* at refrigerated temperatures. *J. Appl. Microbiol.* 68:157-162.

Wells, J.E., Shackelford, S.D., Berry, E.D., Kalchayanand, N. Guerini, M.N. Varel, V.H., Arthur, T.M., Bosilevac, J.M., Freetly, H.C., Wheeler, T.L., Ferrell, C.L., and M. Koohmaraie. 2009. Prevalence and Level of Escherichia coli O157:H7 in Feces and on Hides of Feedlot Steers Fed Diets with or without Wet Distillers Grains with Solubles. *J. Food Prot.* 72: 1624-1633.

Wilson, J. B., McEwen, S. A., Clarke, R. C., Leslie, K. E., Wilson, R. A., Waltner-Toews, D., and C. L. Gyles. 1992. Distribution and characteristics of verotoxigenic *Escherichia coli* isolated from Ontario dairy cattle. *Epidemiol. Infect*. 108:423-439.

Yang, H., P. A. Kendall, L. C. Medeiros, and J. N. Sofos. 2009a. Efficacy of Sanitizing Agents against *Listeria monocytogenes* biofilms on high-density polyethylene cutting boards surfaces . *J. Food Prot.* 72:990-998.

Yang, H., P. A. Kendall, L. C. Medeiros, and J. N. Sofos. 2009b. Inactivation of *Listeria monocytogenes Escherichia coli* O157:H7, and *Salmonella* Typhimurium with compounds available in households. *J. Food Prot.* 72:1201-1208.

Zhao, S., K. Blickenstaff, A.Glenn, S. L. Ayers, S. L. Friedman, J. W. Abbott, and P. F. McDermott. 2009. Lactam Resistance in *Salmonella* Strains Isolated from Retail Meats in the United States by the National Antimicrobial Resistance Monitoring System between 2002 and 2006. *Appl. Environ. Microbiol.*75: 7624-7630.

Zobell, C. E. 1943. The effect of solid surfaces upon bacterial activity. *J. Bacteriol.* 46: 39–56.

Appendix

Table A1 (Data for Figure 3.1). Biofilm formation at 4°C for the seven pathogen groups (selective media counts).

	Days			
	0	1	4	7
Escherichia coli O157:H7*	1.6±0.4 aA	1.7±0.3 abA	1.9±0.3 aA	1.2±0.8 aA
Non-O157 *Escherichia coli* serovars	1.7±0.4 aA	2.1±0.5 abA	2.2±0.5 aA	1.4±0.3 aA
Susceptible *Salmonella* Typhimurium	1.5±0.6 aB	1.7±0.5 bB	2.5±0.3 aA	1.9±0.4 aAB
MDR *Salmonella* Typhimurium	2.0±0.4 aA	1.7±0.7 bA	2.4±0.1 aA	1.7±1.0 aA
MDR-AmpC *Salmonella* Typhimurium	2.0±0.5 aA	1.9±1.0 abA	2.2±0.3 aA	1.5±0.3 aA
Susceptible *Salmonella* Newport	2.1±0.8 aA	1.8±0.6 abA	2.3±0.5 aA	1.4±0.6 aA
MDR-AmpC *Salmonella* Newport	2.4±0.6 aAB	2.8±0.2 aA	2.5±0.1 aAB	1.9±0.8 aB

* Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table A2 (Data for Figure 3.1). Biofilm formation at 15°C for the seven pathogen groups (selective media counts).

	Days			
	0	1	4	7
Escherichia coli O157:H7	1.4±0.6 aC	2.3±0.4 aBC	3.0±0.9 aB	4.8±0.6 abA
Non-O157 *Escherichia coli* serovars	1.7±0.8 aC	2.4±0.3 aBC	3.3±1.2 aB	4.9±0.6 aA
Susceptible *Salmonella* Typhimurium	1.3±0.8 aB	2.7±0.8 aA	3.3±0.6 aA	3.8±0.5 bA
MDR *Salmonella* Typhimurium	2.3±0.4 aC	2.8±0.3 aBC	3.3±0.7 aAB	4.2±0.6 abA
MDR-AmpC *Salmonella* Typhimurium	1.9±0.3 aC	2.3±0.3 aC	3.3±0.5 aB	4.7±0.6 abA
Susceptible *Salmonella* Newport	2.1±0.4 aC	2.8±0.5 aBC	3.1±0.5 aB	4.5±0.4 abA
MDR-AmpC *Salmonella* Newport	2.0±0.2 aC	2.5±0.3 aBC	2.9±0.5 a B	4.5±0.8 abA

* Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table A3 (Data for Figure 3.1). Biofilm formation at 25°C for the seven pathogen groups (selective media counts).

	Days			
	0	1	4	7
Escherichia coli O157:H7	2.0±0.3 aB	2.1±0.8 aB	3.5±0.4 aA	3.3±0.7 bcA
Non-O157 *Escherichia coli* serovars	2.0±0.8 aB	2.6±1.3 aB	4.1±0.2 aA	2.9±0.5 cAB
Susceptible *Salmonella* Typhimurium	1.5±0.8 aB	3.5±0.9 aA	3.7±0.8 aA	4.2±0.4 abA
MDR *Salmonella* Typhimurium	1.7±0.7 aB	3.2±1.0 aA	4.0±0.7 aA	4.3±0.4 aA
MDR-AmpC *Salmonella* Typhimurium	1.6±0.9 aB	2.7±1.1 aB	4.3±0.4 aA	4.1±1.0 abA
Susceptible *Salmonella* Newport	1.6±0.9 aB	3.3±0.9 aA	4.2±0.8 aA	4.3±0.4 abA
MDR-AmpC *Salmonella* Newport	1.7±1.0 aB	3.6±0.7 aA	4.3±0.4 aA	4.3±0.5 abA

* Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table A4 (Data for Figure 3.2). Biofilm formation at 4°C for the seven pathogen groups and background microflora (non-selective media counts).

	Days			
	0	1	4	7
Escherichia coli O157:H7*	2.2±0.4 aD	2.6±0.1 bC	4.1±0.2 aB	5.5±0.2 aA
Non-O157 *Escherichia coli* serovars	2.2±0.4 aC	2.6±0.2 bC	3.9±0.2 aB	5.5±0.2 aA
Susceptible *Salmonella* Typhimurium	2.2±0.3 aC	2.8±0.2 bC	3.9±0.7 aB	5.7±0.2 aA
MDR *Salmonella* Typhimurium	2.5±0.3 aC	2.8±0.3 bC	4.3±0.2 aB	5.4±0.2 aA
MDR-AmpC *Salmonella* Typhimurium	2.4±0.4 aC	2.9±0.4 bC	4.2±0.5 aB	5.7±0.4 aA
Susceptible *Salmonella* Newport	2.6±0.4 aC	3.0±0.2 abC	4.4±0.1 aB	5.5±0.1 aA
MDR-AmpC *Salmonella* Newport	2.8±0.6 aC	3.3±0.1 aC	4.4±0.6 aB	5.6±0.4 aA

* Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table A5 (Data for Figure 3.2). Biofilm formation at 15°C for the seven pathogen groups and background microflora (non-selective media counts).

	Days			
	0	1	4	7
Escherichia coli O157:H7	2.3±0.3 aD	4.6±0.1 bC	5.9±0.3 aB	6.5±0.2 aA
Non-O157 *Escherichia coli* serovars	2.5±0.4 aD	4.6±0.2 bC	6.0±0.2 aB	6.4±0.3 aA
Susceptible *Salmonella* Typhimurium	2.2±0.2 aD	4.6±0.1 bC	5.8±0.3 aB	6.4±0.3 aA
MDR *Salmonella* Typhimurium	2.6±0.4 aD	4.6±0.0 abC	5.8±0.3 aB	6.4±0.3 aA
MDR-AmpC *Salmonella* Typhimurium	2.2±0.2 aD	4.8±0.2 abC	5.9±0.2 aB	6.6±0.2 aA
Susceptible *Salmonella* Newport	2.4±0.2 aD	5.0±0.3 aC	5.9±0.2 aB	6.5±0.3 aA
MDR-AmpC *Salmonella* Newport	2.4±0.1 aD	4.6±0.2 bC	5.9±0.3 aB	6.3±0.2 aA

* Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table A6 (Data for Figure 3.2). Biofilm formation at 25°C for the seven pathogen groups and background microflora (non-selective media counts).

	Days			
	0	1	4	7
Escherichia coli O157:H7	2.3±0.4 aC	5.2±0.1 aB	6.8±0.3 aA	6.4±0.6 aA
Non-O157 *Escherichia coli* serovars	2.1±0.5 aC	5.1±0.1 aB	6.7±0.4 aA	6.5±0.6 aA
Susceptible *Salmonella* Typhimurium	2.0±0.5 aC	5.2±0.3 aB	6.4±0.6 aA	7.0±0.2 aA
MDR *Salmonella* Typhimurium	2.3±0.4 aD	5.2±0.2 aC	6.5±0.3 aB	7.1±0.4 aA
MDR-AmpC *Salmonella* Typhimurium	2.3±0.5 aC	5.2±0.3 aB	6.3±0.4 aA	6.5±0.5 aA
Susceptible *Salmonella* Newport	2.2±0.4 aC	5.1±0.1 aB	6.6±0.5 aA	7.1±0.4 aA
MDR-AmpC *Salmonella* Newport	2.3±0.4 aC	5.2±0.2 aB	6.7±0.5 aA	7.0±0.1 aA

* Within a column, means with different lowercase letter(s) and within a row, means with different upper case letter(s) are significantly different ($P<0.05$).

Table A7 (Data for Figures 4.1 and 4.2). Survival of parents and rifampicin-resistant *E. coli* O26 strains (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

				Time (minutes)				
Strain	Serotype	Media	Control	0	2	4	6	8
5-strain mix	O157: H7	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
5-strain mix R	O157: H7	TSA+Rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O26 (1)	O26	TSA	6.1±0.0 A	5.3±0.0 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O26 (1) R*	O26	TSA+Rif	6.2±0.1 A	5.0±0.2 B	2.3±0.3 C **	1.2±0.4 D **	1.1±0.2 D **	<1.0 E
O26(2)	O26	TSA	5.8±0.4 A *	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B *
O26(2) R	O26	TSA+Rif	6.3±0.1 A	2.0±0.1 B**	<1.0 C **	<1.0 C **	<1.0 C	<1.0 C
A1-006	O26	TSA	6.2±0.1 A	4.4±0.3 B *	1.7±0.1 C *	<1.0 D *	<1.0 D *	<1.0 D *
A1-006 R	O26	TSA+Rif	6.3±0.1 A**	4.6±0.9 B	2.4±0.1 C**	<1.0 D **	<1.0 D	<1.0 D
A1-007	O26	TSA	6.1±0.2 A	2.4±0.3 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
A1-007 R	O26	TSA+Rif	6.5±0.1 A**	2.3±0.3 B**	<1.0 C **	<1.0 C **	<1.0 C	<1.0 C
A1-008	O26	TSA	6.4±0.2 A *	5.2±0.1 B	3.0±0.0 C *	2.8±0.1 D *	2.6±0.1 D	2.4±0.1 E *
A1-008 R	O26	TSA+Rif	6.5±0.1 A**	5.2±0.2 B	2.7±0.4 C	2.1±0.1 D **	<1.0 E	<1.0 E
A1-009	O26	TSA	6.3±0.2 A	5.6±0.2 B *	2.8±0.5 C *	<1.0 D *	<1.0 D *	<1.0 D *
A1-009 R	O26	TSA+Rif	6.4±0.1 A**	5.0±0.0 B	<1.0 C **	<1.0 C **	<1.0 C	<1.0 C
A1-010	O26	TSA	6.2±0.1 A	3.5±0.7 B	2.6±0.1 C *	1.5±0.1 D *	<1.0 E *	<1.0 E *
A1-010 R	O26	TSA+Rif	6.5±0.3 A**	3.6±0.2 B**	2.7±0.2 C **	1.4±0.7 D	<1.0 E	<1.0 E

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. TSA values within each column followed by * are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7. TSA+rif values within each column followed by ** are significantly (P<0.05) different with TSA+rif values of the control mixture of *Escherichia coli* O157:H7.

Table A8 (Data for Figures 4.1 and 4.2). Survival of parents and rifampicin-resistant *E. coli* O111 strains (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

				Time (min)				
Strain	Serotype	Media	Control	0	2	4	6	8
5-strain mix	O157:H7	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
5-strain mix R	O157:H7	TSA+Rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
A1-001	O111	TSA	6.1±0.1 A	4.5±0.1 B *	2.5±0.8 C *	<1.0 D *	<1.0 D *	<1.0 D *
A1-001 R	O111	TSA+Rif	6.4±0.1 A**	5.2±0.2 B	1.7±0.2 C**	<1.0 D**	<1.0 D	<1.0 D
A1-002	O111	TSA	6.4±0.1 A *	5.2±0.1 B	3.2±0.2 C *	1.7±0.5 D *	<1.0 E *	<1.0 E *
A1-002 R	O111	TSA+Rif	6.2±0.3 A	5.5±0.1 B	2.9±0.4 C**	1.7±0.1 D	<1.0 E	<1.0 E
A1-003	O111	TSA	6.1±0.1 A	5.7±0.0 B	3.3±0.4 *	3.4±0.3	2.3±0.8	1.7±0.1 *
A1-003 R	O111	TSA+Rif	6.3±0.1 A**	5.9±0.1 B**	3.6±0.2 E	3.1±0.1 D**	2.2±0.1 E **	1.9±0.2 F **
A1-004	O111	TSA	6.5±0.1 A *	2.8±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
A1-004 R	O111	TSA+Rif	6.5±0.1 A**	3.8±0.3 B**	1.6±0.1 C**	1.05±0.1 D**	<1.0 D	<1.0 D
A1-005	O111	TSA	6.2±0.1 A	4.7±0.4 B	2.3±0.4 C *	<1.0 ±0.0 D *	<1.0 D *	<1.0 D *
A1-005 R	O111	TSA+Rif	6.4±0.0 A**	4.1±0.32 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C
O111(1)	O111	TSA	6.2±0.1 A	<1.0 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O111(1) R	O111	TSA+Rif	6.1±0.3 A	3.4±0.4 B**	1.8±0.1 C**	<1.0 D**	<1.0 D	<1.0 D
O111(2)	O111	TSA	5.7±0.1 A *	2.1±0.6 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *

O111(2) R	O111	TSA+Rif	6.2±0.1 A	2.1±0.1 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. TSA values within each column followed by * are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7. TSA+rif values within each column followed by ** are significantly (P<0.05) different with TSA+rif values of the control mixture of *Escherichia coli* O157:H7.

Table A9 (Data for Figures 4.1 and 4.2). Survival of parents and rifampicin-resistant *E. coli* O103 strains (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

				Time (min)				
Strain	Serotype	Media	Control	0	2	4	6	8
5-strain mix	O157:H7	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
5-strain mix R	O157:H7	TSA+Rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O103(1)	O103	TSA	6.1±0.1 A	4.3±0.4 B *	1.3±0.4 C *	<1.0 D *	<1.0 D *	<1.0 D *
O103(1) R	O103	TSA+Rif	6.0±0.2 A	1.6±0.2 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C
A1-0103 (2)	O103	TSA	6.2±0.1 A *	4.6±0.6 B *	2.4±0.6 C *	<1.5±0.0 D *	<1.0 E *	<1.0 E *
A1-0103 (2) R	O103	TSA+Rif	6.4±0.1 A**	5.2±0.0 B	3.1±0.1 C**	3.0±0.1 C**	1.6±0.3 D	<1.0 E
A1-011	O103	TSA	6.1±0.1 A	4.9±0.1 B *	2.4±0.5 C *	1.3±0.3 C *	<1.0 D *	<1.0 D *
A1-011 R	O103	TSA+Rif	6.1±0.2 A	5.3±0.2 B	3.7±0.2 C	2.3±0.1 D**	1.7±0.1 E**	<1.0 F
A1-012	O103	TSA	6.5±0.1 A *	3.4±0.3 B *	2.1±0.1 C *	1.7±0.1 D *	<1.0 E *	<1.0 E *
A1-012 R	O103	TSA+Rif	6.5±0.1 A**	1.9±0.3 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C
A1-013	O103	TSA	5.9±0.1 A *	4.9±0.1 B *	2.4±0.5 C *	<1.0 D *	<1.0 D *	<1.0 D *
A1-013 R	O103	TSA+Rif	6.2±0.4 A	3.9±0.6 B**	1.8±0.1 C**	<1.0 D**	<1.0 D	<1.0 D
A1-014	O103	TSA	6.2±0.3 A	3.8±0.1 B *	3.4±0.1 C *	2.6±0.1 D *	2.5±0.1 D	2.5±0.1 D *
A1-014 R	O103	TSA+Rif	6.4±0.1 A**	6.0±0.1 B**	3.6±0.1 C**	2.6±0.3 D**	<1.0 E	<1.0 E
A1-015	O103	TSA	6.3±0.1 A *	2.2±0.0 B *	1.4±0.1 C *	1.0±0.0 D *	<1.0 D *	<1.0 D *
A1-015 R	O103	TSA+Rif	6.4±0.1 A **	3.5±0.1 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. TSA values within each column followed by * are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7. TSA+rif values within each column followed by ** are significantly (P<0.05) different with TSA+rif values of the control mixture of *Escherichia coli* O157:H7.

Table A10 (Data for Figures 4.1 and 4.2). Survival of parents and rifampicin-resistant *E. coli* O45 strains (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

				Time (min)				
Strain	Serotype	Media	Control	0	2	4	6	8
5-strain mix	O157	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
5-strain mix R	O157	TSA+Rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O45(1)	O45	TSA	5.9±0.2 A	<1.0 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O45(1) R	O45	TSA+Rif	6.2±0.2 A	2.1±0.9 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C
O45(2)	O45	TSA	6.1±0.1 A	5.2±0.4 B	<2.1±0.9 C *	<1.0 D *	<1.0 D *	<1.0 D *
O45(2) R	O45	TSA+Rif	6.4±0.1 A**	4.1±0.5 B	1.2±0.4 C**	<1.0 D**	<1.0 D	<1.0 D
O45: H2 05-6545	O45	TSA	5.9±0.1 A	5.4±0.3 B	4.9±0.1 C	3.6±0.3 D	3.7±0.0 D *	2.6±0.0 E *
O45: H2 R 05-6545	O45	TSA+Rif	5.7±0.1 A	5.2±0.3 B	4.1±0.2 C**	2.8±0.4 D**	<1.0 E	<1.0 E
O45: H2(2) 96-3285	O45	TSA	6.0±0.0 A	5.5±0.1 B	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O45: H2(2) R 96-3285	O45	TSA+Rif	6.0±0.2 A	2.1±0.4 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. TSA values within each column followed by * are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7. TSA+rif values within each column followed by ** are significantly (P<0.05) different with TSA+rif values of the control mixture of *Escherichia coli* O157:H7.

Table A11 (Data for Figures 4.1 and 4.2). Survival of parents and rifampicin-resistant *E. coli* O121 strains (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

				Time (min)				
Strain	Serotype	Media	Control	0	2	4	6	8
5-strain mix	O157:H7	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
5-strain mix R	O157:H7	TSA+Rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O121(1)	O121	TSA	5.8±0.2 A *	1.0±0.0 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O121(1) R	O121	TSA+Rif	6.2±0.4 A	2.3±0.0 B**	2.2±0.0 C**	1.8±0.2 D	1.1±0.1 E	<1.0 E
O121(2)	O121	TSA	6.0±0.1 A	3.0±0.3 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O121(2) R	O121	TSA+Rif	6.4±0.1 A**	3.5±0.5 B**	1.1±0.2 C**	<1.0 D**	<1.0 D	<1.0 D
O121:H19 97-3068	O121	TSA	5.7±0.0 A *	5.4±0.1 B	4.9±0.1 C *	2.9±0.1 D *	1.6±0.1 E *	<1.0 F *
O121:H19 R 97-3068	O121	TSA+Rif	6.1±0.4 A	4.5±0.1 B	2.0±0.9 C**	<1.0 D**	<1.0 D	<1.0 D
O121:NM 03-4064	O121	TSA	5.8±0.1 A *	5.0±0.2 B	2.7±0.7 C *	<1.0 D *	<1.0 D *	<1.0 D *
O121:NM R 03-4064	O121	TSA+Rif	6.2±0.1 A**	3.3±0.1 B**	3.2±0.1 B**	2.4±0.7 C**	1.8±0.5 D **	<1.0 E
O121:H19 08023	O121	TSA	5.7±0.1 A *	<1.0 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O121:H19 R 08023	O121	TSA+Rif	5.9±0.33 A	4.4±0.3 B	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. TSA values within each column followed by * are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7. TSA+rif values within each column followed by ** are significantly (P<0.05) different with TSA+rif values of the control mixture of *Escherichia coli* O157:H7.

Table A12 (Data for Figures 4.1 and 4.2). Survival of parents and rifampicin-resistant *E. coli* O145 strains (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

				Time (min)				
Strain	Serotype	Media	Control	0	2	4	6	8
5-strain mix	O157:H7	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
5-strain mix R	O157:H7	TSA+Rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O145(1)	O145	TSA	6.2±0.1 A	1.3±0.4 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O145(1)R	O145	TSA+Rif	6.5±0.1 A**	4.0±0.1 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C
O145(2)	O145	TSA	6.3±0.1 A	4.2±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O145(2)R	O145	TSA+Rif	6.3±0.1 A	4.9±0.3 B	1.5±0.4 C**	<1.0 D**	<1.0 D	<1.0 D
O145:NM 03-499	O145	TSA	5.9±0.1 A	5.4±0.3 B	4.9±0.0 C *	4.1±0.1 D	<1.0 E *	<1.0 E *
O145:NM R 03-499	O145	TSA+Rif	6.0±0.3 A	5.1±0.3 B	3.1±0.8 C**	<1.0 D**	<1.0 D	<1.0 D
O145:NM 83-75	O145	TSA	6.1±0.2 A	4.7±0.3 B *	<1.0 C *	<1.0 C *	<1.0 C *	<1.0 C *
O145:NM R 83-75	O145	TSA+Rif	5.9±0.4 A	1.7±0.3 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C
O145:H18 07865	O145	TSA	5.4±0.1 A*	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B *
O145:H18 R 07865	O145	TSA+Rif	5.8±0.4 A	2.4±1.3 B**	<1.0 C**	<1.0 C**	<1.0 C	<1.0 C

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. TSA values within each column followed by * are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7. TSA+rif values within each column followed by ** are significantly (P<0.05) different with TSA+rif values of the control mixture of *Escherichia coli* O157:H7.

Table A13 (Data for Figure 4.3 and 4.4). Survival of antibiotic (drug) susceptible *Salmonella* Newport (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

Strain	Media	Control	Time (min)				
			0	2	4	6	8
O157:H7 mix R	TSA+rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O157:H7 mix	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
CVM N4505	XLD	5.8±0.1 A *	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.8±0.1 A	1.3±0.4 B **	<1.0 B **	<1.0 B**	<1.0 B **	<1.0 B**
CVM N18445	XLD	5.9±0.1 A	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.8±0.2 A**	3.2±0.1 B**	<1.0 B**	<1.0 B**	<1.0 B**	<1.0 B**
CVM N1509	XLD	6.1±0.1 A	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.9±0.1A	1.3±0.3 B**	1.1±0.2 B**	<1.0 B**	<1.0 B**	<1.0 B**
FSL S5-639	XLD	5.8±0.3 A	<1.8±0.1 B *	<1.0 ±0.1 C *	<1.2±0.1 C *	<1.0 C	<1.0 C
	TSA	5.9±0.2 A	3.5±0.1 B**	<1.9±0.1 C**	<1.1±0.2 D**	<1.0 D**	<1.0 D**

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. XLD (xylose lysine deoxycholate) values within each column followed by * are significantly (P<0.05) different with TSA+rif (tryptic soy agar+ rifampicin) values of the control mixture of *Escherichia coli* O157:H7. TSA (tryptic soy agar) values within each column followed by ** are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7.

Table A14 (Data for Figure 4.3 and 4.4). Survival of MDR-AmpC *Salmonella* Newport (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

			Time (min)				
Strain	Media	Control	0	2	4	6	8
O157:H7 mix R	TSA+rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O157:H7 mix	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
FSL R6-531	XLD	6.0±0.1 A	2.9±0.1 B	<1.2±0.3 C *	1.1±0.1 C *	<1.0 C	<1.0 C
	TSA	6.1±0.1 A	4.3±0.1 B**	2.0±0.1 C **	2.0±0.1 C	1.3±0.1 D **	1.2±0.3 D
FSL R8-0104	XLD	5.7±0.1 A *	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.7±0.2 A**	<1.0 ±0.1 B**	<1.0±0.1 B **	<1.0 B **	<1.0 B **	<1.0 B **
FSL S5-413	XLD	5.6±0.1 A *	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.4±0.2 A**	<1.0 ±0.1 B**	<1.0 B**	<1.0 B **	<1.0 B **	<1.0 B **
FSL S5-436	XLD	6.0±0.1 A	<2.4±0.2 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	6.0±0.1 A	3.9±0.1 B**	1.5±0.1 C **	<1.0 C **	<1.0 C **	<1.0 C **
FSL S5-577	XLD	5.8±0.1 A	<1.0 B *	<1.0 B *	<1.0 0B *	<1.0 B	<1.0 B
	TSA	5.7±0.1 A**	2.0±0.1 B**	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
FSL S5-920	XLD	5.8±0.1 A	1.2±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	5.7±0.1 A**	3.8±0.1 B**	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **

FSL R8-2926	XLD	5.9±0.1 A	1.6±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.0±0.1 A	3.8±0.2 B**	<1.0±0.1 C **	<1.0 C **	<1.0 C **	<1.0 C **
FSL R8-2350	XLD	5.9±0.1 A	1.7±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.0±0.1 A	2.7±0.1 B**	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM N635	XLD	6.0±0.1 A	1.3±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	5.9±0.2 A	2.0±0.2 B**	<1.0 C**	<1.0 C **	<1.0 C **	<1.0 C **
CVM 22698	XLD	6.1±0.1 A	1.1±0.2 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.0±0.1 A	3.0±0.1 B**	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM 29461	XLD	5.9±0.1 A	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.9±0.2 A	2.1±0.1 B**	<1.0 B **	<1.0 B **	<1.0 B **	<1.0 B **
CVM 22707	XLD	5.8±0.1 A	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.7±0.1 A**	2.3±0.2 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM N19852	XLD	6.0±0.1 A	1.9±0.2 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.0±0.1 A	3.3±0.1 B**	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. XLD (xylose lysine deoxycholate) values within each column followed by * are significantly (P<0.05) different with TSA+rif (tryptic soy agar+ rifampicin) values of the control mixture of *Escherichia coli* O157:H7. TSA (tryptic soy agar) values within each column followed by ** are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7.

Table A15 (Data for Figure 4.3 and 4.4). Survival of antibiotic (drug) susceptible *Salmonella* Typhimurium (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

Strain	Media	Control	Time (min)				
			0	2	4	6	8
O157:H7 mix R	TSA+rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O157:H7 mix	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
CVM N7300	XLD	6.4±0.1 A *	2.0±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.4±0.1 A **	3.3±0.1 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM N15788	XLD	6.0±0.3 A	1.6±0.2 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.1±0.2 A	3.4±0.2 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM N18534	XLD	6.0±0.1 A	3.1±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	5.9±0.1 A	3.3±0.1 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
FSL S5-536	XLD	6.1±0.2 A	<1.3±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.0±0.1 A	3.7±0.1 B **	<1.0±0.1 C **	<1.0 C **	<1.0 C **	<1.0 C **

R=Rifampicin resistant. Values (mean±SD) within each row, followed by different uppercase letters are significantly (P<0.05) different. XLD (xylose lysine deoxycholate) values within each column followed by * are significantly (P<0.05) different with TSA+rif (tryptic soy agar+ rifampicin) values of the control mixture of *Escherichia coli* O157:H7. TSA (tryptic soy agar) values within each column followed by ** are significantly (P<0.05) different with TSA values of the control mixture of *Escherichia coli* O157:H7.

Table A16 (Data for Figure 4.3 and 4.4). Survival of MDR *Salmonella* Typhimurium (log CFU/ ml during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

Strain	Media	Control	Time (min)				
			0	2	4	6	8
O157:H7 mix R	TSA+rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O157:H7 mix	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
FSL R6-215	XLD	6.0±0.1 A	2.0±0.2 B *	1.4±0.1 C *	<1.0 D *	<1.0 D	<1.0 D
	TSA	6.0±0.1 A	4.0±0.1 B **	2.1±0.1 C **	2.0±0.1 C **	1.6±0.1 D **	<1.0 E **
FSL S9-165	XLD	5.9±0.1 A	1.4±0.3 B *	<1.1±0.1 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.0±0.1 A	2.7±0.1 B **	1.9±0.1 C **	<1.0 D **	<1.0 D **	<1.0 D **
FSL R8-2540	XLD	5.9±0.1 A	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.7±0.1 A**	<1.0 B **	<1.0 B **	<1.0 B **	<1.0 B **	<1.0 B **
CVM N6431	XLD	5.9±0.2 A	3.0±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.0±0.1A	3.6±0.1 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM 30662	XLD	5.9±0.2 A	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.7±0.1 A **	1.1±.2 B **	<1.0 B **	<1.0 B **	<1.0 B **	<1.0 B **
CVM N497	XLD	6.1±0.2 A *	<1.0 B *	<1.0 B *	<1.0 B *	<1.0 B	<1.0 B
	TSA	5.4±0.2 A **	2.4±0.1 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **

Table A17 (Data for Figure 4.3 and 4.4). Survival of MDR-Ampc *Salmonella* Typhimurium (log CFU/ ml) during exposure to 5% lactic acid in 10% (W/W) beef homogenate at 25°C.

Strain	Media	Control	Time (min)				
			0	2	4	6	8
O157:H7 mix R	TSA+rif	5.9±0.2 A	5.0±0.6 B	3.9±0.3 C	1.7±0.5 D	<1.0 E	<1.0 E
O157:H7 mix	TSA	6.1±0.1 A	5.4±0.1 B	4.6±0.1 C	3.8±0.2 D	2.5±0.9 E	1.1±0.2 F
FSL S5-786	XLD	6.2±0.1 A *	3.0±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.1±0.1 A	4.1±0.1 B **	2.5±0.2 C **	1.9±0.1 D **	1.1±0.1 E **	<1.0 E **
FSL S5-916	XLD	5.8±0.1 A	2.5±0.2 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	5.9±0.1 A	4.4±0.1 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
FSL S5-385	XLD	6.0±0.1 A	1.4±0.2 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	6.0±0.1 A	4.3±0.1 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM N176	XLD	5.8±0.1 A *	2.0±.2 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	5.7±0.1 A**	2.7±0.1 B **	1.7±0.1 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM 33831	XLD	5.6±0.1 A *	1.7±0.1 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	5.7±0.1 A**	2.2±0.1 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **
CVM 30034	XLD	5.8±0.1 A	1.9±0.2 B *	<1.0 C *	<1.0 C *	<1.0 C	<1.0 C
	TSA	5.8±0.1 A**	1.8±0.1 B **	<1.0 C **	<1.0 C **	<1.0 C **	<1.0 C **

Table A18 (Data for Figure 5.1). Effects of lactic acid immersion interventions at 25 and 55°C against 7 inoculated serotypes of shiga toxin producing *Escherichia coli* (TSA+Rif counts) on surface of red meat.

Serotype	No Treatment	Lactic Acid at 25°C	Lactic Acid at 55°C
O26	3.2 ± 0.1 Aa	2.4 ± 0.1 Bb	2.1 ± 0.3 Ca
O45	3.2 ± 0.1 Aa	2.5 ± 0.2 Bab	1.8 ± 0.5 Ca
O103	3.2 ± 0.1 Aa	2.7 ± 0.2 Ba	2.1 ± 0.3 Ca
O145	3.3 ± 0.4 Aa	2.4 ± 0.3 Bb	2.2 ± 0.3 Ba
O111	3.2 ± 0.1 Aa	2.5 ± 0.1 Bab	2.2 ± 0.3 Ba
O121	3.1 ± 0.1 Aa	2.4 ± 0.2 Bb	2.0 ± 0.2 Ca
O157:H7	3.2 ± 0.1 Aa	2.5 ± 0.2 Bab	1.8 ± 0.4 Ca

Values (mean ± standard deviation) within each row, followed by different uppercase letter are significantly (P<0.05) different.

Values within each column, followed by different lowercase letters are significantly (P<0.05) different.

Table A19 (Data for Figure 5.2). Effects of lactic acid immersion interventions at 25 and 55°C against background microflora (TSA counts) on surface of red meat.

Serotype	No Treatment	Lactic Acid at 25°C	Lactic Acid at 55°C
O26	3.5 ± 0.2 Aa	2.8 ± 0.3 Ba	2.4 ± 0.6 Ba
O45	3.6 ± 0.2 Aa	2.8 ± 0.4 Ba	2.2 ± 0.5 Ca
O103	3.5 ± 0.1 Aa	2.8 ± 0.2 Ba	2.4 ± 0.3 Ca
O145	3.6 ± 0.2 Aa	2.7 ± 0.5 Ba	2.6 ± 0.6 Ba
O111	3.6 ± 0.5 Aa	2.5 ± 0.6 Ba	2.3 ± 0.5 Ba
O121	3.6 ± 0.4 Aa	2.9 ± 0.4 Ba	2.5 ± 0.5 Ba
O157:H7	3.6 ± 0.4 Aa	2.8 ± 0.3 Ba	2.3 ± 0.5 Ba

Values (mean ± standard deviation) within each row, followed by different uppercase letter are significantly (P<0.05) different.

Values within each column, followed by different lowercase letters are significantly (P<0.05) different.

Table A20 (Data for Figure 5.3). Effects of lactic acid immersion interventions at 25 and 55°C against inoculated serotypes of *Escherichia coli* O157:H7 (TSA+ Rif) and susceptible and resistant *Salmonella* Typhimurium and Newport (XLD counts) on surface of red meat.

Serotype	Media	No Treatment	Lactic Acid at 25°C	Lactic Acid at 55°C
S. Typhimurium, Antibiotic susceptible	XLD	3.2 ± 0.1 Aa	1.9 ± 0.2 Bb	1.6 ± 0.4 Bab
S. Typhimurium, MDR	XLD	3.3 ± 0.0 Aa	2.0 ± 0.2 Bb	1.4 ± 0.5 Cab
S. Typhimurium, MDR, AmpC	XLD	3.0 ± 0.1 Aa	1.7 ± 0.2 Bbc	1.3 ± 0.4 Bb
S. Newport, Antibiotic susceptible	XLD	3.1 ± 0.0 Aa	1.8 ± 0.1 Bbc	1.5 ± 0.4 Bab
S. Newport, MDR, AmpC	XLD	3.1 ± 0.1 Aa	1.6 ± 0.2 Bc	1.2 ± 0.3 Cb
E. coli O157:H7	TSA+Rif	3.2 ± 0.1 Aa	2.7 ± 0.1 Ba	2.0± 0.4 Ca

Values (mean ± standard deviation) within each row, followed by different uppercase letter are significantly (P<0.05) different.

Values within each column, followed by different lowercase letters are significantly (P<0.05) different

Table A21 (Data for Figure 5.4). Effects of lactic acid immersion interventions at 25 and 55°C against inoculated serotypes of *Escherichia coli* O157:H7 (mSMAC counts) and susceptible and resistant *Salmonella* Typhimurium and Newport (XLD counts) on surface of red meat.

Serotype	Media	No Treatment	Lactic Acid at 25°C	Lactic Acid at 55°C
S. Typhimurium, Antibiotic susceptible	XLD	3.2 ± 0.1 Aa	1.9 ± 0.2 Bb	1.6 ± 0.4 Ba
S. Typhimurium, MDR	XLD	3.3 ± 0.0 Aa	2.0 ± 0.2 Bab	1.4 ± 0.5 Ca
S. Typhimurium, MDR, AmpC	XLD	3.0 ± 0.1 Aa	1.7 ± 0.2 Bbc	1.3 ± 0.4 Ba
S. Newport, Antibiotic susceptible	XLD	3.1 ± 0.0 Aa	1.8 ± 0.1 Bbc	1.5 ± 0.4 Ba
S. Newport, MDR, AmpC	XLD	3.1 ± 0.1 Aa	1.6 ± 0.2 Bc	1.2 ± 0.3 Ca
E. coli O157:H7	mSMAC	3.0 ± 0.1 Aa	2.2 ± 0.0 Ba	1.5 ± 0.5 Ca

Values (mean ± standard deviation) within each row, followed by different uppercase letter are significantly ($P<0.05$) different.

Values within each column, followed by different lowercase letters are significantly ($P<0.05$) different

Table A22 (Data for Figure 5.5). Effects of lactic acid immersion interventions at 25 and 55°C against background microflora (TSA counts) on surface of red meat.

Serotype	No Treatment	Lactic Acid at 25°C	Lactic Acid at 55°C
S. Typhimurium, Antibiotic susceptible	4.2 ± 0.5 Aa	3.1 ± 0.4 Ba	2.5 ± 0.4 Ca
S. Typhimurium, MDR	3.9 ± 0.1 Aa	3.0 ± 0.2 Ba	2.5 ± 0.4 Ca
S. Typhimurium, MDR, AmpC	3.9 ± 0.3 Aa	3.1 ± 0.4 Ba	2.7 ± 0.4 Ba
S. Newport, Antibiotic susceptible	3.9 ± 0.4 Aa	3.0 ± 0.2 Ba	2.7 ± 0.4 Ba
S. Newport, MDR, AmpC	4.1 ± 0.3 Aa	2.8 ± 0.3 Ba	2.6 ± 0.3 Ba
E. coli O157:H7	4.0 ± 0.3 Aa	3.2 ± 0.2 Ba	3.0 ± 0.3 Ba

Values (mean ± standard deviation) within each row, followed by different uppercase letter are significantly (P<0.05) different.

Values within each column, followed by different lowercase letters are significantly (P<0.05) different.

Table A23 (Data for Figure 6.1). *Listeria monocytogenes* counts (mean ± standard deviation) of cooked chicken during a 7 day aerobic storage at 7°C before (control) and after 30, 60, and 90 seconds of domestic microwave oven reheating (1100 W).

Storage (Day)	*Listeria monocytogenes* counts (log CFU/g)			
	Control	30 s	60 s	90 s
0	3.7±0.1 D a	3.4±0.1 D b	2.7±0.1 D c	<0.4±0.1 C d
1	3.8±0.1 D a	3.6±0.1 D b	2.8±0.1 D c	0.4±0.1 C d
2	4.6±0.1 C a	4.3±0.1 C b	3.5±0.1 C c	0.4±0.1 C d
4	6.2±0.1 B a	4.8±0.1 B b	4.2±0.1 B c	1.8±0.1 B d
7	7.8±0.2 A a	7.3±0.3 A b	5.6±0.1 A c	2.6±0.2 A d

Values within a column followed by different uppercase letters, and values within a row followed by different lowercase letters are significantly (P<0.05) different.

Table A24 (Data for Figure 6.2). Effects of domestic microwave reheating against aerobic plate counts (log CFU/g) of cooked chicken during a 7 day aerobic storage at 7°C (mean ± standard deviation).

Storage (Day)	**Microwave Reheating Times**			
	Control	30 s	60 s	90 s
0	3.3±0.5 E a	3.0±0.3 D a	2.3±0.1 D b	0.8±0.4 C c
1	4.0±0.2 D a	3.8±0.4 C a	2.6±0.1 D c	1.3±0.1 B c
2	4.7±0.3 C a	4.7±0.0 B a	2.5±0.1 B b	1.1±0.4 BC c
4	6.0±0.1 B a	4.9±0.1 B b	2.6±0.0 B c	0.8±0.3 C d
7	7.8±0.7 A a	7.0±0.4 A b	3.9±0.1 A c	2.6±0.2 A d

Values within a column followed by different uppercase letters, and values within a row followed by different lowercase letters are significantly (P<0.05) different.

Table A25 (Data for Figures 6.3 and 6.4). *Listeria monocytogenes* counts (mean ± standard deviation) of cooked chicken during a 7 day aerobic storage at 7°C before (control) and after reheating to internal temperatures of 50, 60, and 70°C using domestic oven and stove-top methods.

Storage (Day)	*Listeria monocytogenes* counts (log CFU/g)			
	Control	50°C	60°C	70°C
Domestic Oven				
0	3.7±0.1 D a[b]	2.9±0.1 E b	2.7±0.1 E b	1.4±0.3 E c
1	3.8±0.1 D a	3.7±0.1 D b	3.1±0.0 D c	1.8±0.2 D d
2	4.6±0.1 C a	4.5±0.4 C a	4.0±0.1 C b	2.6±0.2 C c
4	6.2±0.1 B a	5.7±0.3 B b	4.9±0.1 B c	4.0±0.1 B d
7	7.8±0.2 A a	6.7±0.1 A b	6.6±0.4 A b	5.9±0.1 A c
Stove-top Reheating				
0	3.7±0.1 D a	1.7±0.1 E b	1.1±0.4 D c	<0.5±0.4 D d
1	3.8±0.1 D a	1.8±0.1 D b	1.3±0.2 D c	<0.4±0.1 D d
2	4.6±0.1 C a	2.6±0.1 C b	2.1±0.1 C c	1.5±0.1 C d
4	6.2±0.1 B a	4.6±0.1B b	3.8±0.1B c	3.0±0.1 B d
7	7.8±0.2 A a	6.8±0.0 A b	5.6±0.1 A c	4.8±0.0 A d

Values within a column (for domestic oven and stove-top methods separately) followed by different uppercase letters, and values within a row followed by different lowercase letters are significantly (P<0.05) different.

Table A26 (Data for Figures 6.5 and 6.6). Effects of domestic oven reheating against aerobic plate counts (log CFU/g) of cooked chicken during a 7 day aerobic storage at 7°C (mean ± standard deviation).

		Samples Internal Temperatures		
Storage (Day)	Control	50°C	60°C	70 °C
Domestic Oven				
0	3.3±0.5 E a	2.9±0.0 E b	2.7±0.1 D b	2.1±0.1 D c
1	4.0±0.2 D a	3.9±0.1 D a	3.6±0.1 C b	2.2±0.3 D c
2	4.7±0.3 C a	4.4±0.2 C b	3.8±0.1 C c	3.3±0.2 C d
4	6.0±0.1 B a	5.3±0.1 B b	4.8±0.1 B c	4.7±0.1 B d
7	7.8±0.7 A a	6.9±0.1 A b	5.7±0.2 A c	6.7±0.1 A b
Stove Top				
0	3.3±0.5 E a	2.7±0.1 E b	2.5±0.1 D b	1.9±0.1 E c
1	4.0±0.2 D a	3.8±0.2 D a	2.8±0.1 D b	<0.4±0.0 D c
2	4.7±0.3 C a	4.1±0.5 C b	3.0±0.4 C c	2.2±0.2 C d
4	6.0±0.1 B a	4.8±0.1 B b	4.5±0.3 B b	4.1±0.4 B c
7	7.8±0.7 A a	7.0±0.2 A b	6.7±0.1 A b	5.9±0.1A c

Internal temperature of cooked chicken samples. For each reheating methods, values with a column followed by different uppercase letters, and values within a row followed by different lowercase letters are significantly (P<0.05) different.

Table A27 (Data for Figure 6.7). Time and temperature profiles (mean±SD) of samples reheated by domestic oven and stove top methods.

	Oven			Stove-top		
	Internal Temperatures			Internal Temperatures		
Time (min)	50°C	60°C	70°C	50°C	60°C	70°C
0	9.22±0.10	9.12±0.10	9.07±0.10	9.00±0.10	9.10±0.10	7.03±0.10
1	10.47±0.10	10.47±0.10	10.47±0.10	10.97±0.10	11.33±0.10	10.97±0.10
2	12.89±0.10	11.28±0.10	10.95±0.10	11.27±0.10	12.33±0.10	11.27±0.10
3	16.28±0.10	12.89±0.10	11.36±0.10	12.28±0.10	13.06±0.10	12.28±0.10
4	18.65±0.10	13.54±0.10	12.89±0.10	14.23±0.10	14.65±0.10	13.65±0.10
5	20.92±0.10	14.29±0.10	13.06±0.10	16.87±0.10	16.67±0.10	14.23±0.10
6	23.65±0.10	16.28±0.10	14.36±0.10	20.69±0.10	17.65±0.10	15.36±0.10
7	26.40±0.10	17.63±0.10	15.63±0.10	26.24±0.10	19.38±0.10	16.87±0.10
8	31.73±0.10	18.39±0.10	16.28±0.10	42.82±0.10	23.63±0.10	19.65±0.10
9	36.84±0.10	20.92±0.10	18.63±0.10	49.65±0.10	26.68±0.10	23.64±0.10
10	41.48±0.10	21.36±0.10	20.92±0.10	50.09±0.10	30.15±0.10	26.24±0.10
11	45.64±0.10	22.63±0.10	26.40±0.10		33.25±0.10	42.82±0.10
12	49.40±0.10	25.63±0.10	27.65±0.10		41.32±0.10	50.51±0.10
13	50.12±0.10	27.36±0.10	28.63±0.10		47.50±0.10	51.73±0.10
14		31.73±0.10	29.45±0.10		57.12±0.10	51.53±0.10
15		34.61±0.10	30.60±0.10		60.11±0.10	57.03±0.10
16		38.26±0.10	31.73±0.10			63.94±0.10
17		41.48±0.10	36.84±0.10			68.47±0.10
18		49.40±0.10	45.64±0.10			70.05±0.10
19		60.07±0.10	53.31±0.10			
20			60.98±0.10			
21			61.63±0.10			
22			64.12±0.10			
23			66.29±0.10			
24			68.17±0.10			
25			70.03±0.10			

Table A28 (Data for Figure 7.3). Effects of microwave reheating for 45 seconds on reduction of inoculated *Listeria monocytogenes* (log CFU/g) during a 7 day aerobic storage at 7°C.

* Values are mean ± standard deviation		Storage days at 7°C				
		0*	1	2	4	7
Non-marinated control	Log reduction	2.1 ± 0.3 A Z	2.6 ± 0.3 A Y	2.6 ± 0.2 A X	3.4 ± 0.1 AB W	4.1 ± 0.5 A V
	Survivors	0.6 ± 0.2 a z	0.7 ± 0.2 a y	1.8 ± 0.1 a x	2.3 ± 0.3 b w	3.4 ± 0.0 b v
Commercial Tomato-based	Log reduction	2.0 ± 0.2 A Z	2.2 ± 0.1 B Y	2.1 ± 0.2 BC X	3.7 ± 0.2 A W	4.1 ± 0.4 A V
	Survivors	0.6 ± 0.2 a w	0.6 ± 0.2 abc w	0.7 ± 0.2 b w	1.0 ± 0.3 de w	1.8 ± 0.2 f v
Commercial Soy-based	Log reduction	2.2 ± 0.2 A X	2.2 ± 0.1 AB X	2.5 ± 0.2 AB W	2.7 ± 0.1 D W	3.8 ± 0.1 AB V
	Survivors	0.5 ± 0.2 a x	0.3 ± 0.1 bc x	0.7 ± 0.3 b wx	0.9 ± 0.2 e w	3.1 ± 0.1 c v
Commercial Lemon-based	Log reduction	1.9 ± 0.2 A X	1.9 ± 0.2 B X	1.8 ± 0.2 C X	3.2 ± 0.2 BC W	3.5 ± 0.1 B V
	Survivors	0.6 ± 0.3 a y	0.7 ± 0.2 ab xy	0.9 ± 0.2 b x	1.5 ± 0.1 c w	2.1 ± 0.1 e v
Tomato-based	Log reduction	2.0 ± 0.3 A W	2.2 ± 0.3 B VW	2.5 ± 0.4 AB V	3.0 ± 0.3 CD V	2.9 ± 0.1 CD V
	Survivors	0.5 ± 0.2 a y	0.8 ± 0.2 a y	1.6 ± 0.4 a x	3.3 ± 0.1 a w	4.0 ± 0.3 a v
Soy-based	Log reduction	2.0 ± 0.2 A X	2.2 ± 0.4 AB X	2.3 ± 0.1 AB X	3.0 ± 0.1 CD W	3.4 ± 0.1 BC V
	Survivors	0.7 ± 0.2 a y	0.7 ± 0.3 ab y	1.0 ± 0.1 b x	2.3 ± 0.1 b w	3.1 ± 0.0 c v
Lemon-based	Log reduction	2.1 ± 0.2 A X	2.2 ± 0.1 AB X	2.1 ± 0.3 BC X	2.4 ± 0.2 E W	2.7 ± 0.5 D V
	Survivors	0.5 ± 0.2 a xy	0.3 ± 0.0 bc y	0.7 ± 0.3 b x	1.4 ± 0.4 c w	2.6 ± 0.2 d v

Table A29 (Data for Figure 7.4). Effects of microwave reheating at 45 seconds on reduction of inoculated aerobic plate counts during a 7 day aerobic storage at 7°C.

		Storage days at 7°C				
		0	1	2	4	7
Non-marinated control	Log reduction	2.5 ± 0.1 A X	2.4 ± 0.3 AB X	2.7 ± 0.2 A X	3.5 ± 0.2 A W	4.0 ± 0.2 A V
	Survivors	0.7 ± 0.2 a z	1.1 ± 0.2 a y	1.8 ± 0.1 a x	2.5 ± 0.2 b w	3.8 ± 0.1 b v
Commercial Tomato-based	Log reduction	2.3 ± 0.2 AB X	2.6 ± 0.2 A X	2.0 ± 0.2 CD Y	2.9 ± 0.2 BC W	4.2 ± 0.5 A V
	Survivors	0.6 ± 0.2 a x	0.5 ± 0.2 c x	1.1 ± 0.2 b w	1.2 ± 0.3 cd w	2.7 ± 0.3 d v
Commercial Soy-based	Log reduction	2.0 ± 0.1 B X	2.1 ± 0.3 B X	2.6 ± 0.2 AB W	2.4 ± 0.1 D W	4.1 ± 0.4 A V
	Survivors	0.7 ± 0.1 a x	0.7 ± 0.2 bc x	0.6 ± 0.2 c x	1.0 ± 0.2 d w	3.3 ± 0.1 c v
Commercial Lemon-based	Log reduction	2.1 ± 0.3 AB W	2.2 ± 0.2 AB W	1.9 ± 0.2 D W	2.1 ± 0.1 D W	3.7 ± 0.5 AB V
	Survivors	0.7 ± 0.3 a y	0.7 ± 0.1 bc y	1.0 ± 0.1 b x	1.5 ± 0.2 c w	3.5 ± 0.1 c v
Tomato-based	Log reduction	2.1 ± 0.4 AB X	2.2 ± 0.3 AB X	2.2 ± 0.4 BCD X	2.8 ± 0.1 C W	3.0 ± 0.1 C V
	Survivors	0.7 ± 0.3 a y	0.9 ± 0.2 ab y	2.0 ± 0.2 a x	3.5 ± 0.1 a w	4.3 ± 0.1 a v
Soy-based	Log reduction	2.0 ± 0.1 B Y	2.0 ± 0.1 B Y	2.4 ± 0.1 ABC X	3.2 ± 0.2 AB W	3.3 ± 0.2 BC V
	Survivors	0.9 ± 0.1 a x	1.0 ± 0.0 ab x	1.0 ± 0.1 b x	2.4 ± 0.1 b w	2.9 ± 0.2 d v
Lemon-based	Log reduction	2.1 ± 0.2 AB W	<2.2 ± 0.2 AB W	2.0 ± 0.2 CD W	2.1 ± 0.4 D W	2.3 ± 0.3 D V
	Survivors	0.8 ± 0.2 a x	<0.5 ± 0.1 c y	0.9 ± 0.2 bc x	2.1 ± 0.2 b y	2.8 ± 0.2 d v

*Values are mean ± standard deviation of two repetitions with three samples per treatment. Log reduction values followed by different uppercase letters of A, B, C, D, E, F, or G within each column, and followed by different uppercase letters of V, W, X, Y, or Z within each row, are statistically (P<0.05) different. Survivor values followed by different lowercase letters of a, b, c, d, e, f, or g within each column, and followed by lowercase letters of w, z, y, or z within each row are statistically (P<0.05) different.

Table A30 (Data for Figure 7.). Effects of microwave reheating for 90 seconds on reduction of inoculated *Listeria monocytogenes* during a 7 day aerobic storage.

		Storage days at 7°C				
		0	1	2	4	7
Non-marinated control	Log reduction	<2.4 ± 0.1 A X	<3.0 ± 0.2 A X	3.5 ± 0.2 A W	4.3 ± 0.3 AB V	4.4 ± 0.4 AB V
	Survivors	<0.3 ± 0.0 a y	<0.3 ± 0.1 a y	0.9 ± 0.1 a x	1.9 ± 0.3 a w	2.6 ± 0.2 a v
Commercial Tomato-based	Log reduction	<2.3 ± 0.2 A X	<2.5 ± 0.1 BC X	<2.5 ± 0.1 B W	4.3 ± 0.2 AB V	4.6 ± 0.4 AB V
	Survivors	<0.3 ± 0.0 a w	<0.3 ± 0.0 a w	<0.3 ± 0.1 b w	0.5 ± 0.2 c w	1.3 ± 0.2 cd v
Commercial Soy-based	Log reduction	<2.4 ± 0.0 A X	2.2 ± 0.2 BCD X	<2.8 ± 0.1 B W	4.3 ± 0.3 AB V	4.1 ± 0.3 BC V
	Survivors	<0.3 ± 0.0 a x	0.4 ± 0.1 a x	<0.3 ± 0.1 b x	0.6 ± 0.2 c w	1.5 ± 0.3 bc v
Commercial Lemon-based	Log reduction	<2.1 ± 0.3 A X	2.0 ± 0.3 D X	2.3 ± 0.3 C X	4.3 ± 0.2 BC V	3.5 ± 0.3 C W
	Survivors	<0.3 ± 0.0 a x	0.5 ± 0.3 a xw	0.5 ± 0.2 b x	0.7 ± 0.2 c w	1.8 ± 0.2 b v
Tomato-based	Log reduction	<2.2 ± 0.1 A Z	<2.6 ± 0.2 ABY	3.7 ± 0.2 A X	4.3 ± 0.1 AB W	5.0 ± 0.5 A V
	Survivors	<0.3 ± 0.0 a x	<0.4 ± 0.3 a x	0.5 ± 0.2 b x	1.3 ± 0.6 b w	2.6 ± 0.2 a v
Soy-based	Log reduction	<2.3 ± 0.3 A Y	<2.5 ± 0.2 BC X	2.9 ± 0.3 B X	4.6 ± 0.4 A W	5.0 ± 0.2 A V
	Survivors	<0.4 ± 0.3 a x	<0.3 ± 0.1 a x	0.4 ± 0.2 b x	0.8 ± 0.2 bc w	1.6 ± 0.4 bc v
Lemon-based	Log reduction	<2.3 ± 0.1 A X	<2.2 ± 0.1 CD X	2.4 ± 0.3 C X	4.0 ± 0.3 B V	3.8 ± 0.1 C W
	Survivors	<0.3 ± 0.0 a w	<0.3 ± 0.0 a w	0.4 ± 0.1 b w	0.3 ± 0.0 c w	0.9 ± 0.3 d v

*Values are mean ± standard deviation of two repetitions with three samples per treatment. Log reduction values followed by different uppercase letters of A, B, C, D, E, F, or G within each column, and followed by different uppercase letters of V, W, X, Y, or Z within each row, are statistically (P<0.05) different. Survivor values followed by different lowercase letters of a, b, c, d, e, f, or g within each column, and followed by lowercase letters of w, z, y, or z within each row are statistically (P<0.05) different.

Table A31 (Data for Figure 7.6). Effects of microwave reheating at 90 seconds on reduction of inoculated aerobic plate counts during a 7 day aerobic storage.

		Storage days at 7°C				
		Day 0	Day 1	Day 2	Day 4	Day 7
		Storage (days)				
Non-marinated control	Log reduction	2.8 ± 0.3 A Y	<2.6 ± 0.3 A Y	3.4 ± 0.3 A X	4.0 ± 0.1 ABC W	4.4 ± 0.2 BC V
	Survivors	0.4 ± 0.2 a y	<0.9 ± 0.3 a x	1.0 ± 0.2 a x	2.1 ± 0.1 a w	3.2 ± 0.1 a v
Commercial Tomato-based	Log reduction	2.5 ± 0.2 AB W	2.6 ± 0.2 A W	2.6 ± 0.2 BCD W	4.1 ± 0.2 AB V	4.5 ± 0.5 B V
	Survivors	0.4 ± 0.2 a x	0.4 ± 0.3 b x	0.5 ± 0.2 b x	0.8 ± 0.2 cd w	1.5 ± 0.2 c v
Commercial Soy-based	Log reduction	2.3 ± 0.3 B Y	2.5 ± 0.1 A Y	2.8 ± 0.3 B X	3.8 ± 0.2 C W	4.5 ± 0.4 B V
	Survivors	0.4 ± 0.1 a x	0.3 ± 0.0 b wx	0.4 ± 0.2 b w	0.5 ± 0.2 d w	1.9 ± 0.2 bc v
Commercial Lemon-based	Log reduction	<2.6 ± 0.1 AB X	<2.5 ± 0.1 A X	2.4 ± 0.2 CD X	3.4 ± 0.1 D W	4.2 ± 0.4 BC V
	Survivors	<0.3 ± 0.0 a y	<0.4 ± 0.1 b xy	0.6 ± 0.2 b x	1.0 ± 0.3 bc w	2.2 ± 0.2 b v
Tomato-based	Log reduction	2.5 ± 0.2 AB Y	2.5 ± 0.2 A Y	3.5 ± 0.3 A X	3.9 ± 0.1 BC W	5.4 ± 0.1 A V
	Survivors	0.3 ± 0.1 a y	0.5 ± 0.3 b xy	0.7 ± 0.2 ab x	1.2 ± 0.1 b w	3.2 ± 0.1 a v
Soy-based	Log reduction	<2.6 ± 0.1 AB X	<2.6 ± 0.2 A X	2.7 ± 0.3 BC X	4.3 ± 0.1 A W	4.7 ± 0.2 B V
	Survivors	<0.3 ± 0.1 a y	<0.4 ± 0.2 b xy	0.6 ± 0.3 ab x	1.1 ± 0.1 bc w	1.9 ± 0.1 b v
Lemon-based	Log reduction	<2.6 ± 0.1 AB W	<2.4 ± 0.1 A W	2.2 ± 0.1 CD W	3.9 ± 0.3 BC V	3.8 ± 0.4 C V
	Survivors	<0.3 ± 0.0 a x	<0.3 ± 0.1 b x	0.6 ± 0.1 b w	0.7 ± 0.2 d w	1.1 ± 0.4 d v

*Values are mean ± standard deviation of two repetitions with three samples per treatment. Log reduction values followed by different uppercase letters of A, B, C, D, E, F, or G within each column, and followed by different uppercase letters of V, W, X, Y, or Z within each row, are statistically (P<0.05) different. Survivor values followed by different lowercase letters of a, b, c, d, e, f, or g within each column, and followed by lowercase letters of w, z, y, or z within each row are statistically (P<0.05) different.

Table A32 (Data for Figures 7.1 and 7.2). Survival and multiplication of L. monocytogenes and background microflora on marinated cooked chicken stored aerobically at 7°C.

	Storage (days)				
(Selective Counts)	0	1	2	4	7
Non-marinated control	2.7±0.1	3.3±0.1	4.4±0.2	6.3±0.2	6.9±0.3
Commercial Tomato-based	2.6±0.1	2.8±0.1	2.9±0.1	5.0±0.2	5.5±0.3
Commercial Soy-based	2.7±0.1	2.6±0.1	3.1±0.1	4.7±0.2	5.8±0.3
Commercial Lemon-based	2.4±0.2	2.5±0.1	2.7±0.1	5.0±0.3	5.3±0.2
Domestic Tomato-based	2.5±0.1	2.9±0.1	4.2±0.1	6.2±0.2	6.9±0.3
Domestic Soy-based	2.7±0.1	2.9±0.1	3.3±0.1	5.7±0.2	6.1±0.3
Domestic Lemon-based	2.6±0.2	2.5±0.1	2.8±0.1	4.1±0.3	4.9±0.2
(Non-selective Counts)					
Non-marinated control	3.2±0.1	3.5±0.1	4.4±0.1	6.5±0.2	7.3±0.3
Commercial Tomato-based	2.9±0.2	3.0±0.2	3.1±0.2	5.3±0.2	5.7±0.3
Commercial Soy-based	2.7±0.2	2.8±0.2	3.2±0.1	5.0±0.3	5.7±0.3
Commercial Lemon-based	2.9±0.1	2.8±0.2	2.9±0.2	5.2±0.3	5.6±0.3
Domestic Tomato-based	2.8±0.1	3.1±0.1	4.2±0.2	6.5±0.3	7.1±0.2
Domestic Soy-based	2.9±0.1	3.0±0.1	3.4±0.1	5.7±0.3	6.2±0.3
Domestic Lemon-based	2.9±0.1	2.7±0.1	2.8±0.1	4.5±0.3	5.0±0.1